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Controlled release in inhalation

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Doctor of Philosophy

2007

Inhalation has become an increasingly viable alternate route to oral dosing, with the advantage of treating local disease with minimal systemic side effects (Hickey, 1992). However, increasingly complicated medication regimens associated with the necessity of the repeated dosing of multiple agents used in treating pulmonary disease has been shown to compromise both disease management and patient convenience (Gumbelton, 2001; Gutierrez et al., 1996; Vermaelen et al., 2001).

In this study the viability of spray drying to introduce controlled release vectors into dry powders for inhalation was investigated. The first experimental section highlights the use of leucine in producing highly respirable spray dried powders, with *in vitro* respirable fractions (Fine particle fraction, FPF: $F < 5\mu m$) exceeding 80% of the total dose. The second experimental chapter introduces the biocompatible polymer chitosan (mw 190 – 310 kDa) to formulations containing leucine with findings of increased FPF with increasing leucine concentration (up to 82%) and the prolonged release of the active markers terbulataline sulfate (up to 2 hours) and beclometasone dipropionate (BDP: up to 12 hours) with increasing chitosan molecular weight.

Next, the thesis details the use of a double emulsion format in delivering the active markers salbutamol sulfate and BDP at differing rates; using the polymers poly-lactide co-glycolide (PLGA 50:50 and PLGA 75:25) and/or chitosan incorporating leucine as an aerosolisation enhancer the duration of *in vitro* release of both agents reaching 19 days with FPF exceeding 60%. The final experimental chapter involves dual aqueous and organic closed loop spray drying to create controlled release dry powders for inhalation with *in vitro* sustained release exceeding 28 days and FPF surpassing 55% of total loaded dose.

In conclusion, potentially highly respirable sustained release dry powders for inhalation have been produced by this research using the polymers chitosan and/or PLGA as drug release modifiers and leucine as an aerosolisation enhancer.

Key words: Dry powder inhaler, spray drying, leucine, chitosan, PLGA.

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List of abbreviations

AC	Adenylate cyclase
ACI	Andersen cascade impactor
AFM	Atomic force microscopy
ANOVA	Analysis of variance statistical test
ARG	Arginine
ASP	Aspartic acid
av	Average
BDP	Beclometasone dipropionate
BP	British Pharmacopoeia
CE	Chitosan emulsion
CFC	Chloroflourocarbon
COPD	Chronic obstructive pulmonary disease
COX	Cyclo-oxygenase
δ_{ae}	Primary particle diameter
$\delta_{ae}:\omega$	Primary particle diameter: Thermal efficiency
DPPC	Diphosphatidylcholine
DSC	Differential scanning calorimetry
ED	Emitted dose
FPD	Fine Particle dose
FPF	Fine Particle Fraction
GRAS	Generally regarded as safe
GSK	GlaxoSmithKline
HEPA	High efficiency particulate air (filter)
HFA	Hydroflouroalkane
HMW	High molecular weight (chitosan)
HPC	High performance cyclone
HPLC	High performance liquid chromatography
HPMC	Hydroxy-propyl methylcellulose
Ld	Loaded dose
LD:δ_{ae}	Aggregation ratio
LEU	Leucine
LMW	Low molecular weight (chitosan)
LMW/MMW	Low / medium molecular weight (chitosan)
LT	Leukotriene
Ltd	Limited (company)
m	Gradient
MMAD	Mass median aerodynamic diameter
MMW	Medium molecular weight (chitosan)
MMW/HMW	Medium / high molecular weight (chitosan)
MSLI	Multistage liquid impinger
P <	Probability less than
P >	Probability greater than
PBS	Phosphate buffered saline
PEG	Polyethylene glycol

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PG	*Prostaglandin*
PHE	*Phenylalanine*
PLA	*Polylactic acid*
PLA$_2$	*Phospholipase A$_2$*
PLGA	*Polylactide co-glycolide*
PVA	*Polyvinyl alcohol*
PVP	*Polyvinyl pyrollidone*
SA	*Surface area*
SC	*Standard cyclone*
sd	*Standard deviation*
SEM	*Scanning electron microscopy*
SS	*Salbutamol sulfate*
TD	*Total dose*
TGA	*Thermogravimetric analysis*
THR	*Threonine*
TMC	*Trimethyl-chitosan*
TXA	*Thromboxane*
UK	*United Kingdom*
USA	*United States of America*
USP	*United States Pharmacopoeia*
UV	*Ultraviolet*
V	*Volume*
VMD	*Volume weighted mean diameter*
ω	*Thermal efficiency*

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Chapter 1

General Introduction

1.1 Delivery to the lung.

1.1.1 A brief History

The lung has been used as either a point of systemic entry or as a target for medicinal purposes since inhalation therapy was first described as Ayurvedic Medicine more than 400 years ago and *Atropa belladonna* leaves were smoked to relieve diseases of the throat and chest (O'Callaghan et al., 2002). Much later the realisation of the lungs as a target for pharmacotherapy, by Bennett's TB inhalation therapy (1664) followed by Potter's asthma cigarettes containing *Datura strammonium* leaves (c.1800), was recognised by western medicine (O'Callaghan et al., 2002). Modern day inspiratory medicine was potentially kick started through the introduction of preliminary nebulisers in the late 1820's (O'Callaghan et al., 2002), preliminary dry powder inhaler (DPI) formulations later rivalling the nebuliser with formulations such as the 'Carbolic Smoke Ball' of 1889; the modern day DPI eventually transpiring as the Intal® sodium cromoglicate Spinhaler® after clinical trials in 1967 (Edwards, 2005; Howell, 2005; Greiner & Meltzer, 2006). However since its introduction in 1956 the pressurised meter dose inhaler (pMDI) has proven to be the main vehicle of drugs to be dispersed into the lungs with global sales exceeding 500,000,000 pMDI units per annum in 1994 (Dalby et al., 1996).

1.1.2 Why pulmonary delivery?

The lung is a vital organ with a relatively large surface area (over 180 m²), the membranes of the pulmonary surface allowing the diffusion of macromolecules with local therapy through inhalation limiting the possibility of systemic side effects and metabolism (Gumbelton, 2001; Gutierrez et al., 1996; Vermaelen et al., 2001). Aside from the delivery of agents to the surface of the pulmonary airways, systemic delivery is achievable through appropriate size classification. The

lung can provide a non-invasive route of delivery with reduced venous transport to the first pass

mechanics of the liver; the pulmonary circulation feeding directly in to the right aorta (Corcoran,

2006; Sakagami, 2006; Scheuch et al., 2006).

1.1.3 The structure of the lung
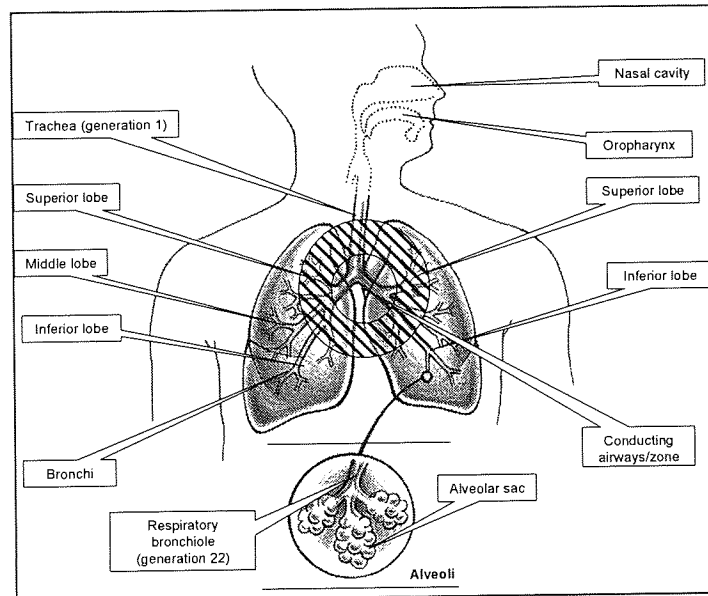
Figure 1.1: Representational cross-section of the human lung. Representation of the cross-section of the human lung with the two left sided pulmonary lobes and the three right pulmonary lobes (based on Mikola, 2007).

The human lung consists of 23 generations of airways and two major zones (Figure 1.1); the

conducting airways of the first 16 pulmonary generations and the respiratory zone of the alveolar

bronchioles consisting of the alveolar ducts at generation 20 and the site of gaseous exchange

the alveolar sacs at generation 23. The inspiration and inflation of the lung is instigated by the

phrenic nerve of the diaphragm and movement of the rib cage via internal and external

intercostal muscles (Frey et al., 2004; Sauiret et al., 2002.; Atwal, 1999; Kemp & Olver, 1996;

Beck et al., 2006).

After the point of entry air is humidified and filtered by the nasal passages or the oropharynx depending on breath type, the air travels to the trachea, a semi rigid airway surrounded by C-shaped cartilage rings with posterior connection to smooth muscle, which divides at the base into two left and three right-sided lung lobes (Terzano, 2001; Koumellis et al., 2005). The airways then repeatedly bifurcate, the lumen of the airways gradually decreasing from the average trachea diameter of 1.8 cm to a 16th generation average diameter of 0.024 cm with an exponentially increasing number of airways (c. 50,000 respiratory bronchioles at generation 22 with a 1000 cm^2 x-sectional area), the velocity of inspired air decreasing from 100 cm/sec at point of *in vivo* entry to zero at the alveoli (Vial et al., 2005).

The lung is considered an aerosol filter by design with patient dynamics, breathing patterns and the physicochemical properties of the formulation determining the deposition of inspired particles (Nazir et al., 2005; Kim & Jaques, 2005; Sosnowski et al., 2006; Bennett & Zeman, 2005; Sturm & Hofmann, 2005; Rabbani & Seville, 2005). The deposition of the particulates in the lung differ depending on particle size, density and inspired flow rate; gravitational and inertial impaction in the airway lumen increasing with particle size and density and reduced velocity with particles less than 1 µm undergoing Brownian motion dependent on diffusional displacement and breath holding for settling. Other factors that have been researched which influence lung drug deposition include surface roughness and porosity (Acerbi et al., 2006; Mahesh Kumar & Misra, 2006; Fiegel et al., 2004; Musante et al., 2002).

Generally speaking, particles of less than 5 µm are considered small enough to navigate the airway divisions post trachea (Martonen & Yang, 1996). Such particles will encounter differing

cell types depending on the site of deposition with lung epithelia varying in type and function throughout the pulmonary tree (Figure 1.2: Fischer et al., 2003; McCann et al., 1989; Bernhard et al., 1997).

The largest population of cells among the airways is that of the ciliated columnar epithelial cells whose primary function is the transport of debris via coordinated cilia movement and mucosal secretion from the airway lumen and out of the trachea where the fluid can be swallowed. The columnar cells also secrete an amount of glycoprotein used in the production of mucus and are present from the trachea throughout the respiratory system to the terminal bronchioles of airway generation 22 (Vaughan et al., 2006; Vermeer et al., 2006; Park et al., 2006; Cullen et al., 2000).

Goblet cells (Figure 1.2), crammed with granules, increase in presence from the higher generations of the respiratory zones through the conducting zone and have a major function of maintaining cilia mucus coverage to a depth of around 6 μm (affected by inflation and disease) for reasons of lung clearance and foreign body protection; goblet cells are also a progenitor for ciliated columnar cells (Long et al., 2006; Kim & Follinsbee, 1997). Goblet cells gradually replace the serous secreting cells of the respiratory bronchioles that provide less viscous fluids for the alveolar regions of gaseous exchange (O'Callaghan et al., 2002; Shimizu et al., 2003).

Basement basal cells are also present anchoring ciliated columnar cells, cilia beating at an estimated 1000 beats per minute, along with sparse brush transitional cells and glycoprotein secreting Clara cells throughout the bronchioles (Broody, 2005; Sbarbati & Osculati, 2005; Hickey & Thompson, 1992; Altiere & Thompson, 1996; Kim & Follinsbee, 1997; Perl et al., 2005).

The structure of the lung airway epithelium therefore provides a significant obstacle in addition to airway geometry for the local delivery of therapeutic particulates. Clearance rates reaching 1 mm/min in the deep lung increasing to 12 mm/min in the trachea. The total lung clearance of inspired particulates in healthy subjects, independent of accelerated proximal cough reflex expulsion, ranging from 24 to 48 hours (Evans et al., 2003; Langenback et al., 1990; Hickey & Thompson, 1992; Altiere & Thompson, 1996; Kim & Follinsbee, 1997; Stocks & Hislop, 2002).
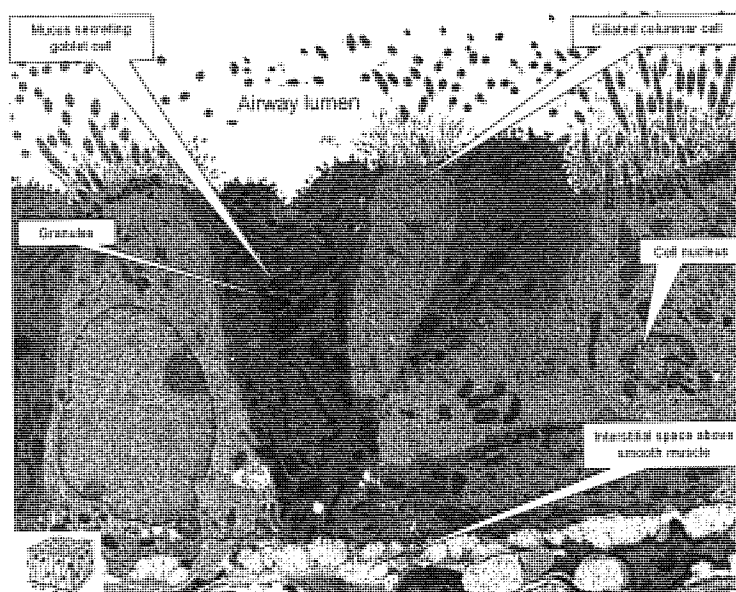
Figure 1.2: A cross sectional light microscope image of the bronchi epithelia (based on Junquiera et al., 1998).

The entry point of gaseous molecules and systemic active agents to the blood, the alveoli, consists of type I (accounting for 97% of total gaseous exchange) and type II pneumocytes, which are responsible for surfactant secretion of the 80% phosphatidylcholine, 9% phosphatidylglycerol coverage and the production of type I cells. There is a high presence of mobile phagocytic alveolar macrophage and alveolar structure differs largely from the bronchial tree airways in terms of porosity and clearance. Clearance, which can take anything up to one year dependent upon endocytic and phagocytic transport out of the alveoli, is usually instigated by macrophages (Abbate et al., 2006; Stocks & Hislop, 2002; Dubourdeau et al., 2006 Wustneck

et al., 2005). The uptake method of active agents targeted at the alveoli is vesicular and differs largely from lung bronchi mechanisms that include diffusive, active and vesicular transcellular and paracellular absorption (Stocks & Hislop, 2002; Kumar et al., 2006).

1.2 Pulmonary disease management.

1.2.1 Systemic disease

The delivery of therapeutic agents to the lung can be broadly split into two sub categories depending on the disease therapy and the pulmonary target area. Generally speaking the sub categories are local delivery, and with the recent advent of novel methods of dry powder formulation and improved devices, the sedimentation of agents in the far-reaching alveoli for access to the cardiac venous circulation determining systemic delivery. A recent example of the treatment of a systemic disease via the lungs is the delivery of recombinant human insulin via the Exubera® device to diabetics (De Galan et al., 2006).

1.2.2 Pulmonary disease and therapy

The focus of the research undertaken during the production of this thesis involves the production of sustained release powders designed for local delivery to the bronchi; the introduction will now compliment this focus. Examples of compounds delivered for local use include sympathomimetic bronchodilators, glucocorticosteroids, anticholinergics (e.g. ipratropium), antibiotics (e.g. kanamycin), antivirals (e.g. Ribavarin), anti-allergenics (e.g. sodium cromoglicate), surfactant replacements, the anticoagulant heparin and enzyme inhibitors (e.g. α_1-antitrypsin and catalyse) (Hiller, 1992; Smith & Bernstein, 1996; Derom & Thorsson, 2002).

Local delivery to the lung is largely concerned with the symptomatic treatment and prevention of pulmonary diseases such as reversible obstructive airway disease (asthma), chronic obstructive pulmonary disease (COPD), and those implicated in the condition cystic fibrosis. Obstructive airway disease, or asthma, was the model disorder advocated in this research, with encompassing standard therapies of the chronic form of the disease relating the use of bronchodilators and anti-inflammatory agents (Janssen & Killian, 2006; Beck et al., 1999; Jarjour et al., 2006; Morice et al., 2006).

1.2.2.1 Asthma pathology

On a histological level the pathology of asthma is ultimately associated with smooth muscle cell contraction and a reduction in the calibre of the airway lumen (Stocks & Hislop, 2002). Irritant receptors, hypothetically located in the cilia mucosal columnar cells of the bifurcating airways epithelia for protection, provide the initial innervation (Hickey & Thompson, 1992). A foreign body in close proximity to an irritant receptor within the bronchi lumen induces receptor conformational change with the G protein α subunit of the associated receptor that signals activation of adenylate cyclase (AC) and phospholipase A_2 enzymes. PLA_2 activation cleaves arachidonic acid from cellular membrane phospholipids revealing substrate for cycloxygenase (COX) and 5-lipoxygenase in the production of prostaglandins (PG) and leukotrienes (LT) respectively. The resulting metabolites PGD_2 and $PGF_{2\alpha}$ give direct smooth muscle contraction possibly through a cholinergic reflex, PGF_2 increasing mucus secretion. LT metabolites LTC_4, LTD_4 and LTE_4 induce idiopathic constriction and gland secretion with thromboxane A_2 potentiating direct contraction via the influx of Ca^{2+} into the smooth muscle cells of the bronchi. Not all the produced mediators are broncho-constrictors; PGE_2 being a potent bronchodilator

(Hickey & Thompson, 1992, Altiere & Thompson, 1996; Kim & Folinsbee, 1997; Stocks & Hislop, 2002; Mukhopadhyay et al., 2006).

Stimulation of the irritant receptor can also be triggered following lumen mast cell destabilisation by foreign antigens resulting in the release of the broncho-constrictor Histamine H1, potentiator tryptase and of goblet cell agonists Histamine H2 and chymase (Hickey & Thompson, 1992, Altiere & Thompson, 1996; Stocks & Hislop, 2002; Fujimori, 1996). Other cells present within the lung proximity capable of delivering the same biologically active inflammatory mediators include eosinophils, macrophages, neutrophils and platelets (which deliver bradykinin from α^2 globulins), all have the potential of causing bronchoconstriction (Stocks & Hislop, 2002; Ying et al., 2006; McKinley et al., 2006; Abraham et al., 2006).

1.2.2.2 B₂ receptor agonists

Of the agents developed to combat restrictive airway disease, β_2 receptor agonists, which antagonise choline induced bronchoconstriction, are a series of bronchodilators (e.g. salbutamol, terbutaline) built around a phenolic ring with a nitrogen substituted side-chain at the 9 position to give β_2 receptor selectivity (Figure 1.3); the increasing number of β_2 receptors in the airways of the lower lung giving a large degree of specificity (Thompson, 1992). The typical duration of bronchodilation resulting from the administration of relatively high doses (e.g. 100 µg terbutaline hemisulfate) of simple β_2 agonists to the airway epithelia ranges from 3 to 6 hours (Anderson et al., 1998), however modification of the side chain (Figure 1.3C) has given greater latent receptor affinity with the long acting β_2 agonist salmeterol having up to a 12 hour duration of action (100 µg dose: Lotvall et al., 2006). Despite the pharmacological improvement, the indication of a short

acting β_2 agonist in phase three asthmatics for symptom breakthrough is still recommended in

conjunction with a long acting β_2 agonist (Tashkin, 2006). The side effects of chronic β_2 agonist

administration and subsequent toxic plasma levels include tremor and headache (Broadley,

2006).
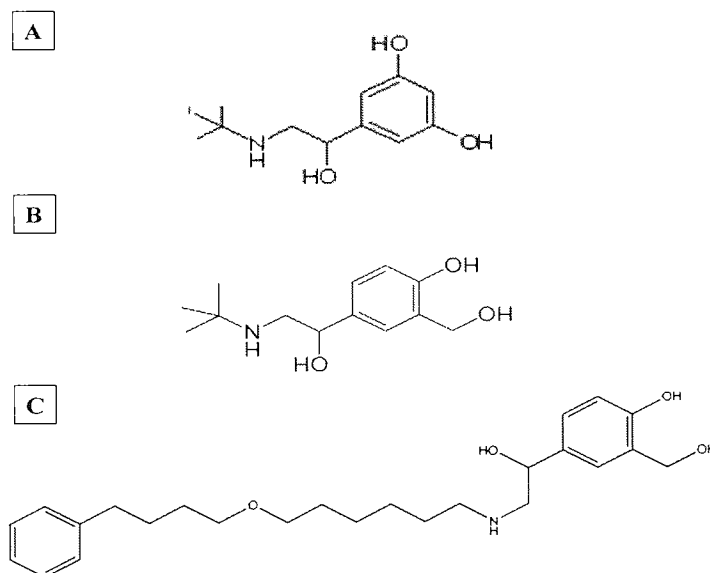
Figure 1.3: The chemical structures of: A. terbutaline; B. salbutamol; C. salmeterol.

1.2.2.3 Corticosteroids

Corticosteroids have been previously described as the most effective drugs in treatment of

asthma (Derom & Thorsson, 2002). The mode of action of corticosteroids in the treatment of

asthma remains ambiguous. It is thought that they reduce mucosal oedema via reduced capillary

permeability, suppress inflammatory response through inhibition of mast cell mediator release,

exert action by inhibiting COX and lipoxygenase enzymes involved in the arachidonic acid

cascade (Levy et al., 2005), stabilise membrane permeability and decrease extra neuronal

uptake of catecholamines (Bisgaard et al., 2002). The potency of corticosteroids administered

via the lung is not in doubt, 400 ug of inhaled beclometasone dipropionate (BDP) equilibrating to

the same local pulmonary action as 7.5 mg of orally administered prednisolone sodium (Derom & Thorsson, 2002). It is thought severe side effects such as Cushing's syndrome, osteoporosis in the elderly and growth retardation manifest from prolonged oral corticosteroid administration (Collice, 2006).

The structures of corticosteroids are loosely based on that of a fluorinated cholesterol molecule, beclometasone being the only non-fluorinated member of the family (Figure 1.4). Beclometasone was the first inhaled steroid used in the treatment of acute respiratory distress and is utilised as a model active agent in this research (Wilcox & Avery, 1973). Beclometasone delivery to the lung is via the pro-drug beclometasone dipropionate (BDP) converted on absorption in to the lung tissues to 17-beclometasone mono-propionate by enzyme reduction at the 17 position, the active metabolite increasing 26 fold the binding affinity to glucocorticoid receptors (Wang & Hochhaus, 2004; Wurthwein & Rohdewald, 1990).

Other agents used in the treatment of restrictive airway disease include the muscarinic receptor antagonists Ipratropium and Tiatropium and the mast cell stabiliser sodium cromoglicate.
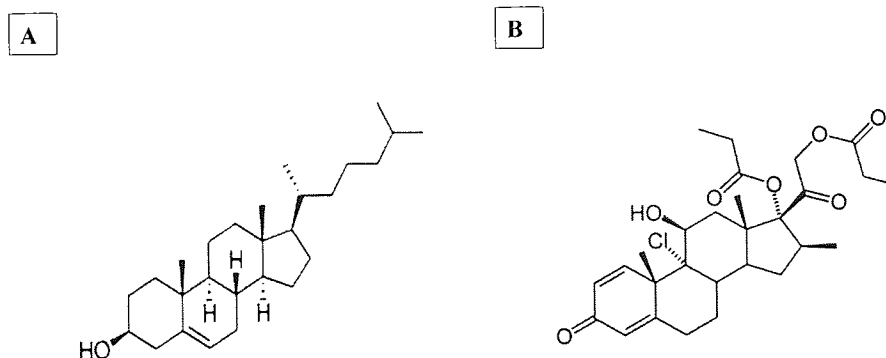
Figure 1.4: The chemical structures of: A. cholesterol, B. beclometasone dipropionate.

1.3 *Formulation and device*

1.3.1 Nebulisers

To date the delivery of active agents can be divided into three subsections with formulations being delivered via nebulisers, pressurised metered dose inhalers (pMDI) and dry powder inhalers (DPI) (Dolovich et al., 2005). Nebulisers are concerned with the delivery of liquid droplets, traditionally using face-masks linked to a condenser. Despite requiring minimal patient breath co-ordination the nebulisers have traditionally been cumbersome and wasteful of loaded drug concentrations (Dolovich et al., 2005; Cates et al., 2006). The design of new nebuliser technology centres around handheld units for portability using minimal volumes of vapour to ensure the aerosol is delivered only during inhalation; one example being jet systems with electromagnets such as the Omron NE-UI4 (Dennis & Nerbrink, 2002), another using ultrasonics to deliver an inhalable mist (e.g. MABISMist™ II Ultrasonic Nebuliser Model 40-270-000: Figure 1.5; Lentz et al., 2006).
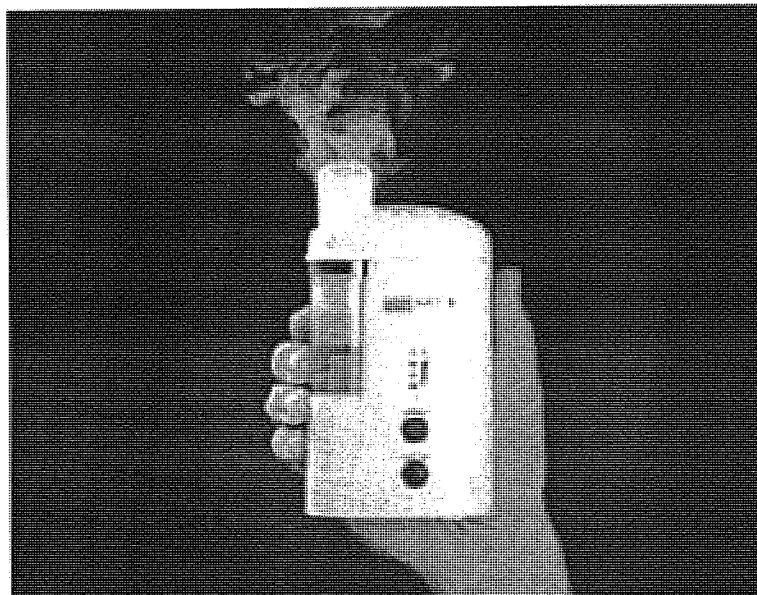
Figure 1.5: Image of the MABISMist™ II Ultrasonic Nebuliser Model 40-270-000 (Mabis Healthcare., 2007).

1.3.2 Pressurised metered dose inhalers (pMDI)

The delivery of particulates to the lung is dominated by pMDI and DPI formulations, which require 4 basic features; a dose metering mechanism, an aerosolisation mechanism, a de-aggregation mechanism and an adaptor to direct the aerosol into the patient's mouth (Hickey, 1992). A potential pMDI device (Figure 1.6) involves the use of micronised drug suspended in a pressurised canister of propellant with possible co-solvents and surfactants to aid dispersion (Smyth, 2003). The potential propellants are usually hydroflouroalkane (HFA) derivates following the cessation in the production of chlorofluorocarbons (CFC's) with ethanol the common co-solvent (Seville et al., 2000). Metered dose inhaler demand exceeds 500,000,000 per year however high velocities that impinge large fractions of the emitted dose at the back of the oropharynx and poor patient breath/actuation coordination vastly reduce the efficiency of this form of pulmonary delivery, although the use of spacer devices can reduce MDI losses from greater than 90% to just over 70% (Dalby et al., 1996; O'Callaghan & Wright, 2002). However, high performance has been achieved with the QVAR pMDI which with a low particulate mass median aerodynamic diameter of 1.3 µm has been shown to deliver beyond 50% of the emitted dose to the respiratory tract of adolescents (Van Schayck & Donell, 2004).

Figure 1.6: Image of the GlaxoSmithKline (GSK) Seretide® pMDI combination inhaler, containing 25 µg of the long acting beta-2 agonist salmeterol xinafoate and 50 µg of the corticosteroid fluticasone propionate.

1.3.3 Dry powder inhalers (DPI)

Marketed pMDI formulations are largely mirrored by marketed DPI formulations. Typical DPI formulations are based around the use of a micronised drug and large carrier particle, invariably lactose (Steckel et al., 2006). The production of the micronised drug largely depends on jet milling which leads to the generation of amorphous areas with in the crystalline drug powder, giving wide poly-disperse populations of differing electrostatic charge induces cohesive and adhesive forces which hamper the flow and therefore the delivery of the majority of the dose to the lung making the use of a carrier particle necessary (Steckel & Brandes, 2004; French et al., 1996; Feeley et al., 1998; Elamin et al., 1995; Buckton, 1997). The micronised drug is compounded until the diameter of particles is below 5 µm (a size regarded as respirable), and is then blended with the carrier which usually has a particle size range of 60 to 70 µm (Dalby et al., 1996). On inspiration, as is the case with pMDI formulations, the force of inspiration (a recommended 28.3 L/min for pMDI's and 60 L/min for DPI's *in vitro*) overcomes some of the surface free energy (consisting mainly of electrostatic, van der Walls and capillary forces) involved in forces of adhesion between micronised drug and carrier particles liberating a minority of the drug (Dalby et al., 1996). The minority is delivered to the lung via the trachea whilst the adhered drug and carrier particles are usually subject to higher gravitational forces and fail to navigate the oropharyngeal bend at the inspired air velocity and impinge at the back of the throat.

The delivery of DPI powders is heavily dependent up on device and formulation (Zeng et al., 2001). The utilisation of a higher resistance device with air baffles to liberate adhered/cohered particles can have a profound effect on the respirability of a DPI formulation (Ehtezazi et al.,

2005). The Spinhaler was the first mass produced DPI in the late 1960's (Figure 1.7D), the device is relatively low in resistance with a basic propeller to instigate turbulent airflow, as a consequence comparatively low performance is seen compared to newer higher resistance devices such as the Turbohaler® (Figure 1.7A), which is used to administer pure micronised drug formulations such as the Bricanyl® terbutaline hemisulfate dry powder (Howell, 2005; Schlimmer, 2002). Indeed a substitution of the Bricanyl® pMDI formulation for the Bricanyl® Turbohaler DPI formulation has shown the effect a device can have on aerosol performance, with an improvement of 6.4% to 18.2% of the emitted dose being regarded as respirable *in vitro* (Dalby et al., 1996). A further advantage of the Turbohaler® over devices such as the Rotahaler® and the Spinhaler® (Figures 1.7B, 1.7D) is the administration of the dose from a reservoir preventing the physical loading of a dose into the DPI unit improving portability and patient convenience (Atkins, 2005). The GSK Diskhaler® is a further popular device of 4 to 60 preloaded doses (Figure 1.7C: Atkins, 2005).

DPI formulations generally show better deposition compared to their pMDI competitors and show better *in vivo* / *in vitro* correlation with a lower fraction of the dose impinging in the oropharynx (Atkins et al., 1992). Extra-thoracic deposition of agents, particularly inhaled glucocorticosteroids, may contribute to the development of systemic side effects if not completely inactivated by 1st pass metabolism of the liver; it is estimated some 20% of inhaled BDP from pMDI's reaches the systemic circulation (O'Callaghan & Wright, 2002). Powder impinging at the back of the throat may also irritate and give a responsive cough reducing the fraction of the dose reaching the mid or deep lung (Buhl, 2006).

A Mouthpiece with spiral shaped channels
Extra air inlets
Inhalation channel
One metered dose
Rotating dosing disk
Drug reservoir
Air inlet
Turning grip
Scrapers
Drying agent

B Twist Barrer
Mouthpiece

C Mouthpiece cover
Mouthpiece
White wheel
Diskhaler lid
White tray
Plastic needle
Cleaning brush
Medication disk

D
Capsule containing 25 mg spray dried powder
Piercing chamber of 2 steel prongs/ site of capsule emptying
Propeller adjacent to capsule fill
Plastic mouth piece site of exit into patient/ impactor

Figure 1.7: Illustration of the internal structures of: A. Astrazeneca® Turbohaler®; B. GSK Rotahaler®; C. GSK Diskhaler® D. Fisons® Spinhaler®. The turbohaler uses a baffled mouthpiece for turbulence and de-aggregation of the airborne formulation where as the rota and diskhalers use a plastic mesh in the mouthpiece and the spinhaler a propeller to disperse particulates (adaptation of: Atkins, 2005).

A major deterrent in the use of DPI devices has been the higher flow rates required to activate the devices compared to the pMDI. The higher flow rate regarded as being unachievable for groups such as infants and the mentally ill, although studies have highlighted that patients suffering from dyspnoea (maximum average inspiratory flow rate c. 188 l/min) can reach the higher flow rate (Zeng et al., 2000a; Atkins, 2005). The lack of visible plume is also a disadvantage for the DPI devices, with patient perceived "lack of benefit" resulting in 40% under use and 20% overuse in one large scale corticosteroid study (Atkins et al., 1992). However, DPI delivery of β-agonists do not have the same problems of patient perceived benefit, with deposition of DPI's out performing pMDI's during asthma attacks (O'Callaghan et al., 2002).

The deposition of liberated drug particles from a DPI is largely dependent upon particulate size and aggregation. Particles or aggregates less than 5 μm generally deposit on the side of the conducting and respiratory airways with particles 1 μm or less in diameter diffusing into the alveoli, dependent on patient inspiratory flow (with the 60 L/min required for DPI activation: Atkins, 2005) and breath holding (O'Callaghan et al., 2002; Lalor & Hickey, 1997). The inspired particle of theoretically >5 μm gradually transforms into an isotonic droplet as it travels down through the pulmonary airway network. The dry particulate transformed by the increasing humidity and temperature from ambient conditions to 37°C and 100 RH at the site of the alveoli (Gonda, 1988). The particle gradually increases in size whilst reaching a state of isotonicity with the lung fluids through the acquisition of moisture from the increasing humidity, growth which can affect the site of deposition of the inspired particulate/ droplet (Gonda, 1988).

1.3.3.1 Novel methods of DPI formulation

Novel methods of dry powder production have lead to increasing possibilities in the formulation and application of DPI's. Over the past 30 years numerous technologies have emerged capable of producing micron size particles:

- Supercritical fluid methods, involving a solute dissolved in a solution either close to its boiling point where the solution is boiled and micronised particles precipitate or where either gas expansion, anti solvents, or high pressures are used for precipitation (Kerc et al., 1999).
- Solvent evaporation techniques, utilising low temperatures (Ungaro et al., 2006).

- High gravity methods, using high gravity precipitation reactors and volatile solvents (Chiou et al., 2006).

- Freeze drying methods, immersing solutes into a volatile solvent (e.g. liquid nitrogen) and snap drying the liquid via a freeze drier has previously been explored as a method of producing respirable particles with varying success (Seville et al., 2002; Van Drooge et al., 2005).

- Spray drying, which will be explored in detail.

A major problem associated with the production of microparticles via these novel approaches of particle micronisation is the presence of associated large surface free energies that drive agglomeration. The upper aerodynamic size limit of the aggregates being dependent upon the relative strength of the surface free energy compared to the gravitational forces exerted during aerosolisation (Saccetti & van Oort, 1996).

1.3.3.2 Spray drying

The focus of dry powder manufacture in this thesis will be spray drying mainly because of the ability of the process to enable a degree of microparticulate design (Masters, 1991). By definition spray drying is a one-step continuous particle processing operation transforming feed from a fluid state into a dry particulate form by spraying the feed in to a hot drying medium (Masters, 1991). For accessibility reasons the spray dryer employed in this research was the Büchi B290 mini lab spray drier (Figure 1.8).
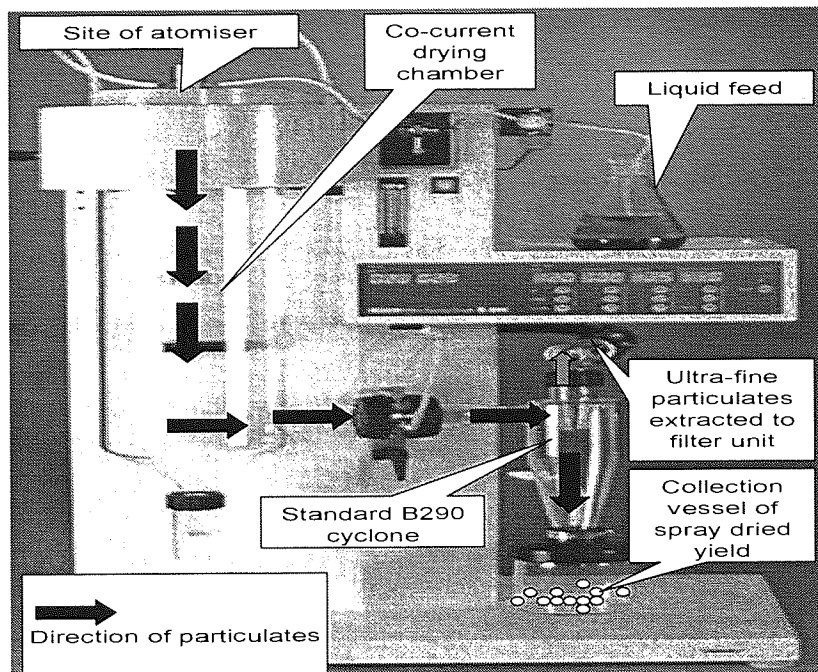
Figure 1.8: Image of the Büchi B290 mini lab spray drier in open cycle format, highlighting the flight of suspended droplets/particulates during production.

Spray drying has applications far wider reaching than the production of dry powders for inhalation, the first significant industrial usage of spray drying occurred in the 1920's with the spray drying of milk and certain detergents to improve the shelf life and handling ease of the relative products. Plastics, ceramics, foods stuffs and hardware products have now all been generated using spray drying (Masters, 1991).

The use of spray drying in the pharmaceutical industries dates back to around 1940 when *Bullock et al.* first spray dried infusions and adrenaline for reconstitution (Masters, 1991). In recent years antibiotics for reconstitution and tablet excipients have been spray dried and spray driers fitted with HEPA (high efficiency particulate air) filters have been used to create sterile products.

The process of spray drying can be roughly split into four generic stages, stage 1 involving the atomisation of liquid feed trough the top of the drying chamber by an atomiser, which in the case of the B290 is a nozzle atomiser. Feed is then projected into a narrow co-current drying chamber (Figure 1.8) of which the dimensions affect drying efficiencies and the particle size generated. Generally wider, shorter drying chambers give higher thermal efficiency through lower atmospheric heat losses (Formula 1.1) and the potential of a finer particulate yield (depending on spray projection).

Thermal efficiency (ω) can be surmised as the heat of evaporation over the heat of input or as:

$$\omega = \left(\frac{T_1 - T_2}{T_1 - T_0} \right) 100$$

Formula 1.1: Equation relating thermal efficiency to atmospheric and spray drying temperature. Where: T_0, is the atmospheric temperature; T_1, is the inlet temperature; T_2, the outlet temperature (Masters, 1991).

The aperture of the atomiser, the viscosity of the liquid feed and the rate at which the feed reaches the atomiser are also important variables on particle size (Figure 1.9). The finer the aperture the higher the kinetic forces at the base of the atomiser and the finer and wider reaching the spray. This improves distribution of the feed and the thermal efficiency of the process (if input temperatures are reduced accordingly), thus producing a finer product potentially increasing any subsequent lung deposition. However, it should be noted that a reduction in the thermal efficiency through excessive heat input could also improve lung deposition via decreased particulate aggregation as a result of particulate fracture. Although, little was understood about the role of thermal efficiency in producing respirable spray-dried powders at the time of writing (Alexander et al., 1988; Masters, 1991).

The viscosity and rate of liquid feed projection during spray drying is a contentious point with regard to the particle size produced, size being an all important factor in lung delivery as previously discussed. Certain sources have concluded that more viscous liquid feeds create finer spray dried products (Bosquillon et al, 2004b; Rabbani & Seville, 2005), whereas more established literature would argue otherwise (Saccetti & van Oort, 1996; Formula 1.2: Masters, 1991). There is a possibility that more viscous liquid feeds and higher liquid feed rates create a larger back-pressure in a co-current spray drying atomiser (Figure 1.9), which result in large longitudinal kinetic forces and these drive the liquid feed in to the drying chamber, creating a finer spray. There may also be a link between reducing liquid feed concentration/viscosity and increased spray dried particle corrugation (Alexander et al., 1988; Chew et al., 2005a). The work in this thesis aims to bring greater understanding to the relationship between liquid feed properties and spray dried product.
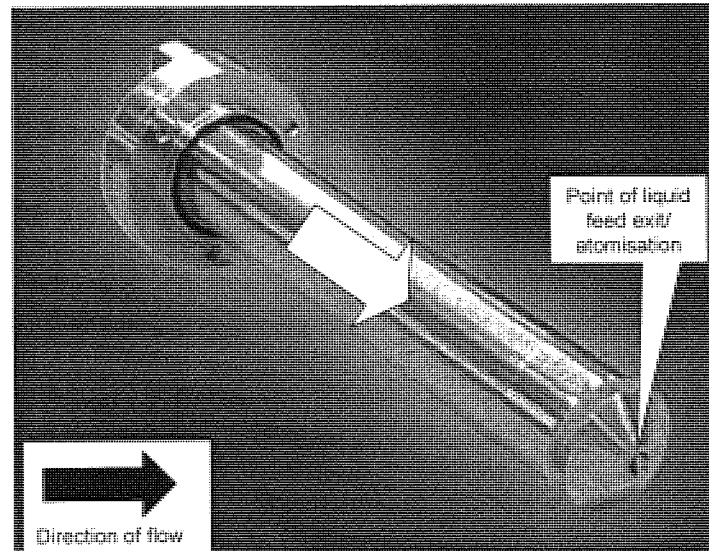
Figure 1.9: Image of the co-current fluid nozzle atomiser as used in the Buchi B290 mini lab spray drier.

Established studies indicate that:

$$D = \frac{A}{\left(V_{rel}^{2}\rho_{a}\right)^{x}} + B\left(\frac{M_{air}}{M_{liq}}\right)^{-\beta}$$

Formula 1.2: Proposed equation relating liquid feed property to particle size. D is the suspended droplet size post-atomisation where V_{rel} is the relative difference in velocity between air and liquid at the atomiser interface, M_{air}/M_{liq} is the mass ratio of air to liquid, P_{a} is the partial pressure of the air flow and β and χ are functions of nozzle design; A and B relate the nozzle design to the liquid properties (Masters, 1991).

Formula 1.2 defines that lower feed rates, reducing M_{liq}, and increasing the relative air volume M_{air} at the atomiser interface, gives a finer spray dried product. However Formula 1.2 offers no direct correlation between droplet size and the diameter of particles collected in the cyclone collection chamber (Masters, 1991). The theoretical size of any dry particulates collected could theoretically be calculated using an approximation derived from the condensation growth equation (Formulae 1.3: Groom et al., 1980; Gonda, 1988). Using the particle porosity ratio of ρ/ρ_{c}: the tapped density ratio of spray dried material over the same pre-process crystalline material to account for the porous nature of the spray dried particulates assuming proportional inter-sample inter-particulate spacing after tapping.

$$d_{ae} = \left(1 + \frac{\rho_{c} - \rho}{\rho_{c}}\right)\frac{D_{e}}{\sqrt[3]{\frac{p}{p_{e}}\left[1 + \frac{1000}{M.m_{e}}\right]}}$$

Formula 1.3: Equation attempting to relate spray dried particle dehydration to atomised droplet size. The theoretical relationship between an immediately suspended droplet post atomiser and the resulting spray dried particulate: where the theoretical primary particle size d_{ae} is related to theoretical droplet size D_{e} via the density of the dry particle ρ, and the density of the liquid feed ρ_{e}, M is the relative molecular mass and m_{e} the molarity of the liquid feed, related to porosity by ρ_{c}, the tapped density of the crystalline material pre-spray drying.

The theoretical particle size derived takes into account heat expansion caused by the migration of solutes and the production of the characteristic hollow spray dried core particulates are

accounted for by the particle porosity ratio. In addition rough surface textures caused by any thermal inefficiency, which are almost incidental when spray drying formulations containing aerosolisation enhancers, can add to the actual particle size, such textures are recognised by an increase the particle porosity ratio (Masters, 1991).

Stage 2, spray air contact is reliant upon the inlet temperature of the air being fed through the top of the drying chamber; lower inlet temperatures improve thermal efficiency but higher temperatures and faster evaporation (reduced thermal efficiency) could theoretically create more fragmented particles with better aerosolisation properties. A shift in the thermal efficiency due to reduced liquid feed load heat requirements could be a reason for previous researchers noting improvements in the aerosolisation properties of spray dried products due to particles having a greater level of surface fracture (Rabbani & Seville, 2005; Chew et al., 2005a).

Rabbani and Seville (2005) recognised increased surface fracture and FPF with ethanol blends, the aerosolisation enhancement was attributed to minor increases in viscosity however it is more likely that the increase in the proportion of a more volatile solvent (ethanol) gave reduced thermal efficiency. Increased viscosity has previously been recognised as detrimental to spray dried particulate properties (Alexander et al., 1988; Masters 1991). The reduction in thermal efficiency in the study could be the reason for a greater degree of particle surface fracture due to excessive inlet heat energy. The surface fracture preventing close contact between adjacent particles, which increases the interparticulate distance (*H*), reducing interparticulate van der Waals forces of attraction and improving aerosolisation property (Formula 1.4: Chew et al., 2005a).

Chew et al (2005a) utilised reducing liquid feed loadings accompanied by reduced rates of atomisation in the production of corrugated spray dried particulates, no reason was offered in the article for the corrugation but a link of decreasing liquid feed loads giving decreased thermal efficiency and increased particle corrugation could be assumed. The reduction in drying airflow rate may have simply prolonged the contact between the spray and the excessive inlet heat increasing particle surface fracture. The effect of thermal efficiency on spray-dried powders for inhalation is explored in detail in the subsequent chapters.

$$FvdW = \frac{Ar}{12H^2}$$

Formula 1.4: Relation of the van der Waal forces of inter particulate attraction to inter particulate distance and size; relating van der Waal's force *F*vdw, Hamaker constant A, particle radius r, and inter-particulate distance H (Chew et al., 2005a).

Stage 3, the drying of the spray: Generally the slower the air flow the more efficient the drying of the liquid feed, however the longer residency time is also associated with shallow yields as larger particles with greater gravitational energies sediment out of the air flow before the cyclone collection vessel further downstream. In addition, the reduction in air-flow rate gives longer residency time to the liquid feed at the nozzle of the atomiser. The increased residency combined with lower kinetic forces instigated by the air stream creating larger particles (Masters, 1991).

Stage 4, the separation of dried product from the air is a critical step with the geometry of the collection cyclone important. High performance cyclones with smaller radii of the cyclone chamber and exit duct causing greater resistance to airflow through the cyclone compared to wider standard cyclones (Figure 1.10); this creates a greater pressure drop across the collection unit aiding the recovery of finer particulates that would otherwise be extracted by the filter unit.

Hence, an increase in particle size on scale up is witnessed in industrial settings (Masters, 1991).

The recovery by specific cyclones is represented by Barth's classic model (Formula 1.5: Maury et

al., 2005).
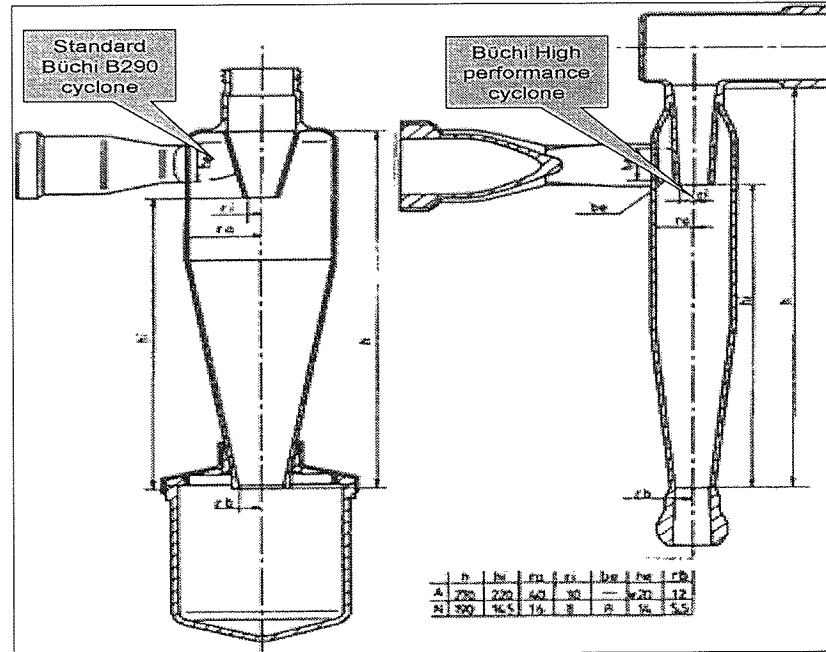
Figure 1.10: Illustrations of Büchi B290 mini lab spray drier standard and high performance cyclones (Maury et al., 2005).

$$\overline{d}^{2} = \frac{9\eta Q}{\left(\overline{v}\dfrac{\rho}{t}\right)^{2} p_{p}\pi h_{i}}$$

Formula 1.5: Barth's classic model; Q represents volumetric airflow rate, d is the smallest particle diameter collected on the cyclone wall, V_{t} velocity of air at the exit duct, η is the air viscosity, ρ_{p} particle density, and h_{i} vortex height (Maury et al., 2005).

Research into producing spray dried powders for inhalation holds many areas of interest with

research groups focusing on the spray drying process variables of powder production (Maa et al.,

1997, 1998; Stahl et al., 2002; Maury et al., 2005) and the physical attributes of simple spray

dried formulations (Bosquillon et al., 2001a; Elversson et al., 2006). With the greater

understanding of the generic principles of spray drying an evolving complexity of research into

spray dried powders involving the addition of agents for improved aerosolisation, bioadhesion or dissolution property is being investigated (e.g. Oster & Kissel, 2005).

1.4 Controlled release

The aim of the controlled delivery of any agent via any route is to achieve a concentration either locally or systemically that falls into the therapeutic window of that agent avoiding periods of sub-therapy and toxicity. The controlled delivery of the active agents may require a reduction in the dissolution times of poorly soluble drugs to avoid to avoid toxicity or an increase in the duration of release to extend the half-life of a rapidly absorbed or metabolised agent.

In general there are two approaches to achieve controlled release *in vivo*:

- Alter the microenvironment of the target (by altering pH and viscosity of the lung lining fluid in the pulmonary system)

- Alter the drug substance (via pro-drugs, salt forms etc.) or formulation (via encapsulation etc.)

The alteration of the microclimate of the lungs could have profound detrimental effects on pulmonary cell integrity so the spotlight of research into controlled release entities for inhalation usually focuses on the manipulation of agents and formulations.

For example, with regard to the longevity of agent action the use of pro-drugs or derivation of shorter acting substances to agents with greater residency at the receptor has been explored using the sodium isoniazid methanesulfonate pro-drug of the anti-tuberculosis agent isoniazid

and by the conversion of salbutamol into salmeterol respectively (Zhou et al., 2002; Smith & Bernstein, 1996).

As for the delivery of controlled release microparticulates to the lungs, formulations can be broadly split into two broad sub categories; liposomal based and encapsulation based microspheres.

1.4.1 Liposomal based controlled release

The use of liposomal technology in the delivery of active agents to the lungs is an increasing area of exploration commonly utilising resident lung surfactants to assemble nano to micron-sized particles; frequently phosphatidylcholine and phosphatidylglycerol derivatives (Lu & Hickey, 2005; Vermehren et al., 2006). The use of liposomes has also been explored as a method of pulmonary DNA and macromolecule delivery (Birchall et al., 2000; Huang & Wang, 2006). Controlled release via vesicular delivery from liposomes of respirable size has been achieved *in vivo* by *Chougule et al.* (2006) through the sustained release of Amiloride from 55% diphosphatidylcholine (DPPC) formulations. DPPC formulations have also been used to achieve highly respirable particles of 1.3 µm which can deliver beclometasone to the lungs with a residency of over 24 hours (Saari et al., 1999).

1.4.2 Encapsulation based controlled release

Polymeric microspheres of natural and synthetic polymers intended for pulmonary delivery have been largely manufactured using two processes to date; solvent evaporation (Huo et al., 2005; Jaspart et al., 2006), and spray drying.

The co-spray drying of dissolution enhancing polymers with poorly water-soluble drugs has been explored by a few select groups; Corrigan et al. (2003) co-spray dried the poorly water-soluble thiazide diuretic bendroflumethiazide with polyethylene glycol (PEG 4000) to good effect producing improved dissolution of the active agent via respirable sized particles.

Polyvinyl alcohol (PVA) is also known to improve the dissolution of poorly water soluble drugs, for example the enhanced dissolution of co-spray drying PVA with the poorly water soluble hormone progesterone (Orienti et al, 2002). Work in the inhalation field has also shown PVA to improve in vitro aerosolisation deposition via delivery from both DPI and pMDI devices (Jones et al., 2006a; Liao et al., 2005). Polyvinyl pyrollidone (PVP) has shown similar in vitro improvements in the aerosolisation and dissolution of poorly water soluble drugs improving the dissolution of phenytoin and the aerosolisation of other spray dried systems (Muhrer et al., 2006; Jones et al., 2006b).

Hydroxypropyl cellulose (HPC) spray dried systems have also shown promise in delivering poorly soluble agents in animal studies. Beclometasone dipropionate (BDP) delivered in a spray dried formulation to guinea pigs showing lung residency over 24 hours by increasing the proportion of BDP dissolved in the lung fluids and resisting mucociliary clearance (Sakagami et al., 2002).

Relatively few groups have focused on the sustained delivery of active agents to the lung from either a local or systemic viewpoint. Co-spray dried rifampicin and polylactide co-glycolide (PLGA) has been explored as a potential respirable powder with a prolonged duration of release (O'Hara & Hickey, 2000), as has the same polymer spray dried with the highly water soluble 5-

flurouracil (Hitzman et al., 2006a, 2006b). Minimal increases in duration of release have also been achieved *in vitro* with the release of bovine serum albumin from hydroxypropyl methylcellulose (HPMC) spray dried systems, although the respirability of such powders are in question (Elversson & Millqvist-Fureby, 2006). Additional research includes ipratropium delivery by polylactic acid (PLA) microspheres to guinea pigs and the use of fatty acids to reduce the release rate of leuprolide *in vitro* (Taylor et al., 2006; Alcock et al., 2002)

A major problem with the delivery of encapsulated microparticulates for controlled delivery to the central lung is the necessity of bioadhesives. The mucociliary escalator as previously discussed reduces the residency time of a controlled release agent in the lung to between 0 to 48 hours depending on site of deposition (Kim & Folinsbee, 1997). The greatly reduced contact time between a microparticulate and the lung lining requires the incorporation of an 'anchor-like' substance to improve drug delivery. Bioadhesives commonly work via multi-molecular non-covalent bonding (such as hydrogen bonding), by physical interlocking, being sequestered by epithelial cells or by biochemical adherence. A bioadhesive which could be potentially delivered to the lung includes carbopol, which have been incorporated into buccal delivery systems and research into the efficacy of carbopol inclusion in calcitonin loaded liposomes for pulmonary delivery has also been conducted (Kockisch et al., 2003; Takeuchi et al., 2003). The Nasal delivery of bioadhesives has encompassed the use of chitosan and chitosan cross-linked derivatives such as trimethyl-chitosan (TMC) which have greater penetration properties than the parent polymer via increased cationic charge groups that associate with polar groups (such as phosphates) on mucus membrane cell surfaces (Illum, 1998; Illum et al., 1994; Thanou et al., 2001). The use of lectin and HPC as mucoadhesives has also been explored in human tissue culture and animal tests respectively (Lehr, 2000; Sakagami et al., 2002).

Like the controlled release entity the bioadhesive must be biocompatible with the lung tissue, and as the mucostatic is required to be at the surface of the particulate the surface energies of the substance in the solid state must be low to minimise any aggregation which would be to the detriment of the formulation. A further consideration is the potential increase in the primary particle diameter with the addition of an increasing number of excipients.

1.5 Research aims and objectives

Drawing on the previous subsections of this preliminary chapter it can be recognized that improvements in the management of pulmonary disease are achievable through greater efficiency in the local delivery of pre-existing active agents e.g. terbutaline and BDP. With the demand for more simplified convenient regimens in the treatment of restrictive airway disease, and the advances made in the formulation of DPI devices, the aim of this thesis is to produce highly respirable sustained release dry powders which could potentially improve patient compliance and the disease management of obstructive airway disease.

The thesis objectives are therefore as follows:

- To explore spray drying as a method of aerosol particle engineering

- To produce highly reproducible stable dry powder formulations using spray drying

- To produce highly respirable spray dried powders using spray drying

- To produce highly respirable spray dried powders with a degree of sustained release

- To produce multiple drug loadings within the same highly respirable controlled release powder

- To gain greater insight into the factors which govern the spray drying of respirable powders

Complement of these aims with prior consideration of the lung environment and the natural mechanisms of absorption and clearance in the respiratory tract could potentially offer a viable alternative to the frequent dosing associated with both chronic and acute respiratory disease. In addition, with the acquisition of a deeper understanding of the spray drying process further advances in the production of spray dried powders for inhalation could be made.

Chapter 2

The influence of spray dried powder amino acid modification.

2.1 Introduction

As previously discussed, spray drying has been utilized as an efficacious way to prepare dry particles within the respirable size range. Unfortunately spray-dried particles have a tendency to aggregate or "stack" into larger species, which consequently decrease the air-flow properties of the powders and their subsequent deposition into the deep lung (Sacchetti & van Oort, 1996; Seville et al., 2002). The interactions between particles which are responsible for such aggregation are mainly dependent up on the physicochemical characteristics of the interacting particles such as, particle size, shape and surface morphologies, contact area and hygroscopicity (Prime et al., 1997; Maa et al., 1998). To modify such parameters and prevent the aggregation of future spray dried drug-polymer particles the addition of a suitable dispersibility enhancer may be required.

Several attempts have been made to enhance the dispersibility of spray-dried powders for pulmonary delivery, by involving the use of carrier-based formulations loosely based on the principle of adhesion-cohesion balance between drug-drug and drug-carrier forces of attraction (Nakate et al., 2004, Flament et al., 2004, Begat et al., 2004), altering particle density (Edwards et al., 1997) or incorporating modified components (Bot et al., 2000; Li et al., 2003, 2005a, 2005b; Maa & Prestrelski, 2000). However, in previous studies great promise has been represented by the addition of various amino acids to significantly improve the *in vitro* deposition of spray-dried sodium chromoglycate and non-viral gene delivery vectors via dry powder inhalers, findings that may aid the delivery of more complex controlled release vectors in subsequent research (Najafabadi et al., 2004; Chew et al., 2005; Li et al., 2003, 2005a).

Although amino acids have performed well in enhancing the lung deposition of the afore mentioned entities, their performance in influencing the physical properties and aerosolisation of spray-dried powders with the employed equipment and set parameters needs to be validated to demonstrate whether enhancement of *in vitro* deposition via amino acid modification is a ubiquitous approach. Furthermore, little is understood about the mechanism of action of performance enhancing amino acids in spray drying.

Here, the amino acids arginine (ARG), aspartic acid (ASP), leucine (LEU), phenylalanine (PHE) and threonine (THR), selected due to diverse properties such as hydrophobicity (Tabibzadeh, 1995), were investigated as potential 'dispersibility enhancers' in spray dried powders employing lactose as a primary excipient (diluent) and salbutamol sulphate (SS) as a low molecular weight model drug (marker). The influence of amino acid on the physical property of the spray dried formulation was investigated, exploring multiple particle diameters (using theoretical primary, Fraunhofer, aerodynamic and visual assessment of particulate size), surface morphology, *in vitro* deposition and the thermal characteristics. The aim was to determine the utility of amino acids in enhancing the deposition of low molecular weight drugs delivered via dry powder inhaler with a view to their incorporation into subsequent potential controlled release formulations.

2.2 Materials and Methods

2.2.1 Materials

α-Lactose monohydrate, l-arginine (ARG), l-aspartic acid (ASP), l-leucine (LEU), l-phenylalanine (PHE) and l-threonine (THR) were obtained from Sigma-Aldrich Chemicals (Poole, UK). Salbutamol sulphate was kindly donated by Allchem International Ltd (Maidenhead, UK). HPLC grade methanol was obtained from Fisher Scientific UK Ltd (Loughborough, UK).
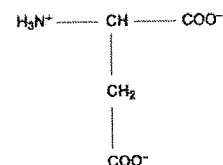
l-arginine (ARG)

H_3N^+ ——— CH ——— COO⁻
            |
         (CH₂)₃
            |
           NH
            |
            C ═══ NH₂⁺
            |
           NH₂

l-aspartic acid (ASP)

H_3N^+ ——— CH ——— COO⁻
               |
              CH₂
               |
              COO⁻

l-leucine (LEU)

H_3N^+ ——— CH ——— COO⁻
            |
           CH₂
            |
           CH ——— CH₃
            |
           CH₃

l-phenylalanine (PHE)

H_3N^+ ——— CH ——— COO⁻
            |
           CH₂
            |

l-threonine (THR)

H_3N^+ ——— CH ——— COO⁻
            |
           HC ——— OH
            |
           CH₃

Figure 2.1: The chemical structures of the amino acids: l-arginine, l-aspartic acid, l-leucine, l-phenylalanine and l-threonine.

2.2.2 Preparation of spray-dried powders

Aqueous solutions containing salbutamol sulphate (model drug), lactose (bulking agent) and a selected amino acid (ARG, ASP, LEU, PHE or THR; Figure 2.1), employed as a potential dispersibility enhancer, were prepared with a total powder mass of 2% w/v. The prepared formulations (100 mL) were subsequently spray-dried using a Büchi B-290 mini spray-dryer equipped with a high performance cyclone (Büchi Labortechnik AG, Switzerland) and a 0.7 mm

two-fluid nozzle, using the following standard operating conditions: inlet temperature, 180°C; spray flow rate, 600 L/h; pump setting, 10% (3.2 mL/min); aspirator setting, 85% (34 m³/h). These conditions resulted in an outlet temperature of 85-103°C, the temperature of the apparatus pre-process and relative inlet, outlet temperatures mid-process were recorded and the thermal efficiency calculated as in Equation 1.1. A total of 20 powders were prepared, each containing 4% w/w salbutamol sulphate, 5-20% w/w amino acid and 91-76% w/w lactose dissolved in double distilled water at 2% w/v. In addition, a powder containing 4% w/w salbutamol and 96% w/w lactose (i.e. no amino acid modifier) was produced as a control from a 2% w/v aqueous solution.

2.2.3 Powder characterisation

2.2.3.1 Spray-drying yield and drug content

The yields of spray-dried powders were quantified as a percentage mass of anticipated total powder yields. The salbutamol content of each prepared powder was measured in triplicate, with analysis by HPLC, and expressed as the percentage of nominal load.

2.2.3.2 Scanning electron microscopy

Spray-dried powders were mounted onto separate, adhesive-coated, 12.5 mm diameter aluminium pin stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle-free compressed gas across each. The specimen stubs were sputter coated with a thin (approximately 10 nm) layer of gold in a Polaron SC500 coating unit at 10 mA for 2 min using an argon gas purge. The specimens were examined using a Topcon SM-300 scanning electron microscope (SEM: LG Philips, Blackburn, UK). The SEM was operated at high

vacuum with an accelerating voltage of 5 kV and a specimen working distance of 12 mm. Secondary electron images were recorded digitally at a magnification 5000×.

2.2.3.3 Amorphous nature and water content

Determination of the degree of amorphous material and the water content in the spray-dried powders was performed using differential scanning calorimetery (DSC) and thermogravimetric analysis (TGA), respectively. DSC (Pyris Diamond DSC and Intracooler 2P: Perkin Elmer, Wellessey, USA) was performed on 2 mg samples in aluminium pans using a nitrogen purge at 20 mL min $^{-1}$ (range ambient -300°C, heating rate 20°C per minute), after calibration at the same rate with an Indium test sample. TGA analysis (Pyris 1 TGA: Perkin Elmer) was performed on 10 mg samples in platinum pans using a nitrogen purge at 20 mL min $^{-1}$ (range ambient -360°C, heating rate 50°C per minute). Measurements were performed in triplicate.

2.2.3.4 Particle size, powder density and primary aerodynamic diameter

The particle size of the spray-dried powders was measured by laser diffraction (HELOS particle size analyser incorporating VIBRO/RODOS dry dispersion system: Sympatec® GmbH System-Partikel-Technik, Clausthal-Zellerfeld, Germany). Approximately 100 mg of each powder was used to achieve the required obscuration of 5%, and each sample was measured in triplicate at 1 bar air pressure. The data obtained were expressed as the volume weighted mean particle size (VMD).

The poured density of the spray-dried powder was determined by pouring a mass of approximately five grams under gravity into a calibrated measuring cylinder and recording the

volume occupied by the powder. The tapped density of the same samples was subsequently determined using a tamping volumeter (Tapped Density Assessor: Copley Scientific Ltd., Nottingham, UK) to displace powder until no further change in the powder volume was observed. Measurements were performed in triplicate.

The percentage porosity of the spray-dried material was also calculated using the ratio of porosity (Formula 2.1).

$$\phi = \frac{100(\rho_c - \rho)}{\rho_c}$$

Formula 2.1: Proposed equation for the spray dried percentage particle porosity ϕ, where ρ_c is the tapped density of the crystalline blend pre-spray drying and ρ is the tapped density of the spray dried blend.

Carr's Index values for each spray-dried powder were derived from poured density and tapped density data, according to Formula 2.2.

$$Carr's\ Index(\%) = \frac{100(Tapped\ Density - Poured\ Density)}{Tapped\ Density}$$

Formula 2.2: Carr's index.

Theoretical estimates of particle primary aerodynamic diameter (δ_{ae}) were derived from the particle sizing (d) and tapped density (p) data, according to Formula 2.3. (Bosquillon et al., 2004a):

$$d_{ae} = d\sqrt{\frac{p}{p_1}}$$

Formula 2.3: Formulae for primary particle aerodynamic diameter. Where $p_1 = 1$ g cm^{-3}, by definition the density of a water droplet of the same size that has the same aerodynamic and physical diameter (i.e.1 g/cm). δ_{ae} is defined as the diameter of an assumed sphere of unit density that has the same settling velocity in air as the aerosol particle being measured (Gonda, 1988).

2.2.3.5 In vitro powder aerosolisation (MSLI)

The aerosolisation properties of the spray-dried powders were investigated using a Multi Stage Liquid Impinger (MSLI: Copley Scientific). HPLC mobile phase (20 mL) was introduced to Stages 1-4 of the MSLI, and a filter paper (Whatman GF-A) placed at Stage 5. The flow rate through the MSLI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific). Powder aliquots (25 mg) were loaded into size 2 HPMC capsules (Shionogi Qualicaps) and placed into a Spinhaler® dry powder inhaler (DPI), attached to the MSLI via a stainless steel USP throat. The capsule was pierced and the liberated powder drawn through the MSLI at a flow rate of 60 L/min for a 5 second aspiration using a pressure calibrator (Model TPK: Copley Scientific), the same capsule was then excited for a further 5 seconds after a 10 second interval. Under these conditions, the effective cut-off diameters are Stage 1: 13.0 μm; Stage 2: 6.8 μm; Stage 3: 3.1 μm; Stage 4: 1.7 μm; with Stage 5 as a terminal filter. Each deposition experiment was repeated in triplicate.

The emitted dose (ED), defined as the percent of total loaded powder mass exiting the capsule following simulated inhalation, was determined gravimetrically. The size 2 capsule weighed pre-fill, filled and exhausted after MSLI liberation of the loaded content. Subsequently, the mobile phase at each stage of the MSLI was removed for analysis. The inhaler, throat and filter were each washed with 20 mL mobile phase. HPLC was used to quantify the fraction of salbutamol recovered from the inhaler, throat, stages 1-4 and filter of the MSLI. The fine particle dose (FPD), defined as the mass of drug less than 5 μm, was calculated by interpolation from a plot of cumulative mass vs. effective cut-off diameter of the respective stages. The fine particle fraction (FPF) was calculated as the ratio of FPD to total loaded dose, expressed as a percentage and corrected for actual salbutamol content in each powder, comparing drug content against three

randomly selected 25 mg samples. The mass median aerodynamic diameter (MMAD) of the powders was also derived, defined as the particle size at the 50% mark of a plot of cumulative fraction vs. effective cut-off diameter (BP, 2004; Zeng et al., 2006).

The geometric standard deviation σ_g is the square root of the 84th percentile over the 16th percentile and is used as a parameter of poly-dispersity (Gonda, 1988). A factor of $\sigma_g > 1.2$ indicates the existence of a particulate size range. The sample produced by the heterogeneous spray drying method, negating the creation of multiple populations through aggregation, is a normal distribution created by minor fluctuations in air flow rate and viscosity at the atomiser aperture (Masters, 1991). All spray dried samples produced in the entireties of this research are assumed polydisperse ($\sigma_g > 1.2$), the theory compounded by normal distribution curves produced by Fraunhoffer dry dispersion laser diffraction (Sympatec illustrations: Appendix 1).

2.2.3.6 HPLC analysis of salbutamol sulfate

Salbutamol concentration was determined using reverse-phase HPLC (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6 x 150 mm Phenomomex La Luna column (Phenomomex, USA) and 20 µL injection volume with UV detection at 275 nm. The mobile phase (1 mL/min) consisted of 15% v/v aqueous methanol, providing elution of salbutamol around a retention time of 2.5 min (Sample HPLC trace: Appendix 2). With the limit of detection (LOD) determined as 0.77 mcg/ml and the limit of quantification (LOQ) 2.3 mcg/ml with standard samples complying to 99% confidence limits.

2.2.3.7 Statistical analysis

The drug loading, emitted dose, FPD and FPF of the amino acid-modified spray dried powders were statistically compared to those of the control powder using one-way analysis of variance (ANOVA) with Dunnett multiple comparison test. Where appropriate, the aerosolisation properties of the amino-acid modified powders were compared against each other using one-way analysis of variance with Tukey-Krammer multiple comparisons test. The significance level where not stated was 0.05.

2.3 Results and Discussion

2.3.1 Powder characterisation

All 21 spray-dried powders, 20 amino-acid modified powders and one control, were successfully spray dried. The yield of the control powder (4% salbutamol, 96% lactose) was a reasonable 52% of the anticipated product, in line with previous investigations (e.g. Li et al., 2005a; Rabbani and Seville, 2005). The addition of the amino acid dispersibility enhancers caused considerable variation in the spray drying yields of the powder produced (range 31-81%, Table 2.1), with phenylalanine giving the greatest deviation in spray-drying yield from that of the control powder with a maximum yield of 81% at 20% amino acid concentration (n=1). This is in agreement with a previous study on amino acid-modified spray-dried powders reporting the greatest spray-dried yield from phenylalanine containing powders (Li et al., 2005a). Only those formulations modified with leucine showed an overall increase in powder yield at all the amino acid concentrations tested, i.e. 5%, 10%, 15% and 20%.

Low spray drying yields are indicative of a formulation which is cohesive in nature as large agglomerates carry greater gravitational forces and are displaced from the air flow of the spray

drying process pre-collection vessel (Masters, 1991; Maury et al., 2005). In keeping with this theory, it has previously been suggested that low spray-drying yields are indicative of cohesive powders that demonstrate poor aerosolisation properties during *in vitro* testing (Rabbani & Seville, 2005). The addition of formulation excipients may change the physicochemical characteristics of the resultant spray-dried powders resulting in an improved spray-drying yield, as the gravitational forces decrease proportionally with the agglomerate size enhancing formulation aerosolisation properties and the proportion of powder particles reaching cyclone collection.

Spray drying thermal efficiency varied from 48.2 to 62.2% with the LEU-modified spray dried formulations having the lowest thermal efficiencies (Table 2.2).

Analysis of the salbutamol content of the spray-dried powders indicated that drug loading varied from 47% to 106% of nominal load (4% w/w) with the majority of powders containing at least 90% of the anticipated dose (Figure 2.2). The 5% ARG, 10% ARG, 20% LEU, 15% PHE and 20% PHE spray-dried powders contained significantly lower drug content than the control powder (one-way ANOVA/Dunnett: $p < 0.05$). Although it is not clear why spray-drying certain formulations resulted in the loss of variable amounts of salbutamol, it has been suggested that amino acids display surfactant-like properties (Gliniski et al., 2000); a critical amount of a particular amino acid may be required in each formulation to form micelle-like structures within which the drug is encapsulated and protected from relatively harsh spray drying conditions (Rabbani & Seville, 2005). Although it should be noted that far more heat labile entities have been spray dried at high temperatures with less variance (e.g. bovine serum albumin, BSA: Masters, 1991).

Amino acid[b] (%w/w)		SD Yield (%)	Water Content (% mean±SD n=3)	Particle Size (µm mean±SD n=3)	Tapped Density (g cm⁻³, mean±SD n=3)	Degree of Porosity ɛ (% mean±SD, n=3)	Car's Index[a] (%)	Flowability	d_{xc} (µm)	MMAD (µm)
None	0	52.4	1.1±0.0	3.08±0.44	0.36±0.02	52.81±2.92	25.6	Poor, cohesive	1.86	5.52±2.24
ARG	5	66.0	1.0±0.3	2.10±0.02	0.32±0.02	55.71±2.94	14.7	Good	1.18	6.67±0.39
	10	56.6	1.8±0.3	1.73±0.01	0.27±0.01	65.14±5.70	24.4	Poor, fluid	0.91	5.30±2.26
	15	44.8	2.3±0.2	1.95±0.31	0.28±0.01	63.99±1.43	25.5	Poor, cohesive	1.03	6.30±0.71
	20	52.1	1.9±0.9	1.72±0.01	0.30±0.06	60.84±7.19	26.1	Poor, cohesive	0.94	2.33±0.26
ASP	5	50.0	1.8±0.6	1.71±0.02	0.30±0.02	61.38±6.03	12.7	Good	0.94	4.01±3.14
	10	55.4	1.4±0.3	1.71±0.01	0.37±0.08	52.18±9.96	27.3	Poor, cohesive	1.03	7.28±0.55
	15	41.6	1.0±0.3	1.81±0.01	0.30±0.04	61.24±5.25	21.6	Poor, fluid	0.99	7.36±0.51
	20	54.5	1.1±0.5	1.48±0.01	0.33±0.02	55.68±5.75	20.0	Fair	0.85	7.76±0.36
LEU	5	62.2	1.8±1.1	6.91±0.45	0.33±0.00	55.58±1.88	16.2	Good	3.94	4.44±0.24
	10	63.5	1.6±0.2	3.65±0.13	0.31±0.02	52.42±4.43	25.8	Poor, cohesive	2.04	2.80±0.93
	15	68.7	1.5±0.7	1.77±0.02	0.35±0.03	41.66±3.48	13.1	Good	1.05	2.23±0.64
	20	66.2	1.4±0.1	1.70±0.02	0.35±0.01	33.95±1.48	26.4	Poor, cohesive	1.01	0.86±0.40
PHE	5	36.0	1.2±0.4	2.42±0.55	0.33±0.02	52.57±4.01	24.1	Poor, fluid	1.39	4.62±1.97
	10	31.5	2.1±0.1	2.94±0.29	0.34±0.00	55.41±4.49	14.4	Good	1.71	4.46±0.68
	15	52.5	1.9±0.4	1.87±0.01	0.29±0.02	55.17±1.88	32.5	Very poor	1.01	4.07±1.46
	20	80.8	1.7±0.5	2.36±0.45	0.29±0.01	46.07±1.34	28.9	Poor, cohesive	1.28	5.39±2.09
THR	5	41.9	1.7±0.4	1.80±0.01	0.41±0.10	58.76±6.99	31.5	Poor, cohesive	1.15	6.82±0.41
	10	55.2	1.8±0.3	1.77±0.04	0.32±0.05	52.00±2.08	21.1	Fair	0.99	5.93±1.00
	15	52.9	1.8±0.3	1.65±0.02	0.36±0.00	53.45±6.59	19.7	Fair	0.99	7.53±0.45
	20	55.1	1.7±0.1	1.86±0.02	0.36±0.04	52.81±2.92	30.3	Poor, cohesive	1.11	7.21±0.89

[a] Car's Index flowability: 5-12% excellent; 12-18% good; 18-21% fair; 21-25% poor, fluid; 25-32% poor, cohesive; 32-38% very poor; >40% extremely poor (from De Villiers, 2005)

[b] n=1

Table 2.1: Table illustrating the physical properties of amino acid modified spray dried powders. Values are mean ± SD, n=3.

Leucine % w/w	inlet °C			outlet °C			room °C			ω %			av	sd
5	180	180	182	95	96	95	20	19	19	53.13	52.17	53.37	52.89	0.63
10	181	182	181	98	99	99	19	18	17	51.23	50.61	50.00	50.61	0.62
15	181	180	180	100	100	101	18	17	19	49.69	49.08	49.07	49.28	0.36
20	181	180	180	103	102	103	19	19	19	48.15	48.45	47.83	48.14	0.31

Table 2.2: Table of how thermal efficiency reduced with increasing leucine concentration of a spray dried blend.
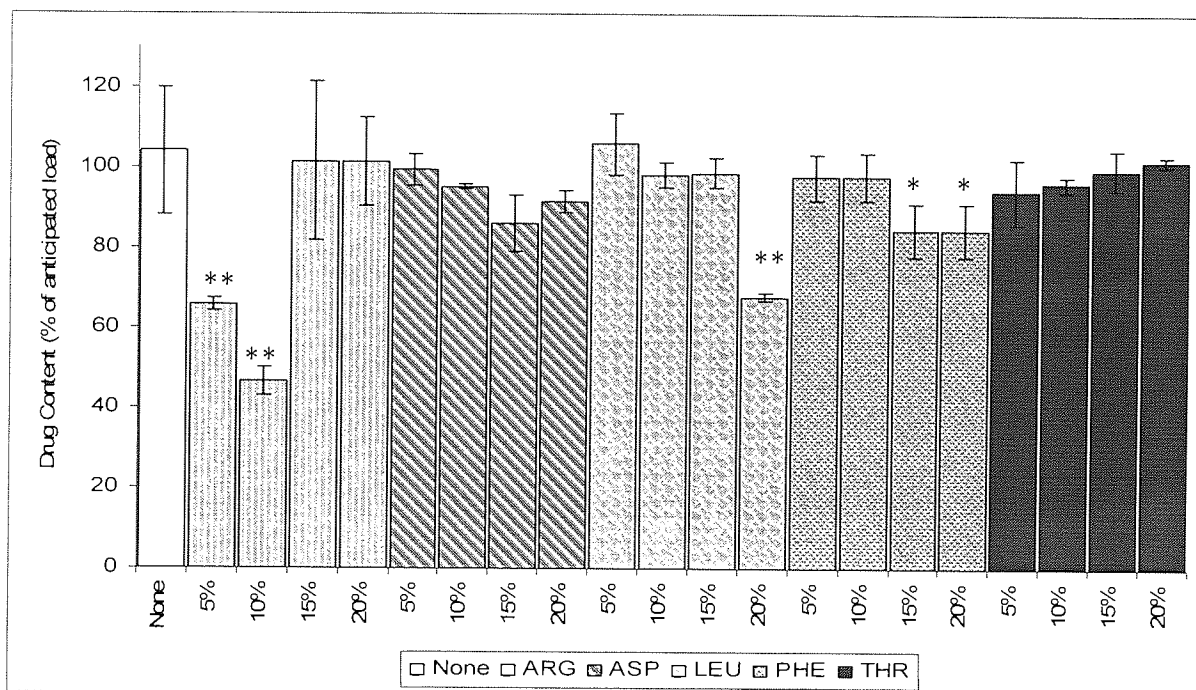
Figure 2.2: Bar chart representing the drug content of amino acid modified spray-dried powders. Expressed as the percentage of anticipated load (mean ± SD, n=3). Statistical difference (one-way ANOVA/Dunnett) from control powder: $^*p < 0.05$, $^{**}p < 0.01$.

Scanning electron microscopy, used to visualise the particle diameter and surface morphologies of the spray-dried powders, indicated that the majority of powders comprised regular spherical particles with a diameter of less than 10 μm, features characteristic of spray dried particulates (Marriott et al., 2007; Figure 2.3). All powders appeared to exhibit some visual cohesion; most apparent with the unmodified lactose powder (Figure 2.3A), less so in the amino acid-modified dry powders, especially those containing arginine, leucine or phenylalanine (Figure 2.3B, D, E).

The leucine and phenylalanine-modified spray-dried formulations displayed higher visual degrees of surface roughness compared with the other powders. The surface roughness could be a result

of low thermal efficiency when spray drying as rapid evaporation from a drying droplet by excessive heat could give surface fractures (Figure 2.3D, E). The surfactant like nature of leucine in particular, with a clearly isolated Zwitterion and hydrophobic side chain (Figure 2.1), could reduce longitudinal forces at the base of the atomiser which transpire into a reduction in the surface active forces of the droplet produced. The droplets would be smaller in size due to the reduction in surface force. The reduction in surface tension allows greater penetration of the heat from the spray dryer inlet into the droplet. What is now excessive heat, due to the reduction in thermal efficiency by the surfactant, transpires into higher evaporative energy that physically manifests as pitting on the surface of the spray dried particles. The 180°C inlet temperature therefore could be the correct parameter for all of the spray dried powders except the LEU and PHE formulations that may require spray drying at a lower temperature to improve thermal efficiency during processing to prevent surface fracture. However this theory remains untested.

Surface roughness has been previously linked to drying rate by Alexander and King (1985) who recognised that excessive inlet temperatures gave increased drying rates resulting in a more wrinkled appearance of spray dried lactose samples. It is important to note that this study into spray dried lactose also found that liquid feed viscosity had no bearing on spray dried particulate surface roughness (Alexander et al., 1985). Research into the corrugation of respirable spray dried powders has found increased particulate surface folding to be beneficial to aerosolisation (Chew et al., 2005a). The LEU and PHE powders also appeared to display the lowest visual degree of interparticulate cohesion, as evidenced by greater inter-particulate spacing. In all SEM images the presence of rounded microspheres, some with textured surfaces, indicated that the powders formed were amorphous in nature (crystalline particulates generally possessing sharp edges and linear morphologies due to a rigid intermolecular organisation within individual

particles). This was as expected as powders generated through spray drying are known to be predominately amorphous in nature due to the heterogeneous arrangement of the molecules precipitated from solution during the process (Corrigan, 1995; Bosquillon et al., 2004b).

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Figure 2.3: Representative scanning electron micrographs of control and amino acid modified spray-dried powders. A) Control powder; B) 20% ARG-modified powder; C) 20% ASP-modified powder; D) 20% LEU-modified powder; E) 20% PHE-modified powder; F) 20% THR-modified powder. Bar = 10 μm.

The amorphous nature of the spray-dried powders was confirmed using differential scanning calorimetry (DSC). Figure 2.4 shows a DSC trace (n=3) obtained from the 20% LEU spray-dried formulation (lower trace) and a 'sister' trace obtained from a physical blend of 4% salbutamol,

20% leucine and 76% lactose (i.e. not spray-dried, upper trace). It is clear from these traces that

the endotherms in the upper trace arising from the fusion of crystalline material in the physical

blend are absent in the DSC trace for the spray-dried material (lower trace), demonstrating the

amorphous nature of these powders. Fusion peaks can be seen in the crystalline trace at 150°C

for lactose/the dehydration of water and 230°C for salbutamol (Larhrib et al., 2003; Sabulal et al.,

1997; Brodka-Pfeiffer et al., 2003), the glass transition decomposition of leucine at around 273 to

293°C can not be seen (Murphy et al., 2005). No crystalline endotherms are viewed on the flat

spray dried trace (lower trace). Given the amorphous nature of the spray dried powders, all

samples were stored at room temperature in a dessicator overnight after spray drying to limit the

probability of any potential crystallisation between powder production and aerosolisation testing.
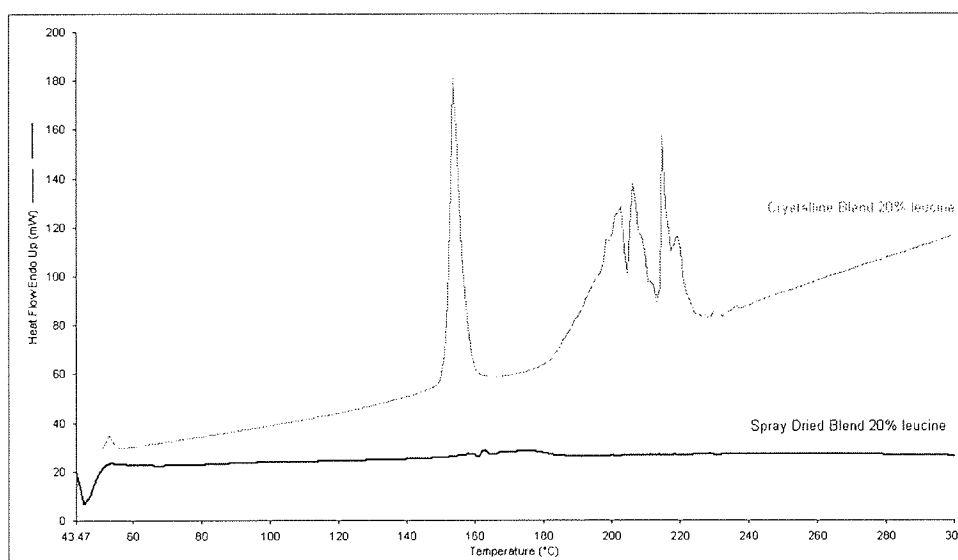
Figure 2.4: Representative differential scanning calorimetry trace. Lower trace obtained from 20% LEU spray-dried powder, showing lack of crystalline content. Upper trace obtained from a physical blend of 4% salbutamol, 20% leucine and 76% lactose, showing endothermic fusion peaks due to presence of crystalline material. Peak at 130°C is the evaporation of water/lactose activity, peak at approx 225°C is a probable lactose endotherm (Zeng et al., 2000a;Larhrib et al., 2003), salbutamol sulfate endotherm can be seen at approx 210°C (Brodka-Pfeiffer et al., 2003) crystalline leucine decompostion at 273°C can not be seen (Rosu et al., 2003).

Thermogravimetric analysis of the spray-dried powders indicated that the moisture content of the

powders ranged between 1.0 - 2.3% w/w (Table 2.1). These values compare favourably with

other studies that indicate moisture content of spray-dried powders in the region of 7.5% w/w (Stahl et al., 2002; Chew et al., 2005). Generally speaking increased inlet temperature as well as the geometry and volume of the glassware in a spray-drying unit can improve solvent evaporation from spray dried material (Masters, 1991). Only the LEU series showed a decrease in water content, although not statistically so, with increasing amino acid concentration (1.76-1.42% w/w). The figures potentially linked to a decrease in thermal efficiency. The surfactant nature of LEU and potentially PHE, a result of the amino acids bipolar molecular structure with distinct hydrophilic and lipophilic areas, allows the congregation of the amino acids at water-air interfaces which transpires to lower the surface tension between the two phases (Gliniski et al., 2000). The reduction in the surface tension of droplets produced by atomisation during spray drying could potentially allow the greater permeation of inlet heat into the atomised liquid feed. The increase in the heat of evaporation driving off more water from the droplet, the reduction in the heat requirements to dry the droplets with the increase in LEU concentration leading to greater outlet temperatures and thus a decrease in thermal efficiency. As the capillary action of residual water can cause spray dried particle aggregation the reduction of water content is advantageous in producing a more respirable powder.

Laser diffraction data are presented in Table 2.1 with the exception of one powder (5% LEU), the mean particle size of the resultant spray-dried powders was considerably less than 5 μm (the size regarded as respirable); excluding this powder, the average size of the spray-dried powders was 2.1 ± 0.6 μm, indicating that the powders were of a small enough size (negating any surface active forces present during aerosolisation) to avoid deposition by inertial impaction in the oropharynx (Atkins, 2005; Larhrib et al., 2003).

The effect of pressure on the dispersion of dry powders undergoing laser diffraction in the Sympatec® device was investigated by using spray dried lactose as a marker of cohesion (Figure 2.5). It was found that by increasing the vacuum from one bar to five bar the mean diameter could be reduced from 10.6 to 8.3 µm, the median diameter from 7.8 to 6.2 µm and the modal diameter from 9.7 to 8.2 µm. This indicates that at 1 bar the Sympatec® operation used for particle sizing through out this research includes aggregates; however to provide insight into the cohesive nature of spray dried powders 1 bar is used as standard throughout this research (Jones et al., 2005). Differing mean, modal and median calculations prove that the population of untreated spray dried lactose is poly-disperse and doesn't tightly follow a normal distribution; where modal, median and mean values are isometric. This is due to the presence of two species, primary particles and agglomerates (Figure 2.5; Gonda, 1988). Figure 2.5 shows only the modal diameter, which is not affected to the same extent as mean and median markers by a dual species population, not to have a strong positive correlation of a reduction in size with increased pressure of dispersion ($R^2 = 0.5$).
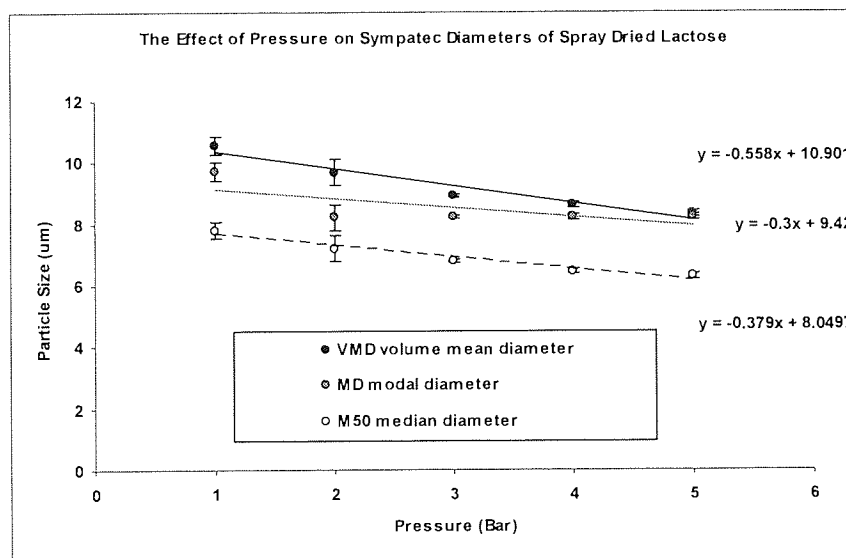
Figure 2.5: A line chart illustrating the effect of Sympatec vacuum pressure on particle measurements, showing the Fraunhoffer particle size of pure spray dried lactose during Sympatec® dry powder laser diffraction using Vibri® and Rodos® Systems (mean ± SD, n=3).

The tapped density of the spray-dried powders ranged between 0.27 g cm^{-3} and 0.41 g cm^{-3} (Table 2.1). These observations are in line with those recently presented by *Bosquillon et al.*, who previously suggested that lower powder tapped density is associated with better aerosolisation properties (Bosquillon et al., 2004a). The theory of *Bosquillon et al.* (2004a) is based on a porous particle holding greater aerosolisation character over a particle of the same size which is non-porous, the derived degree of porosity revealed decreasing porosity with increasing leucine concentration (ϕ= 55.6% to 34%, LEU 5% to LEU 20% respectively: Table 2.1). Theoretical estimates of primary particle diameter (d_{ae}), which are inherently related to tapped density, ranged between 0.85 μm (20% ASP) and 3.94 μm (5% LEU), indicating that all of the powders were of a theoretical primary particle size suitable for pulmonary administration (Table 2.1).

Comparison of dry dispersion laser diffraction sizes assessed at one bar, which from the work done in Figure 2.5 can be assumed as aggregates. Derived d_{ae} can reveal something about the cohesive nature of a spray dried formulation and the influence of any potential aerosolisation enhancer. Figure 2.6 illustrates the reduction in primary particle size (d_{ae}) with increasing LEU concentration visualised as the increasing surface area to volume ratio. The aggregation ratio (LD:d_{ae}) shows a negative gradient signifying lower aggregation (lower LD size) at higher LEU concentrations the trend linked to the dual effect of increasing LEU concentration and the subsequent reduction in thermal efficiency on modified spray dried particulates. Leucine giving reduction in primary particle size (d_{ae}) through lowering the resistance to kinetic forces at the atomiser nozzle via surfactant activity and lowering aggregate size (LD) by increasing particulate surface folding at the lower thermal efficiencies (Table 2.2). The gradient of the primary particle

to thermal efficiency ratio plot (d_{ae}:w) indicates that small shifts in thermal efficiency appear either to have larger effects on the reduction of d_{ae} in LEU modified spray dried powders through heat kinetic size reduction, or transversely, that the effects of LEU-modification are more profound at the atomiser-air interface in reducing d_{ae} than in the spray drying chamber in reducing thermal efficiency.
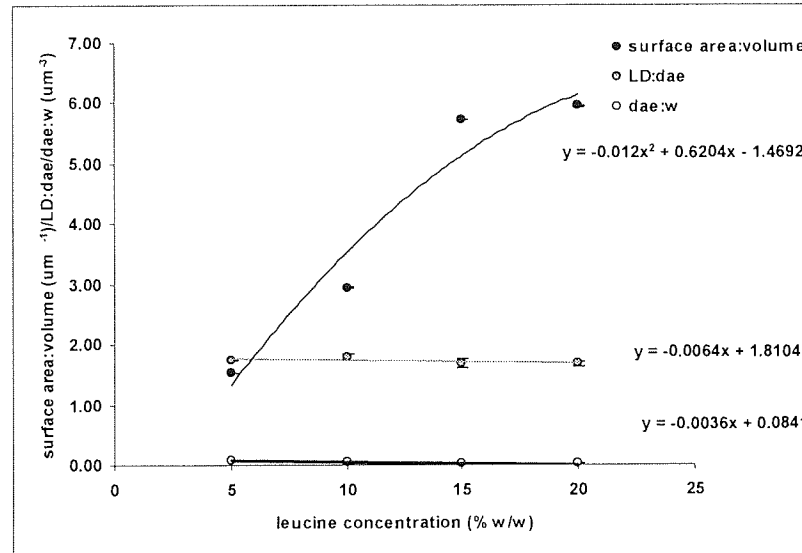
Figure 2.6: Chart illustrating the effect of leucine concentration on surface area:volume, laser diffraction size: primary particle diameter (LD:d_{ae}, aggregation ratio) and primary particle diameter:thermal efficiency (d_{ae}:w).

Plotting the aggregation ratio of LD:d_{ae} against theoretical primary particle diameter can reveal the affect reducing primary particle size has on the aggregation of spray dried blends. Out of the five amino acid series all except the LEU spray dried powders showed the conventional trend of reducing particle size giving increased particulate aggregation through an increase in surface area:volume exemplified by ARG-modified spray dried powders in Figure 2.7. The decreasing particle size of ARG modified powders proved to follow convention with increased surface area correlating to a higher aggregation ratio, visualised by the negative gradient of the LD:d_{ae} plot, the smaller primary particles having a greater surface area for surface active forces to act and

result in aggregation. Aspartic acid, phenylalanine (results not shown) and threonine modified spray dried powders followed suit with increasing aggregation at lower primary particle sizes with threonine having the steepest negative gradient (m= -0.7866) indicating a heavy influence of surface active forces (Figure 2.8).
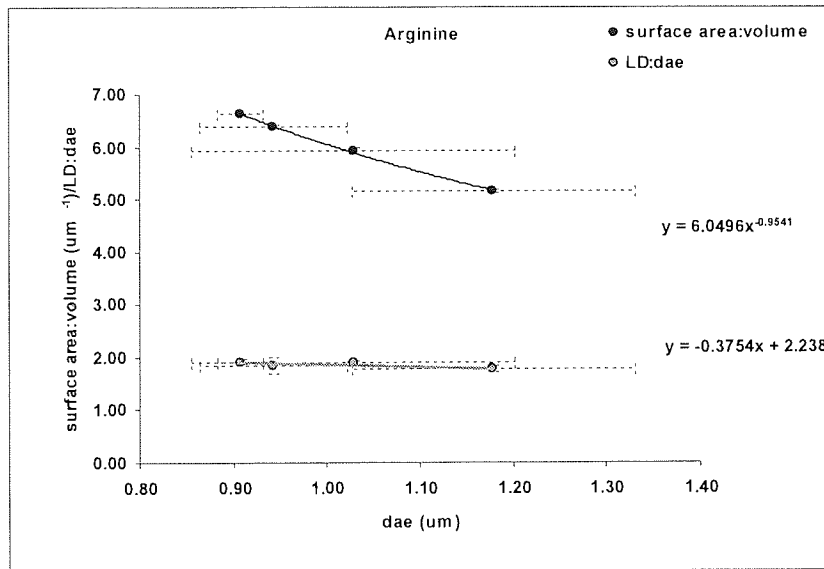
Figure 2.7: Chart illustrating the increase in surface area:volume with decreasing primary particle diameter (d_{ae}) compared to increasing aggregation ratio ($LD:d_{ae}$) in ARG modified spray dried powders.
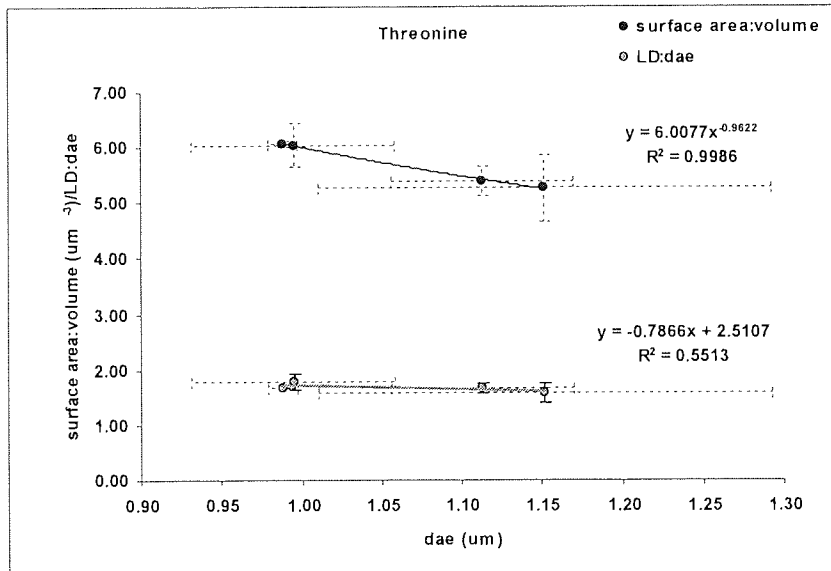
Figure 2.8: Chart illustrating the increase in surface area:volume with decreasing primary particle diameter (d_{ae}) compared to increasing aggregation ratio ($LD:d_{ae}$) in THR modified spray dried powders.

Figure 2.9 represents the unconventional trend of LEU to produce less aggregated powders when d_{ae} is reduced (as a result of increasing LEU concentration). The reduction in aggregation signified by the positive gradient of the aggregation ratio of $LD:d_{ae}$ which following pharmaceutical convention would be expected to follow the negative gradient of the surface area:volume plot, as seen in Figures 2.7 and 2.8 with ARG and THR modified powders, as increased surface area gives greater inter-particulate contact area for surface force to act upon. In addition, smaller particles of the same material possess lower gravimetric force which aids the formation of aggregates. The $d_{ae}:w$ plot shows how thermal efficiency (w) is a either a major effector of primary particle size or how the effects of LEU-modification are more profound at the atomiser-air interface in reducing d_{ae} than in the drying chamber in reducing thermal efficiency, with the proportional increase in d_{ae} being larger than the proportional increase of w at a particular particle size.
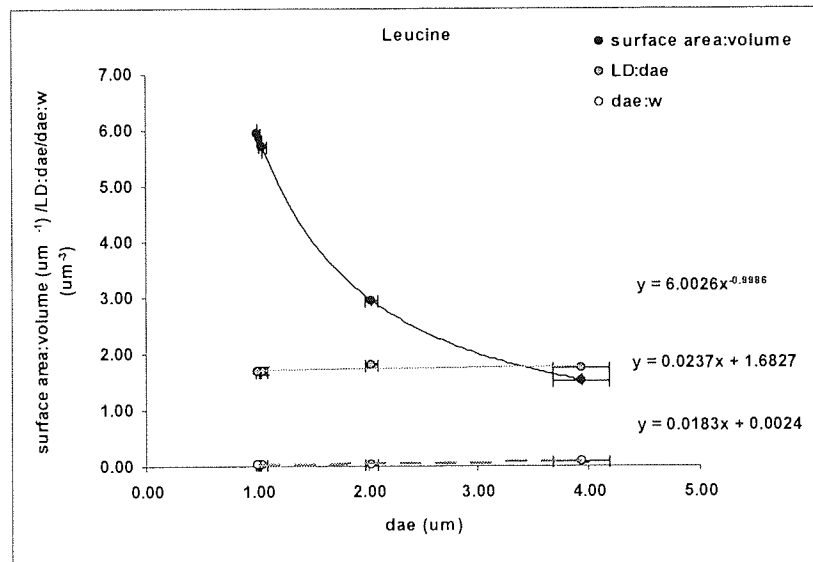
Figure 2.9: Chart illustrating the increase in surface area to volume with decreasing primary particle diameter (d_{ae}) in LEU modified spray dried powders compared to the decreasing aggregation ratio ($LD:d_{ae}$) and the relationship of thermal efficiency with increasing d_{ae}.

The Carr's Index value gives an indication of powder flow properties; a value less than 25% indicating a fluid flowing powder, a value greater than 25% indicating cohesive powder characteristics (De Villiers, 2005). Carr's Index values varied considerably within the spray dried powders, ranging from 12.7% (5% ASP: good flowability) to 32.5% (15% PHE: very poor flowability); interestingly, the addition of some amino acids appeared to produce powders with poorer flowability than the control powder (Carr's Index, 25.6%: cohesive, poor flowability; Table 2.1).

Physical characterisation of the spray-dried powders therefore demonstrated that the amino acid modified spray dried powders were amorphous in nature and contained low moisture content in particles of a size regarded as respirable. Although, there was considerable variation in the flowability of the powders, as evidenced by the Carr's Index values, this showed no correlation with the visual natures of individual spray dried powders. However, given that all the spray dried formulations were generated using identical spray-drying conditions, the difference in flowability must be attributed to amino acid modification, with some amino acids enhancing and others deteriorating the powder flow properties. The alteration of spray drying parameters, such as air flow rate, liquid feed rate and even inlet temperature, however minor, has been show to significantly affect powder flow properties (Masters, 1991; Maury et al., 2005), the maintenance of constant variables eliminating these factors (the reproducibility of the Büchi B290 unit has been validated with a free flowing powder in Appendix 1).

2.3.2 In vitro powder aerosolisation

The gravimetrically assessed emitted dose (ED) was defined as the percent of total loaded powder mass exiting the capsule. The ED of the control powder, i.e. no amino acid, was a

surprising 70%, although as indicated by the high standard deviation, the powder demonstrated a lack of reproducibility in the dispersion from the employed HPMC capsules following aerosolisation (Figure 2.10). The lack of reproducibility in powder output by the control would not provide suitable dosage uniformity if comparative *in vivo* tests were undertaken. Visual characterisation of the control powder during aerosolisation highlighted substantial variable electrostatic attraction of the spray dried formulation to the HPMC capsule wall preventing complete release of the powder during aerosolisation. A large number of the amino acid-modified powders also performed poorly during aerosolisation testing for similar reasons of attraction to the HPMC capsule, with an ED similar to, or on some occasions less than, that of the control. In contrast, dry powders formulated with leucine (5-20% LEU) demonstrated no electrostatic attraction to the HPMC capsules during dispersion resulting in high ED, with at least 95% of the capsule contents released during aerosolisation. The 20% PHE powder also performed encouragingly, with an ED of 91%, although with greater variability in ED compared to the LEU powders.

Due to the high variability in ED demonstrated by the control powder, a statistical improvement in ED in the amino acid-modified powders over the control powder could not be ascertained (one-way ANOVA/Dunnett: not significant). What is apparent, however, is that in comparison to the other spray-dried powders, the ED exhibited by the LEU powders was highly reproducible, as demonstrated by the small standard deviations in Figure 2.10. Statistical comparison of the ED of the LEU powders with non-leucine amino-acid modified powders indicated that they exhibited significantly higher ED than 5% ARG and 20% THR (one-way ANOVA/Tukey-Kramer: $p < 0.05$).
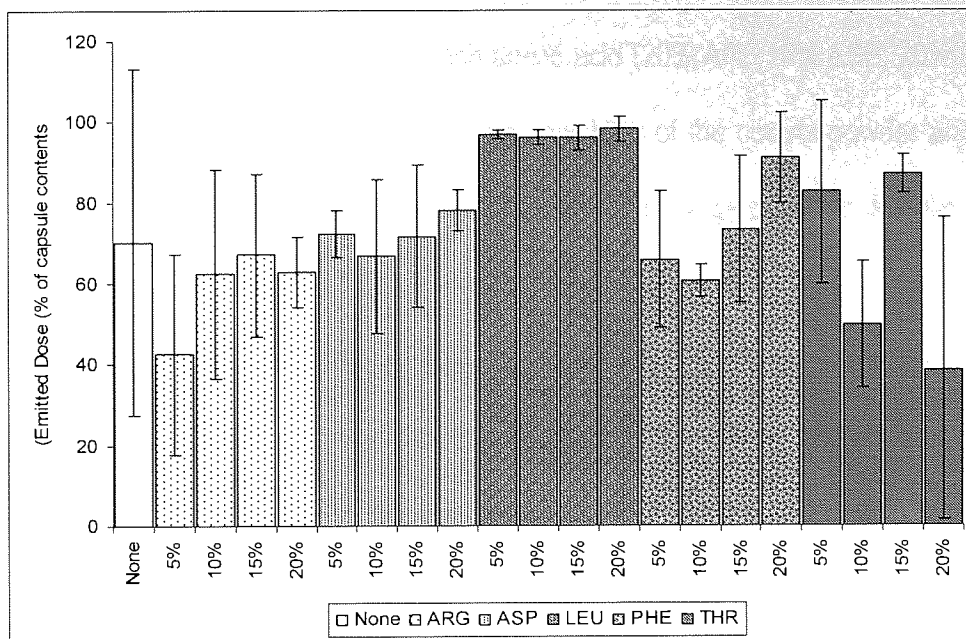
Figure 2.10: Bar chart illustrating the gravitationally assessed emitted dose of amino acid modified spray-dried powders, expressed as the percentage of total capsule content (mean ± SD, n=3).
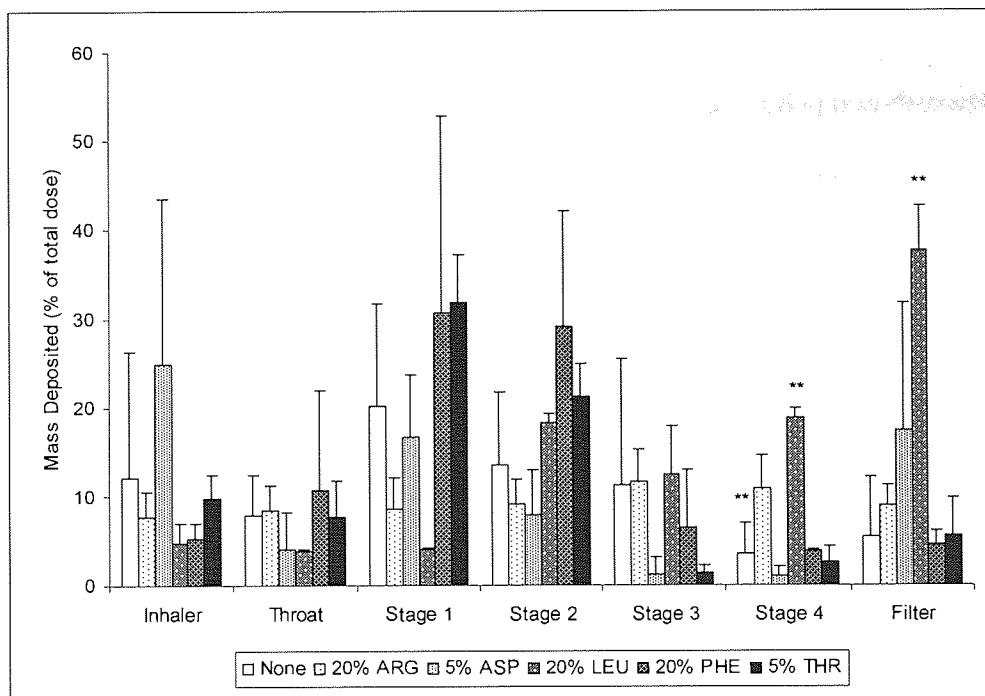
Figure 2.11: Bar chart representation of a Multi-Stage Liquid Impinger (MSLI) deposition profile for selected amino acid modified spray-dried powders, expressed as the percentage of total loaded dose (mean ± SD, n=3). Statistical difference (one-way ANOVA/Dunnett) from control powder at that stage of the MSLI: **$p<0.01$.

Figure 2.11 shows the MSLI deposition pattern of the control powder and the best performing powder (selected on the basis of FPF) for each amino acid (20% ARG, 5% ASP, 20% LEU, 20% PHE and 5% THR respectively). The lack of reproducibility of the control powder and some of the amino acid-modified powders is apparent, demonstrated by large standard deviations.

The powder modified with 20% ARG deposited consistently on each individual stage of the MSLI (one-way ANOVA/Tukey-Kramer: not significant), the USP II throat and in the Spinhaler® therefore not displaying targeting to any particular region of the lung; if *in vitro* aerosolisation proved representative of *in vivo* deposition. The large poly-dispersity of the 20% ARG powder is an indication of substantial differences in surface active force which is acquired via the shape and surface morphology of primary particles, flatter spray dried particles tending to 'stack' due to higher surface areas and the additional forces which coincide (Sacchetti & van Oort, 1996). Furthermore, the slight difference in surface texture or shape (Figure 2.3) is symptomatic of differing interfacial tensions at the atomiser liquid/drying air interface giving poly-dispersity in size which in turn gives varied heating of droplets which produces heterogeneous surface morphologies throughout a spray dried blend giving irregularity in surface active force and degree of aggregation. The high inhaler deposition observed with the 5% ASP powder indicative of poor aerosolisation properties with large aggregates most likely impinging on the propeller of the DPI device (Atkins, 2005). 20% PHE and 5% THR demonstrated high deposition on Stage 2 and above, with little deposition beyond Stage 2 (cut off 6.8 μm); these powders would not be expected to deliver a comparatively large dose to the conducting and peripheral lung following plume inhalation.

In contrast to the other formulations represented in figure 2.11, 20% LEU exhibited an optimal deposition profile, with little deposition on the inhaler, throat or Stage 1, and high deposition on Stages 2-4 and the filter. This powder would be expected to perform well during inhalation, and to deliver a large fraction of the total dose to the central conducting and even peripheral regions of the respiratory system. The proportion of drug reaching Stage 4 (1.7 μm) and the filter was significantly greater (one-way ANOVA/Dunnett: $p < 0.01$) than was observed for the control powder; this suggests that this powder may be useful to deliver active agents to the alveolar region of the lung, potentially for systemic drug delivery dependent up on patient variables and technique (Gonda, 1992).

The hypothetical loaded dose in each aerosolisation test was 1000 μg salbutamol (25 ± 0.25 mg powder per capsule, 4% w/w nominal drug load), yet the FPD for the control powder was only a fraction of the nominal at less than 300 μg salbutamol (Figure 2.12), providing a reflection of the poor aerosolisation characteristics of this unmodified powder. Interestingly, and possibly due to the electrostatic charges acquired by the formulations, the majority of amino acid modified spray-dried powders exhibited poorer aerosolisation properties than the control powder. In comparison, LEU-modified spray-dried powders demonstrated high FPD, with the best performing powder (15% LEU: FPD 673 μg salbutamol) exhibiting a statistically higher (one-way ANOVA/Dunnett: $p < 0.01$) FPD than was obtained with the control powder. Comparison of the 15% LEU formulation with all non-leucine amino acid-modified powders indicated that the FPD obtained for 15% LEU was significantly higher (one-way ANOVA/Tukey-Kramer: $p < 0.01$).

The 5% LEU, 10% LEU and 20% LEU powders also demonstrated significantly higher FPD than all other amino acid modified powders, with the exception of 20% ARG (one-way ANOVA/Tukey-

Kramer: p<0.05). As identified earlier, the drug content of each powder varied between 47-106%

of the nominal load holding a large influence over the FPD despite the relative good deposition of

some powders (e.g. 15% LEU). Figure 2.13 shows the fine particle fraction (FPF) of each

powder, corrected for actual drug content to give greater representation. The 20% LEU spray-

dried powder gave the highest FPF, with a cumulative plot estimation of 78% of total loaded

powder having an aerodynamic diameter <5 µm. Statistical analysis (one-way ANOVA/Dunnett:

p<0.05) indicated that the 10% LEU, 15% LEU and 20% LEU spray-dried powders had

significantly higher FPF than the control powder. In addition, the 15% and 20% LEU powders

had significantly higher FPF than all non-leucine amino acid-modified powders (one-way

ANOVA/Tukey Kramer: p <0.05 and p <0.001, respectively). If the *in vitro* tests proved

representative of *in vivo* conditions, these powders would be expected to deliver the majority of

the emitted dose to the target regions of the central to peripheral lung with minimal deposition in

the oropharynx, thus reducing the potential of local buccal side effects and resulting systemic

absorption and side-effects following the ingestion of material deposited on the back of the

throat.
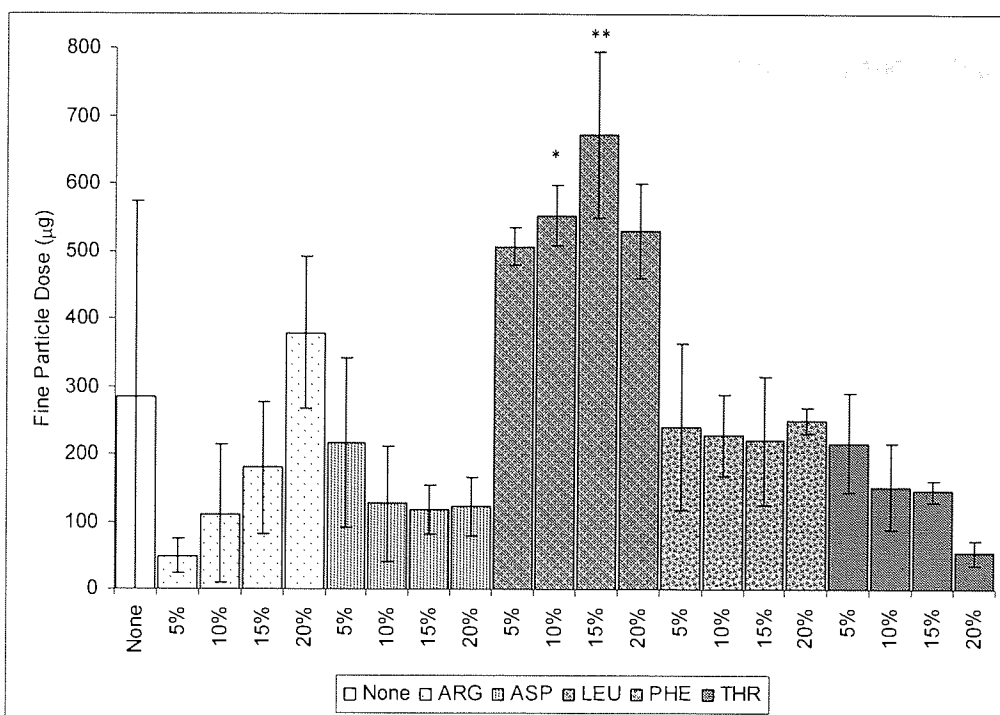
Figure 2.12: Bar chart representation of the Fine Particle Dose (FPD) of the amino acid modified spray-dried powder series, expressed as mass of salbutamol sulphate <5 μm aerodynamic diameter. Values are mean ± SD, n=3. Statistical difference (one-way ANOVA/Dunnett) from control powder: *p< 0.05, **p< 0.01.
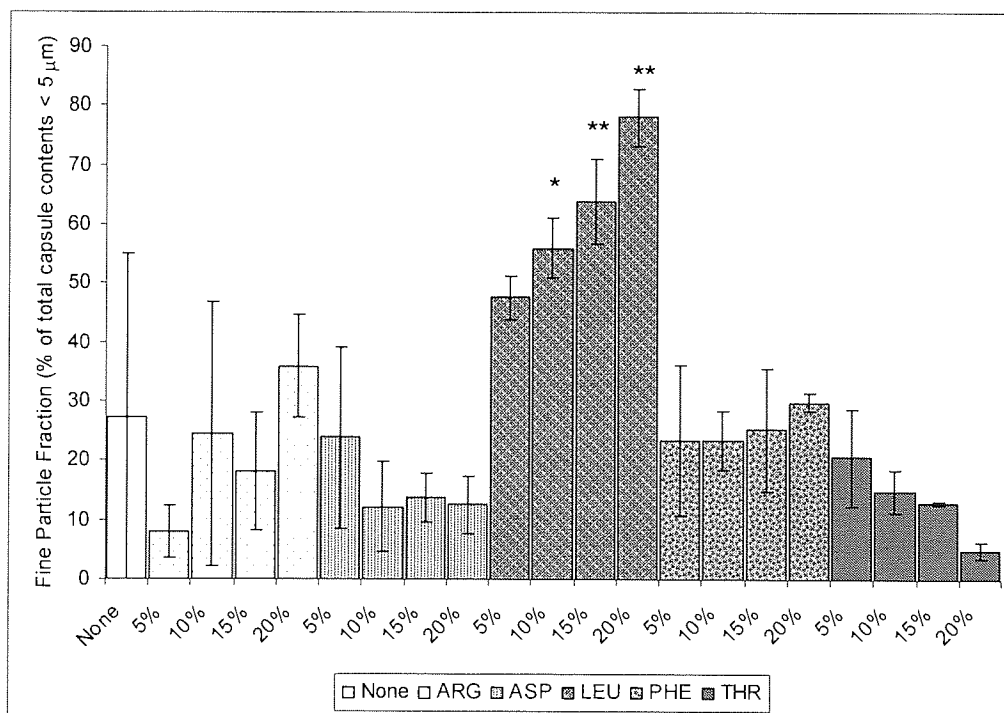
Figure 2.13: Bar chart showing the Fine Particle Fraction (FPF) of amino acid modified spray-dried powders, expressed as the percentage of total loaded dose <5 μm aerodynamic diameter, and corrected for actual drug content. Values are mean ± SD, n=3. Statistical difference (one-way ANOVA/Dunnett) from control powder: *p< 0.05, **p< 0.01.

The MMAD of the spray-dried powders varied between 0.86 µm (20% LEU) and 7.76 µm (20% ASP; Figure 2.4). Despite the theoretical estimates of primary aerodynamic diameter indicating that all powders comprised particles of a suitable size for pulmonary administration, just over half of the powders exhibited a MMAD greater than 5 µm (Table 2.1). This suggests that during aerosolisation, these spray-dried powders did not behave as individual particles, but rather as particle aggregates due to high surface energy. In comparison to individual particles, the larger mass of these aggregates results in a higher gravitational pull during aerosolisation, hence the aggregates fail to navigate the 90° USP II throat angle and the higher MSLI stage cuts depositing via inertial impaction higher in the MSLI, leading to a reduced FPD and FPF.

In contrast, aerosolisation of all leucine-based spray-dried powders resulted in the dispersion of individual particles that due to their lower mass exhibit reduced momentum and gravitational forces, resulting in more prolonged residence in the slip stream of the 60 L/min inspiratory flow rate and in deposition at the lower stages of the MSLI, with effective high FPD and FPF. The reduction of surface tension at the atomiser air interface in the drying chamber of the B290 spray drier by LEU, and theoretically PHE due to structural similarity, could potentially give a finer spray (Wolfenden et al., 1981), the finer spray allowing the greater penetration of heat at the drying air/liquid droplet interface post atomiser. The increased contact between the two phases manifesting as the rougher surface textures of the LEU-modified spray dried powders compared to the other amino acid modified powders. The rougher surface texture a consequence of excess heat of evaporation produced by LEU reducing the amount of heat required to drive off water from the droplet during spray drying to produce a dry particulate, the excess heat transpiring into increased outlet temperatures and reduced thermal inefficiency. The effects of

decreasing thermal efficiency with increasing leucine concentration on particle size are shown in Figure 2.14.
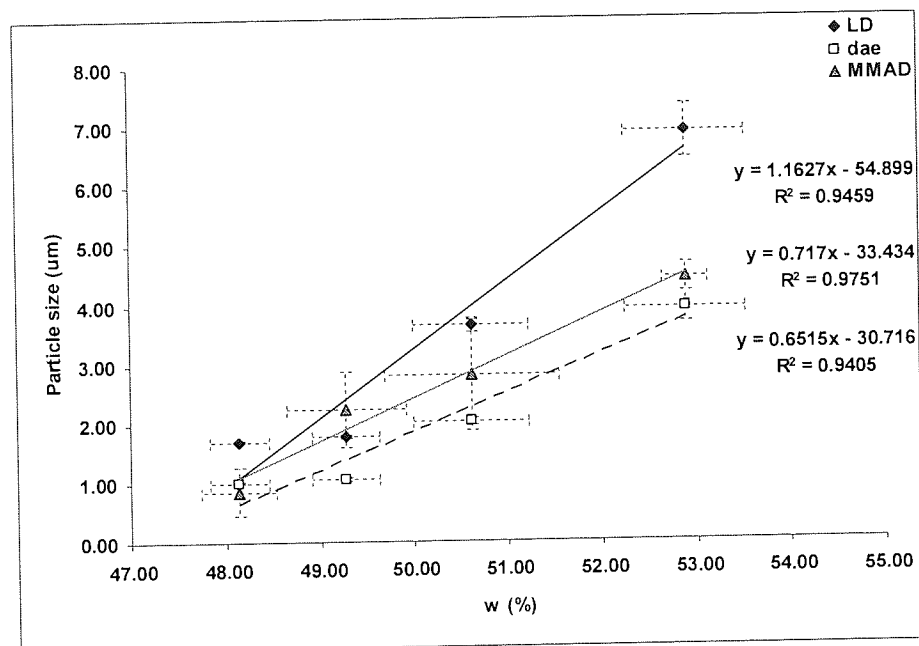
Figure 2.14: Chart illustrating the effect of spray drying thermal efficiency on the Fraunhoffer dry dispersion laser diffraction size (LD), primary particle diameter (δ_{ae}) and mass median aerodynamic diameter (MMAD).

Despite evidence to the contrary it is possible that LEU may also be acting as a surface active agent at the particle surface or be behaving as a glidant lubricating the particles surface, as spray dried leucine has shown to do in the coating of dies in tablet production (Rotthauser et al., 1998), previous research showing the migration of LEU to the surface of spray dried particulates (Chew et al., 2005b). The overall shape and morphology compared well with recent studies which investigated the use of co-spray dried formulations involving amino acids (Li et al., 2005a, Rabbani & Seville, 2005) with the exception of Chew and co-workers (2005b) who produced far more pitted particulates when spray drying leucine with disodium chromoglycate at lower concentrations of amino acid and (according to literature) at only 90°C.

Carr's index flowability data showed little correlation with the aerosolisation performance of the spray-dried powders (Figure 2.15). It was anticipated that those powders demonstrating lower Carr's Index values (i.e. better powder flow) would exhibit enhanced aerosolisation performance, evidenced by increased ED and FPF. However, the aerosolisation performance of the spray-dried powders appeared to be independent of respective Carr's values, possibly due to the way that the index is calculated, the tool essentially measuring the compressibility of the powder relative to powder flow.

Highly compressible powders, those demonstrating a large difference between bulk and tapped density, are considered to have poor powder flow properties according to the Carr's Index parameter whereas better powder flow properties are attributed to powders that exhibit minimal change to powder density following tapping. Commonly applied to the flow of powders comprising of large crystalline particles doubts exist over the use of the Carr's Index tool when considering potentially cohesive porous spray-dried powders with relatively large surface areas and surface active forces, as it has previously been demonstrated that spray-dried powders exhibit high compressibility, and has been suggested that Carr's Index provides an estimate of the interparticulate forces within particle aggregates (Louey et al., 2004a; 2004b).

It is feasible that the spray-dried powders investigated in this study comprised of particle aggregates with weak, intermediate or strong interparticulate forces. For powders with weak aggregates these forces might be easily overcome during the tapped density measurement, leading to the production of primary particulates and particle rearrangement into the

interparticulate voids. A large difference would therefore be apparent between the bulk and tapped density of the powder, prescribing a high Carr's Index value and poor flowability classification. In comparison, a powder with strong interparticulate cohesion would require higher forces to break down the aggregates, and would therefore withstand the tapping forces and remain as aggregates; the result would show minimal difference between bulk and tapped density; the powder therefore receiving a low Carr's Index value and a good flowability classification (Louey et al., 2004a; 2004b).

In direct comparison, powders with weak aggregates would be easily dispersed into primary particles during aerosolisation, and would demonstrate superior aerodynamic properties compared to powders with strong aggregates, which would remain as aggregates during aerosolisation. These data suggest that classification of powder flowability using Carr's Index does not provide a reliable prediction of the aerosolisation performance of spray-dried powders.
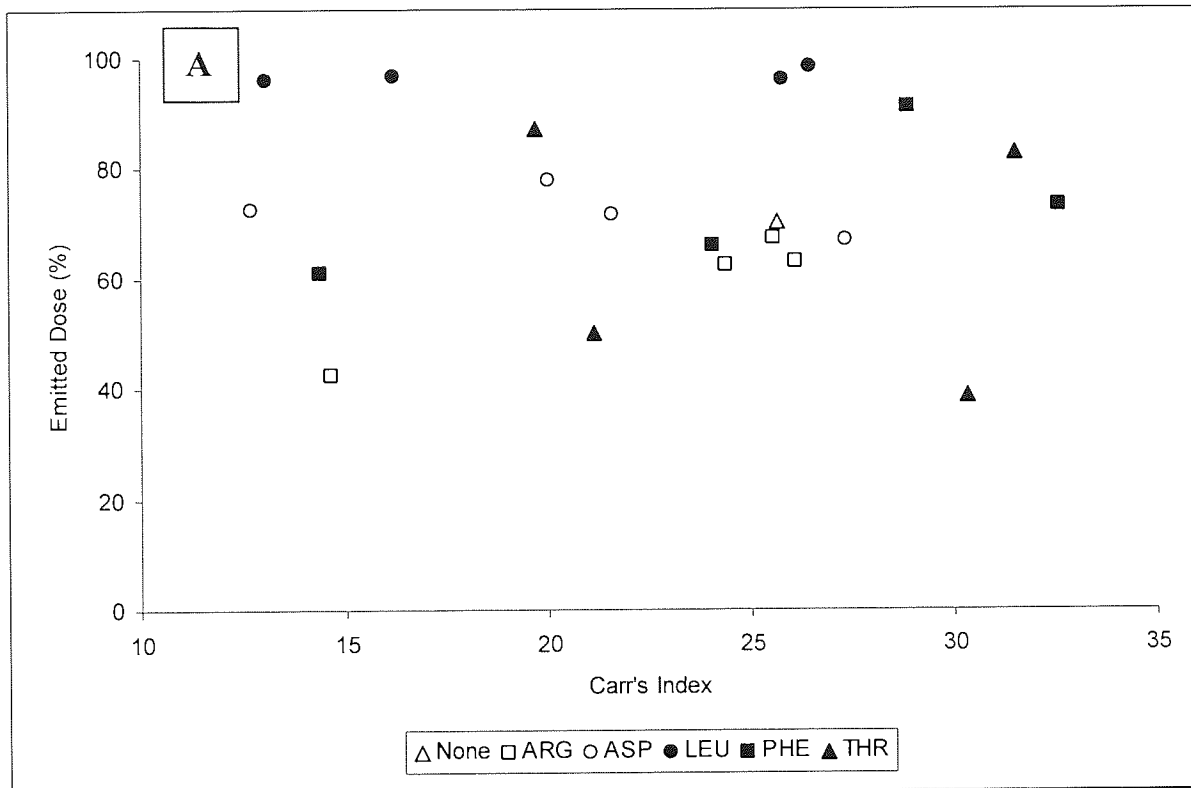
Figure 2.15: Scatter graph comparison of Carr's Index values calculated for each spray-dried powder with emitted dose (A) and fine particle fraction (B).
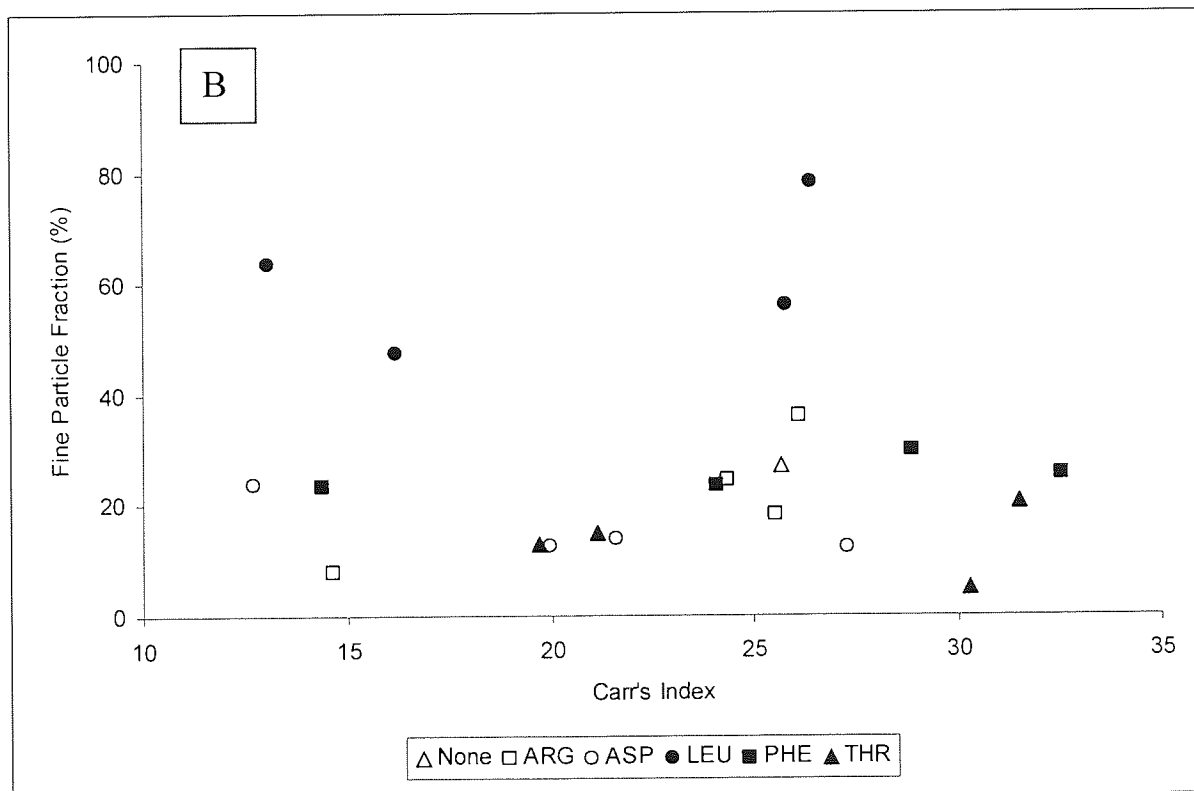
2.4   Conclusions

Through the manipulation of various spray drying parameters such as drying air velocity, liquid feed rates and the careful selection of glassware the primary particle diameter of a formulation can be determined (Maury et al. 2005). However, such reductions in particle size and the complementary increase in surface area and relative surface active forces have proven to cause variable cohesion within spray dried populations reducing the respirability of such formulations (Sacchetti & van Oort, 1996).

Recently it has been demonstrated that the addition of a range of amino acids, including but not exclusive to leucine, to spray-dried formulations of DNA-containing non-viral gene delivery vectors can significantly reduce the interactions between the constituent particulates and enhance dispersibility and *in vitro* deposition (Li et al., 2003; 2005a). The study concluded that phenylalanine also improved *in vitro* deposition, the structural similarities observed between leucine and phenylalanine would suggest that the two amino acids should indeed be similar in action.

In this study, using salbutamol sulphate as the active marker, a significant enhancement in FPF was only observed in leucine-modified powders. The reason for this may possibly include the different analytical methods for determining FPF in these studies, i.e. through the inherently variable biological activity of DNA (Li et al., 2005a) and through HPLC analysis of specific drug quantity (this study).

In addition the use of HPMC capsules for spray dried powder loading in the place of the gelatine capsules utilised by *Li et al.* (2005a) may also have a profound effect on the electrostatic surface active forces of the loaded micro-particulates. Desirable aerosolisation properties may have been lost through the use of potentially electrostatic HPMC capsules, PHE modified powders may not experience such forces when dispersed from gelatine capsules. It should be noted that PHE showed the same wrinkled appearance of constituent micro-particulates as LEU when viewed by SEM. The wrinkled appearance a by-product of overheating during spray drying, potentially due to a change in the thermal efficiency of the system (although no data was recorded for PHE), brought about by the alteration of the formulation being processed. The reduction of surface tension by the relatively bipolar structure of PHE acting as surface active agent could be a root cause although no subsequent reduction in thermal efficiency was noted. In addition, despite the production of particles with more fractured surface morphologies by PHE spray dried powders a reduction in aggregation was not seen with increasing PHE concentrations or with decreasing d_{ae} as was seen with the LEU series.

The consistency of standard spray-drying operating conditions and assay procedures employed during this study enable the effect of amino acids upon the salbutamol lactose spray dried formulation to be derived. Previously, it has been demonstrated that the incorporation of leucine enhances the aerosolisation properties of spray-dried lactose powders (Li et al., 2003). Further to this research a similar investigation involving research in to the effects of 5% w/w arginine, leucine, methionine, phenylalanine and tryptophan on spray dried disodium chromoglycate powders have shown how leucine has less polar interaction and hydrogen bonding compared to the other amino acids tested; the reduction in hydrogen bonding lowering surface active forces. The surface coverage of the amino acids on the microparticulates was explored by the

researchers, and revealed that a lower percentage of less polar amino acids (including LEU) migrated to the surface but that the reduction in surface activity was greater (Chew et al., 2004). A further study by *Najafabadi et al.* also explored the use of 10% w/w leucine in spray dried disodium chromoglycate formulations but with comparatively poor outcomes (Najafabadi et al., 2004).

It has previously been suggested that LEU displays surfactant-like properties (Gliniski et al, 2000), and has the capacity to migrate to the droplet surface during the rapid drying phase in spray-drying, hence influencing the surface characteristics of the resultant particle by affecting surface tension and the thermal efficiency of suspended drying droplets (Columbano et al., 2003; Rabbani and Seville, 2005; Chew et al., 2005). Indeed, a closer look at the structural arrangement reveals that only leucine and phenylalanine out of the five amino acids tested have a bipolar structure where the hydrophilic amine and carboxylic acid groups are in close proximity thus exposing a lipophilic hydrocarbon chain independent of conformation. It has also been suggested that the surface activity of a solute is linked to the solute's hydrophobicity (Gliniski et al., 2000), and that LEU is a particularly hydrophobic amino acid (Black and Mould, 1991).

In addition to surface activity, the enhanced surface roughness of the leucine modified powders caused by a shift in the thermal efficiency during spray drying (as evidenced by scanning electron microscopy) may present fewer sites for interparticulate cohesion, thereby facilitating dispersion of primary particles during aerosolisation. Previous researchers have failed to recognise the link between thermal efficiency and the action of LEU and the parameters effect on the production of respirable particulates from spray drying as a whole.

In conclusion, the use of LEU in a simple spray dried 4% w/w salbutamol in lactose formulation makes an effective co-excipient for the enhancement of *in vitro* aerosolisation with the intention of either local or systemic pulmonary delivery. Therefore, the reduction of aggregation and the enhancement of *in vitro* deposition profiles by LEU show the potential of the amino acid to enhance a drug-polymeric spray dried formulation aimed at producing the overriding theme of this thesis, a respirable controlled release powder.

Chapter 3

The investigation of leucine-modified

chitosan spray-dried powders.

3.1   Introduction

The use of aqueous solutions or suspensions in the production of spray-dried powders that alter the solubility or availability of a drug in a dry powder formulation has been previously documented. For example, with the improvement in solubility of poorly water-soluble drugs, *Ozeki and co-workers (2005)* explored the use of co-spray drying sodium salicylate and flurbiprofen to potentate solubility and *Wong and co-workers (2006)* co-spray dried poloxamer with griseofulvin to enhance the water solubility of the hydrophobic antifungal. Further excipients investigated in spray-dried formulations to modify drug release rates include hydroxypropyl cellulose (Surendrakumar et al., 2003; Cook et al., 2005) and polylactic acid (Taylor et al., 2006).

There are many advantages to developing sustained release formulations in pulmonary drug delivery, including reduced dosing frequency, improved patient compliance and reduction in side effects (Hardy & Chadwick, 2000). When the use of a polymer is employed as a rate-limiting factor to achieve sustained drug dissolution this ultimately indicates the requirement of a partially water insoluble vector (Kim et al., 2006), in addition further specifications of the pulmonary route require that the polymer must be capable of forming particles of less than 5 μm in diameter, and that such particles have sufficient bio-adhesive nature to retard the lung clearance methods discussed in chapter one.

Chitosan, an amino-polysacharide produced by the deacetylation of the naturally occurring polymer chitin (derived from crustacean shells), is insoluble in water until acidified into a gel structure of interlocking chains. A promising excipient with a wide range of applications chitosan can be used to promote sustained release in spray-dried preparations (Illum, 1998). Chitosan

has also been employed in pharmaceutical research as a bio-compatible polymer for nasal delivery (Witschi & Mrsny, 1999; Cerchiara et al., 2005; Martinac et al., 2005a, b), which requires an optimum particle size of 15 μm (Gavini et al., 2005); chitosan also has antifungal (Prapagdee et al., 2006), mucoadhesive (Harikarnpakdee et al., 2006; Sigurdsson et al., 2006; Dang et al., 2006) and penetration enhancing attributes (Gavini et al., 2006; Corrigan et al., 2006b; Rabe et al., 2006).

Given that chitosan not only acts as a drug release modifier but also has mucoadhesive properties (Martinac et al., 2005b; Harikarnpakdee et al., 2006), it would appear to be a useful excipient when preparing sustained release formulations for pulmonary drug delivery. Although substantial research has been aimed at developing such formulations, only a handful of researchers have investigated the viability of chitosan-modified spray-dried powders for pulmonary drug delivery (Huang et al., 2003; Asada et al., 2004; Grenha et al., 2005; Corrigan et al., 2006b), with none apparently having considered the incorporation of dispersibility enhancers such as leucine to improve powder aerosolisation character.

The advent of multiple treatments for chronic obstructive respiratory disease has had a positive affect in the management of a potentially life threatening disease. However, such management is heavily dependent up on patient compliance and concordance with often complex regimens of three or more devices (Holgate & Polosa, 2006). Similarly, patients on medication for asthma can experience breakthrough symptoms and acute exacerbations of the disease over an extended time period, such breakthroughs are often down to poor medication compliance or disease management (Barnes, 2006; Holgate & Polosa, 2006).

The introduction of dry powder "combination" inhalers on to the global market incorporating a long acting β2 agonist with a long acting corticosteroid has aided disease management. However, poor delivery of active agents and subsequent side effects from typical dry powder inhaler (DPI) formulations are still causes for concern with the addition of a short acting β2 agonist to the regimen recommended to treat breakthrough symptoms (Theophilus et al., 2006; Holgate & Polosa, 2006; Balanag et al., 2006). Theoretically, the delivery of a highly efficacious short acting β2 agonist along with a corticosteroid to the bronchi as part of a sustained release formulation could potentially simplify dosing regimens by reducing frequency of administration and thus improve disease management.

In this chapter, formulations of chitosan (employed as a drug release modifier) and leucine (LEU: dispersibility enhancer) were used to generate highly dispersible powders that exhibit sustained drug release properties in the presence of hydrophilic and/or hydrophobic active agents. A detailed investigation was also made in to the effects increasing LEU concentration and chitosan molecular weight have on the physical, aerodynamic and solvate character of the spray dried formulations.

3.2 Materials and Methods

3.2.1 Materials

Terbutaline sulfate, beclometasone dipropionate (BDP), low molecular weight (LMW: <190 kDa), medium molecular weight (MMW: 190-310 kDa) and high molecular weight (HMW: >310 kDa) chitosan, phosphate-buffered saline (PBS) tablets, α-lactose monohydrate and l-leucine were purchased from Sigma Aldrich Chemicals (Poole, UK). The Bricanyl Turbohaler® (Astra Zeneca,

Loughborough, UK) was purchased from AAH pharmaceuticals Ltd (Coventry, UK). HPLC grade methanol and ethanol were purchased from Fisher Scientific Ltd (Loughborough, UK).

3.2.2 Preparation of spray-dried powders

3.2.2.1 The influence of varying leucine concentration

Formulations for spray-drying containing 0 (control), 6, 12, 18, 36% w/w LEU were prepared by the addition of an aqueous solution of terbutaline sulfate, LEU and lactose (bulking agent) to a HMW chitosan gel.

The HMW chitosan gel was prepared by homogenizing (Heidolph, Kelheim, Germany) 2.7 g HMW chitosan in 100 mL glacial acetic acid aqueous solution (0.55% v/v) for two hours. 37.5 ml of the chitosan gel (enough to obtain 1 g of chitosan) was then mixed with 30 mL ethanol. A 33.5 mL aqueous solution of 80 mg terbutaline sulphate, 120 to 720 mg LEU and 200 to 800 mg lactose was then combined with the chitosan ethanol mixture under homogenisation at 1600 rpm for 10 minutes to produce a 100 mL 30% v/v aqueous ethanol solution (Rabbani & Seville, 2005) containing a total solid mass of 2% w/v (50% w/w chitosan).

The prepared formulations were subsequently spray-dried using a Büchi B-290 mini spray-dryer with a 0.7 mm two-fluid nozzle, equipped with either the standard cyclone or the high performance cyclone (Büchi Labortechnik AG, Switzerland) using the standard operating conditions detailed in chapter 2.

The ten formulations, five produced by the normal cyclone, five by the high performance cyclone, were then assessed for physical, aerodynamic and solvation properties.

3.2.2.2 The influence of varying chitosan molecular weight

A total of 18 spray dried formulations where prepared in this section. 15 spray dried formulations were prepared by the addition of an aqueous solution of terbutaline sulfate and/or beclometasone dipropionate (BDP), LEU (aerosolisation enhancer) and lactose (bulking agent) to a chitosan gel, prepared using LMW, MMW, HMW chitosan or combinations thereof. With the addition of three controls of 4% w/w terbutaline and/or 4% w/w BDP, 36% w/w LEU and up to 60% w/w lactose prepared by spray drying as 2% w/w aqueous ethanol (30% w/v) solutions.

LMW chitosan gel was prepared by homogenization of 4 g LMW chitosan in 100 mL glacial acetic acid aqueous solution (1.5% v/v) for 2 hours. MMW chitosan gel was prepared by mixing 2.5 g MMW chitosan in 100 mL glacial acetic acid aqueous solution (0.5% v/v: the minimal amount of acid required for solubilisation) for 2 hours, HMW chitosan gel was as prepared as outlined previously. All preparations were allowed to stand overnight before use.

Sufficient chitosan gel to provide 1 g chitosan was measured and subsequently diluted with 30 mL ethanol to prepare LMW, LMW/MMW, MMW, MMW/HMW or HMW chitosan formulations. For example, to prepare the LMW chitosan formulation, 25 mL LMW chitosan gel (containing 1 g LMW chitosan) was mixed with 30 mL ethanol. An aqueous solution of 80 mg terbutaline sulphate and/or 80 mg BDP, 720 mg LEU and 120 - 200 mg lactose was then combined with the chitosan ethanol mixture under homogenisation at 1600 rpm for 10 minutes to produce 100 mL of

a 30% v/v aqueous ethanol solution containing a total solids mass of 2% w/v (50% w/w of which was chitosan).

The prepared formulations were subsequently spray-dried using a Büchi B-290 mini spray-dryer equipped with a high performance cyclone (Büchi Labortechnik AG, Switzerland) with a 0.7 mm two-fluid nozzle, using the standard operating conditions detailed in chapter two.

3.2.3 Powder characterisation

The powders were characterised as described in Chapter two, to determine spray-drying yield, drug loading, particle morphology, amorphous nature, water content and powder density. In addition, the primary aerodynamic diameter (d_{ae}) and Carr's Index values were calculated as described previously.

3.2.3.1 Particle size

In section 3.3.1, wet dispersion laser diffraction was utilized. Approximately 5 mg of spray-dried sample was suspended in hexane and the suspension ultra-sonicated (Soniprep 150; Curtin Matheson Scientific Ltd, Houston, TX, USA) for 30 s. The particle size of the sample was then measured by laser diffraction (Mastersizer; Malvern Instruments, Malvern, UK) using a 100 mm focal length lens achieving 5% obscuration. Each sample measured in triplicate and the data obtained expressed as the volume weighted mean particle size (D[4,3]).

In section 3.3.2, dry dispersion laser diffraction was used to assess particle size using a Sympatec HELOS particle size analyzer. For Sympatec measurement parameters please refer to chapter 2.

3.2.4 In vitro powder aerosolisation

3.2.4.1 The influence of varying leucine concentration (ACI)

The aerosolisation properties of the spray-dried powders were investigated using an Andersen Cascade Impactor (ACI: Copley Scientific). Stages -1 to 6 of the ACI were lined with pre-weighed Whatman GF-A filter papers, with the stage plates cut side down. The flow rate through the ACI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific). Powder aliquots of 25 mg loaded into nine size 2 HPMC capsules (Shionogi Qualicaps) and placed into a Spinhaler® dry powder inhaler (DPI) were attached to the ACI via a stainless steel USP throat (volume: 66 mL). The capsule was pierced and the liberated powder drawn through the ACI at a flow rate of 60 L/min for a 5 s aspiration using a pressure calibrator (Model TPK: Copley Scientific), the capsule exposed to a further 5 s aspiration after a 10 s interval. Under these conditions, the effective cut-off diameters are Stage -1: 8.6 µm; Stage 0: 6.5 µm; Stage 1: 4.4 µm; Stage 2: 3.2 µm; Stage 3: 1.9 µm; Stage 4 1.2 µm; Stage 5: 0.55 µm; Stage 6: 0.26 µm with Stage F as a terminal filter. Each deposition experiment was performed in triplicate.

The emitted dose (ED), defined as the percent of total loaded powder mass exiting the capsule, was determined gravimetrically as in chapter 2. The filters from each stage were removed and also assessed gravimetrically with the powder brushed from around the preceding stage edges,

to recover the re-bound of appropriately sized particles off the stage. Powder brushed from the inhaler and throat regions was also assessed gravimetrically. The total dose was defined as the total powder mass loaded into the HPMC capsules before aerosolisation. Aerodynamic fractions and parameters were then derived as detailed in chapter 2.

3.2.4.2 The Influence of varying chitosan molecular weight (MSLI)

The aerosolisation properties of the spray-dried powders in this subchapter were investigated using a Multi-Stage Liquid Impinger (MSLI: Copley Scientific). HPLC mobile phase (20 mL) was introduced to Stages 1-4 of the MSLI, and a filter paper (Whatman GF-A) placed at Stage 5. The flow rate through the MSLI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific). Powder aliquots (3 x 25 mg) were loaded into size 2 HPMC capsules (Shionogi Qualicaps) and placed into a Spinhaler® dry powder inhaler (DPI), attached to the MSLI via a stainless steel USP throat. The capsule was pierced and the liberated powder drawn through the MSLI at a flow rate of 60 L/min for 2 x 5 s aspirations using a pressure calibrator (Model TPK: Copley Scientific). Under these conditions, the effective cut-off diameters are Stage 1: 13.0 μm; Stage 2: 6.8 μm; Stage 3: 3.1 μm; Stage 4: 1.7 μm; with Stage 5 as a terminal filter. Each deposition experiment was performed in triplicate.

The emitted dose and MSLI derived aerodynamic parameters were then calculated as outlined in the materials and methods section of chapter 2 with the exception of the six dual loaded formulations containing both terbutaline sulphate and BDP, where the FPF, FPD and MMAD values were calculated as an average of the six individual terbutaline and BDP values.

3.2.5 HPLC analysis of terbutaline sulfate and BDP

The mass of terbutaline deposited on each stage of the MSLI was determined using reverse-phase HPLC (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6 x 150 mm Phenomomex La Luna column (Phenomomex, USA) and 15 µl injection volume with UV detection at 276 nm. The mobile phase (1 mL/min) consisted of 23% v/v aqueous methanol, with terbutaline eluting with a retention time of 2.5 min (Xu et al., 2005; Weda et al., 2004), with LOD to 99% confidence 0.69 µg/ml, LOQ 0.93 µg/ml.

The mass of beclometasone deposited on each stage of the MSLI was determined using reverse-phase HPLC as described for terbutaline with UV detection of BDP at 250 nm eluting at a retention time of around 5 min (Gupta & Myrdal, 2004), with LOD to 99% confidence 0.98 µg/ml, LOQ 1.23 µg/ml. Example trace appendix 1.

3.2.6 In vitro dissolution

Dissolution testing was performed on 200 mg spray dried powder samples using Modified USP II dissolution apparatus (Hanson research SR6 Dissolution Test Station: Hanson Ltd, California, USA; Caleva SG6 and 65G Dissolution apparatus: Caleva Ltd, Dorset, UK, or Sotax A7 Dissolution Apparatus: Sotax, London, UK) with 2 cm diameter stainless steel wire baskets (Copley Scientific Ltd., Nottingham, UK), rotating at 50 rpm in 1000 mL PBS (37°C, pH 6.8) (Shaw et al., 2005). Samples (3 mL) were withdrawn for analysis at specified time points, and assessed for terbutaline and/or BDP content by UV spectroscopy (Jenway 6305 UVvis spectrophotometer: Dunmow, Essex, UK) at 276 nm and 250 nm respectively, the sample was

returned to the bath immediately after analysis. Each dissolution experiment was performed in triplicate.

Further assessment of dissolution rate was performed using the plotted averages of zero (Formula 3.1), first (Formula 3.2) and Higuchi homogeneous matrix (Formula 3.3) rate equations (n=3, Philip & Pathak, 2006; Richards & Aulton, 1988):

$$Q = kt$$

Formula 3.1: Zero order rate equation, where Q (ordinate), is the cumulative total of mass detected during sampling at time t (abscissa) and k (gradient) is the dissolution rate constant (Richards & Aulton, 1988).

$$\log\frac{Co}{C} = \frac{kt}{2.303}$$

Formula 3.2: First order rate equation, where *log (Co/C)* (ordinate) is the logarithm of *Co*, the initial concentration contained in the spray-dried powder, divided by *C*, the concentration remaining after time t (abscissa) and k (gradient) is the rate constant (Philip & Pathak, 2006; Richards & Aulton, 1988).

$$Q = kt^{1/2}$$

Formula 3.3: Higuchi homogeneous matrix rate equation, where Q (ordinate) is the cumulative drug release at time t and k is the rate constant, a plot of $t^{1/2}$ is used to determine appropriateness (Philip & Pathak, 2006).

Plots of the rate equations were then assessed using least squares linear regression lines formulated using the following equations (Formula 3.4):

$$y = a + bx$$

$$b = \frac{\sum xy - n\overline{x}\,\overline{y}}{\sum x^2 - n\overline{x}^2}$$

$$a = \overline{y} - b\overline{x}$$

Formula 3.4: Formula for linear regression.

The squared residual difference between the expected trend line values and the observed values is then subtracted from 1 to give an r^2 value. The closer the r^2 value to 1 the more appropriate the kinetic of the rate order is to the samples dissolution data.

3.2.7 Statistical analysis

The drug loading, emitted dose, FPD and FPF of the chitosan spray-dried powders were statistically compared to those of the control spray-dried powder using one-way analysis of variance with Dunnett multiple comparison test. Where appropriate, the aerosolisation properties of the chitosan powders were compared against each other using one-way analysis of variance with Tukey-Kramer multiple comparisons test. Where not declared the significance level was 0.05.

3.3 Results and Discussion

3.3.1 The influence of varying leucine concentration

3.3.1.1 Powder characterisation

Ten spray dried powders of 0% w/w, 6% w/w, 12 w/w, 18% w/w and 36% w/w LEU concentration were produced using both a high performance (HPC) and standard Büchi B-290 mini lab spray-dryer cyclone (SC); the maximum concentration of LEU being limited by its solubility in the 30% v/v aqueous ethanol blend. Spray drying yields varied from 53.3 to 77.2% of the anticipated dry mass (formulations containing 12% LEU SC and 6% LEU SC respectively: Table 3.1). Unlike the LEU modified salbutamol lactose spray dried powders from the previous chapter where an increase in LEU concentration gave an increase in spray drying yields, LEU modified chitosan spray drying yields appeared to be unaffected by LEU concentration (n=1). However, the use of a high performance cyclone appeared to have an effect on the yield of LEU modified chitosan

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spray powders with increases in yield gained from using the HPC over the standard Büchi B-290 mini lab spray-dryer cyclone. The HPC has previously been shown to increase yield through the collection of finer spray dried particles that are exhausted to the filter unit of the Büchi B-290 mini lab spray-dryer when the SC is employed due to a change in pressure variables and effective cut off diameters, as represented in Barth's classic model in chapter 1 (Maury et al., 2005).

Chitosan preparations of increasing LEU content appeared off-white in appearance and free flowing. Scanning electron microscopy revealed microspheres of 2 to 5 μm in diameter with a textured surface, the spray dried samples could be presumed amorphous due to the lack of defined shape seen in SEM imaging of crystalline materials (Bosquillon et al., 2001b), with the surface becoming more textured with increasing LEU concentration. The texture relating to the surface active phenomena of leucine and its effects on thermal efficiencies in spray drying has been described in chapter 2. The addition of ethanol to the gel pre-spray drying also holds a bearing on a more wrinkled appearance, the use of the ethanol co-solvent reduces the heat required to dry a suspended droplet post atomiser, excess heat manifests as kinetic energy during drying causing a "fizz" effect, the fizz giving greater texture to the resulting dry particle. The presence of wrinkling is thought advantageous as it lowers the inter-particulate contact area and reduces the surface available to forces of aggregation (Alexander et al., 1985; Masters, 1991; Rabbani & Seville, 2005).

Cyclone[a]	Leucine (%w/w)	%Yield	%ED	%FPF	MMAD(μm)	Size (μm)	d_{ae} (μm)
High Performance	0	66.0	94.77±0.80	16.68±1.03	5.68±0.88	8.11±0.22	3.02±0.11
High Performance	6	76.8	97.60±0.98	19.03±3.15	6.12±0.63	5.54±3.68	2.33±1.49
High Performance	12	55.4	96.84±3.05	25.42±1.09	5.86±0.52	8.62±1.35	3.26±0.56
High Performance	18	69.2	96.60±0.95	27.86±0.96	6.63±1.11	5.05±0.96	1.91±0.30
High Performance	36	55.7	97.50±2.20	52.68±6.22	3.19±0.39	5.53±3.02	1.99±1.08
Standard	0	54.2	95.36±2.85	14.72±0.11	6.40±0.23	10.01±0.24	5.11±0.12
Standard	6	77.2	94.10±1.10	17.96±1.64	8.46±0.35	9.72±0.42	4.24±0.19
Standard	12	53.3	97.24±1.81	24.86±0.79	8.58±0.29	6.25±0.38	2.50±0.15
Standard	18	59.0	93.32±0.50	21.87±3.34	7.50±2.41	4.34±0.13	1.68±0.21
Standard	36	55.1	99.38±0.23	50.08±1.91	3.52±0.38	3.58±0.36	1.34±0.18

Table 3.1: Table highlighting the affect of cyclone and leucine modification on spray dried chitosan properties (mean ± SD, n=3), yield [a]n=1.

The appearance of fine fibres was evident in only one image that of the formulation with the highest LEU concentration (36% w/w leucine: Figure 3.1G). It is possible that these flakes are either leucine or chitosan "spears" caused by the saturation and subsequent precipitation of either component, the identity of the fibres is discussed in greater detail in the next sub section. Ruptured microspheres within the spray dried samples were also captured by SEM imaging, indicating that spray dried chitosan microspheres are hollow (Figure 3.1A). A detailed study demonstrating the hollow nature of spray dried microspheres was performed recently by *Hadinto et al.* (2006) who used spray drying for the purpose of generating hollow particulates.

The increase in LEU content from 6 to 36 % w/w was represented by visually decreasing aggregation in the SEM images (Figures 3.1A, C and E). Figures 3.1A and B show a large degree of aggregation present in the 4% w/w terbutaline, 6% w/w LEU, 50% w/w chitosan HMW and 40% w/w lactose formulation. A 12% w/w increase in LEU concentration (from 6% to 18% w/w LEU) to the detriment of lactose showed a visible decrease in aggregation (Figures 3.1A, E).

The series of spray-dried chitosan powders with increasing LEU concentration were found to be relatively stable by DSC with no crystalline fusion peaks evident in the respective spectra (example trace: Figure 3.2). The water contents of the eight powders as determined by TGA ranged from 3.2% (18% LEU HPC) to 4.0% (6% LEU HPC): spectra not shown. Residual water contents appeared to be independent of LEU concentration and the cyclone employed in powder production unlike the trends seen with simple LEU spray dried powders in chapter 2, showing that HMW chitosan may be causing the spray dried particulates to retain a certain amount of solvent.
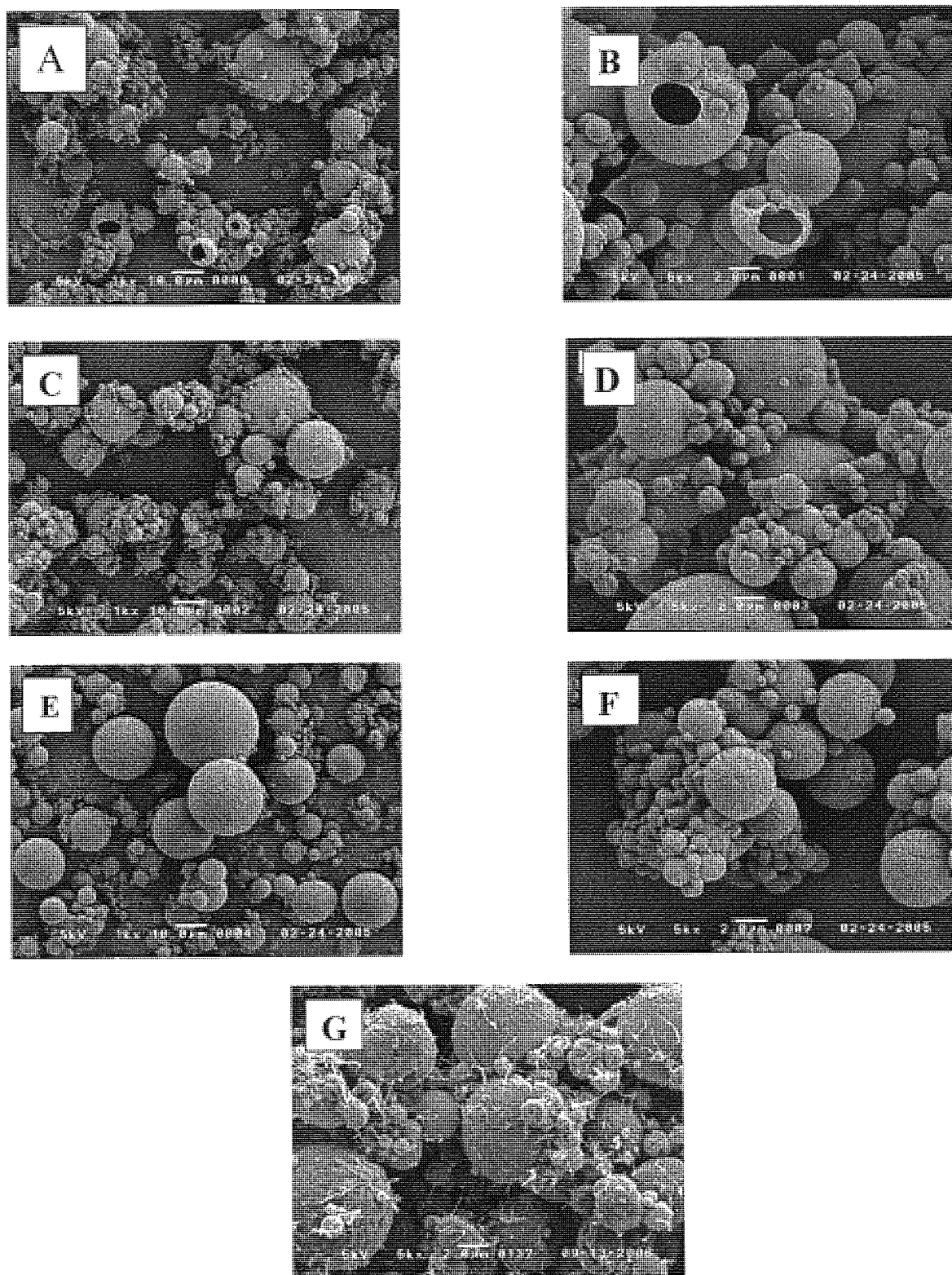
Figure 3.1: Representative scanning electron microscopy images of modified chitosan spray dried powders containing: A. 6% w/w LEU HPC 1000x magnification; B. 6% w/w LEU HPC 5000x magnification; C. 12% w/w LEU HPC 1000x magnification; D. 12% w/w LEU HPC 5000x magnification; E. 18% w/w LEU HPC 1000x magnification; F. 18% w/w LEU HPC 5000x magnification; G. 36% w/w LEU HPC 5000x magnification.
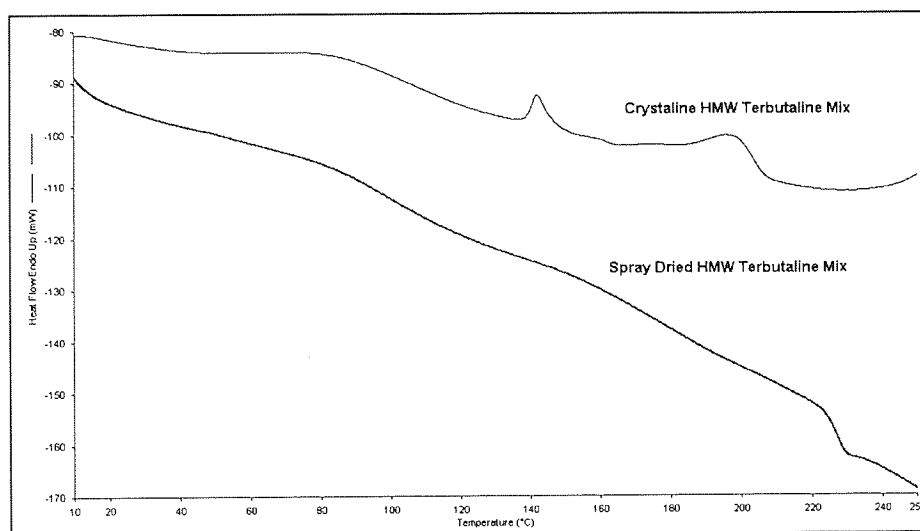
Figure 3.2: A representative comparative DSC trace of a modified chitosan spray dried powder, comparing a crystalline and spray dried formulation containing 4% w/w terbutaline, 36% w/w LEU, 50% w/w high molecular weight chitosan and 10% w/w lactose. The fusion peak at c.140°C on the crystalline trace indicates lactose crystallinity/ the evaporation of water (Sabulal et al., 1997, Larhrib et al., 2003) with the endotherm at c. 200°C terbutaline crystallinity (Brodka-Pfeiffer et al., 2003). The treated partially amorphous raw material HMW chitosan can be seen as a faint endotherm at 80°C on the crystalline trace (Murphy et al., 2005), LEU decomposition at 273°C (Loo et al., 2005; Rosu et al., 2006) can not be seen on the spectra (mean ± SD, n=3).

Particle sizing using wet laser diffraction showed low resolution in determining a physical difference in the size of the particles contained in the five HPC spray dried formulations with sizes ranging from 5.1 μm to 8.6 μm (formulations containing 18% w/w LEU and 12% w/w LEU respectively: Table 3.1). Better resolution was attained with the SC spray dried produce with a defined trend of decreasing particle size with increasing LEU concentration (range 3.6 – 10.0 μm). The surface tension associated with the wet dispersion of dry powders could indicate that aggregates were being focused. The SEM images would encourage this theory as a large reduction in primary particle size was not seen.

Tapped density values ranged from 0.13 to 0.18 g cm^{-3} over the two formulation series (36% w/w LEU HPC and 6% LEU SC products respectively), the values then used to ascertain the theoretical primary particle diameters (d_{ae}) of the formulations (Table 3.1). As d_{ae} is largely

dependent on the laser diffraction values obtained for a formulation (Table 3.1) only the SC produced spray dried powders gave any incidence of a pattern with powder composition, with a decrease in primary particle diameter from 5.11 ± 0.12 μm in the 0% w/w LEU modified control powder to 1.34 ± 0.18 μm in the 36% w/w LEU modified powder. No pattern was seen with the smaller HPC spray dried products; ranging 1.91 ± 0.30 μm to 3.26 ± 0.56 μm in diameter (18% and 12% w/w leucine modified powders respectively: Table 3.1). The smaller theoretical particle sizes compared to laser diffraction sizes, regardless of wet dispersion difficulties indicate a degree of aggregation: the theoretical sizes reflecting the primary particles that can be visualised in the SEM micrographs despite a high degree of polydispersity (Figure 3.1). Further evaluation of the trend between reducing laser diffraction size (LD) and primary particle diameter (d_{ae}) can be viewed in Figure 3.3.
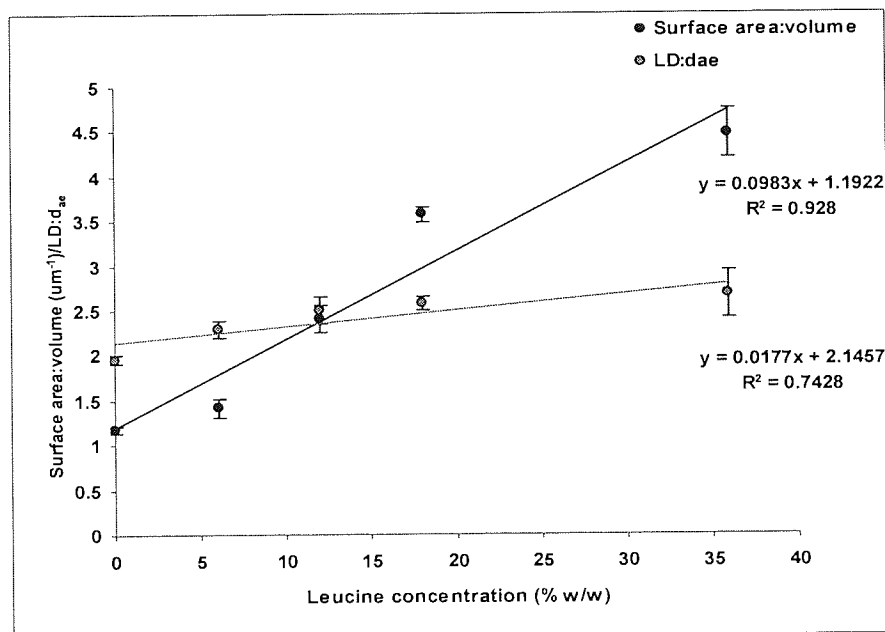
Figure 3.3: Chart of the increase in aggregation (LD:d_{ae}) seen with increasing LEU concentration in the LEU modified HMW chitosan powders compared to the increase in surface area:volume.

Figure 3.3 shows the increase in potential surface area for aggregation (as a function of surface area: volume) to be far greater (m=0.10) than the increase in aggregation of the LEU SC HMW chitosan spray dried powders (m=0.02). It is possible that the trend of decreasing aggregation with decreasing primary particle diameter as seen in chapter two with simple LEU modified spray dried powders is modified by the inclusion of HMW chitosan. The Interaction between the 310 kDa plus chains may mediate the action of LEU in reducing thermal efficiency and increasing the surface fracture of spray dried particles. The surface fracture increases the surface area relative to the presumed spherical surface (Figure 3.3) transversely reducing the surface area available for van der Waal surface forces to act up on (as related to Formula 1.4), by reducing inter-particulate contact points and increasing inter-particulate distance (H).

When compared to reducing particle size it can be seen that particle size is playing the key role in the increase of aggregation with increasing LEU concentration (Figure 3.4). HMW chitosan appears to prevent increasing LEU concentrations giving a proportional decrease in aggregation as seen in chapter 2. However, it should be noted that increasing LEU modification potentially has an effect on reducing surface force through alteration of surface morphology, due to the differences in the gradients of the $LD:d_{ae}$ and surface area:volume.

LEU effects are thought to be two fold. The reduction in surface tension at the atomiser air-interface creating a fine spray of droplets which gives finer spray dried particulates, this effect noted with the LEU SC chitosan powders as related through Formula 1.3, and the reduction in particulate aggregation through the creation of rougher surface textures through reduced system thermal efficiency.
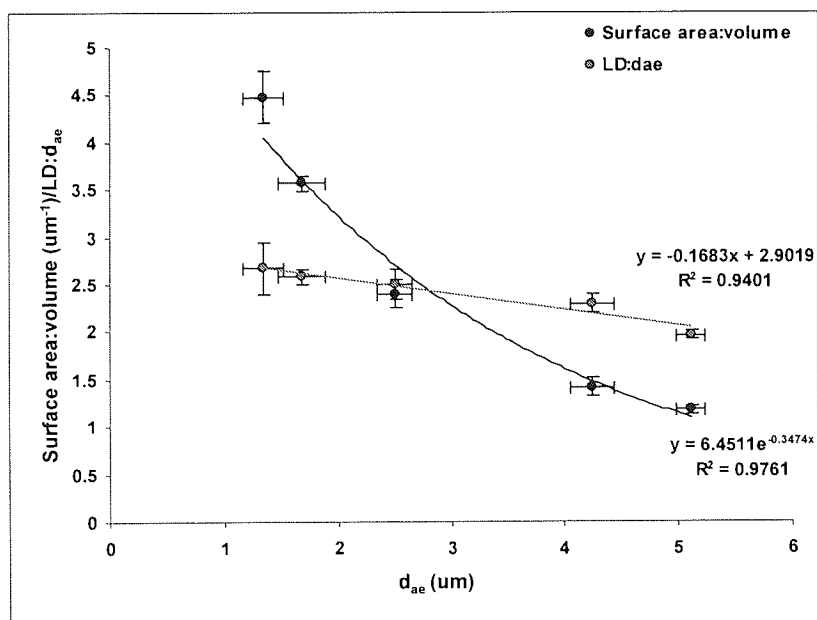
Figure 3.4: Chart of the increase in aggregation (LD:d_{ae}) with increasing particle size as visualised against increasing surface area:volume.

3.3.1.2   In vitro powder aerosolisation

The assessment of aerodynamic deposition of controlled release spray dried powders is limited by the sensitivity of assays and the retention of drug loads by the intimately mixed polymers with in the blend. A further problem is that of the adhesive nature of chitosan, which sticks to the silicone coated aluminium ACI stage plates discouraging media wash off for HPLC analysis. To overcome this difficulty a gravimetric method of ACI assessment was employed using Whatman GF-A filter papers to line cut side down stage plates. The principle of the technique means that homogeneity has to be assumed and no quantification of terbutaline drug loading could be made, furthermore the relative slip factors of individual plates could be affected (Swift, 1996).

The total recovered mass as a percentage of the total loaded dose was low ranging from 39.6 to 84.3% (0% w/w LEU control and 36% w/w LEU SC formulations respectively: Figure 3.5). The

mass recovery increased with increasing LEU concentration but was independent of the cyclone used during spray drying. The low recovery attributed to particle bounce, the powder which rebounded off the stage, coating the ACI in the higher stages where higher powder masses impacted; the thin spray dried powder coating of the ACI apparatus evading detection. The increase in LEU concentration improved aerosolisation and reduced the powder load and particle bounce on the upper stages of the ACI, particularly the throat region to stage 3, increasing the percentage of powder mass impinging on the filter papers.
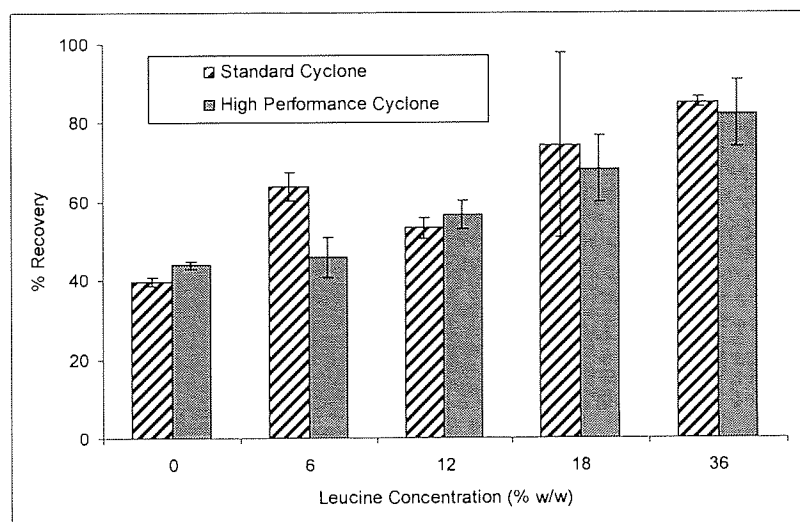
Figure 3.5: Illustration of the percentage mass recovery of leucine modified chitosan preparations from an Andersen Cascade Impactor (ACI) using gravitational assessment (mean ± SD, n=3).

The LEU modified chitosan powders gave a high emitted dose irrespective of the cyclone used in production, range 93.3 to 99.4% (Table 3.1). With the HPC 36% w/w LEU spray dried powder emitting the lowest percentage of total mass and SC 36% w/w LEU the highest. The powders were observed as having low static charges in the hydroxypropyl methylcellulose (HPMC) capsules, with emitted dose independent of LEU concentration and method of production (HPC/SC).

The ACI deposition pattern of the spray-dried powders showed a trend from large deposition in

the Spinhaler device and USP II throat with low LEU concentrations to large deposition in the

lower stages of the ACI with the highest LEU concentration (36% LEU: Figure 3.6). For

maximum benefit *in vivo*, minimal oropharyngeal deposition is required, loosely translating to the

throat region and stages -1 to 1 of the ACI despite significant shape and volume differences

(Srichana et al., 2000). ACI deposition could also be affected by the cyclone employed in the

production of the spray dried powders, spray dried powders produced using the HPC appearing

to show improved ACI deposition characteristics over SC counterparts (Figure 3.6).
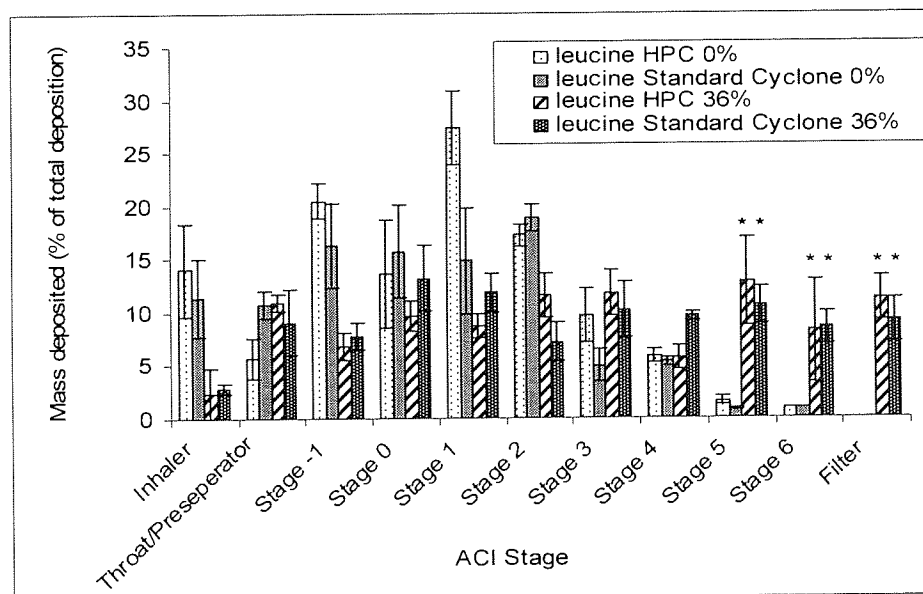
Figure 3.6: Percentage mass deposition of 0% and 36% w/w LEU modified chitosan spray dried powders produced by high and standard performance Büchi B290 mini lab cyclones when inspired through an Andersen Cascase Impactor at 60 Lmin-1. Statistical difference (one-way ANOVA- Tukey/Krammer) between formulation stage deposition: *p <0.05.

The fine particle fraction (F < 5μm) improved from 14.4 to 50.1% (control and 36% w/w LEU

powders respectively) for SC produced powders, and from 16.7 to 52.7% (control, 36% w/w LEU

formulations respectively) for HPC produced powders (Table 3.1). The incremental increases in

FPF were seen for both methods of production (HPC & SC) with increasing LEU concentration (Figure 3.7), the highest concentration of 36% LEU giving a statistically significant increase in FPF over the next concentration of 18% LEU for both cyclones (ANOVA, Tukey Kramer Post Hoc: $P <0.05$). At all LEU concentrations (0, 6, 12, 18 and 36% w/w LEU), formulations produced by the HPC gave higher FPF's compared to those produced by the SC, although the difference was considered not to be statistically significant (students matched paired t-test with Tukey Kramer Post Hoc, $P >0.05$). The use of the HPC possibly collects more particles from the lower end of the poly-disperse distribution ($\sigma< 2$: Maury et al., 2005), the filter stage deposition ($F < 0.26$ μm) being significantly higher in the HPC produced formulations (students matched paired t-test, $P <0.05$).
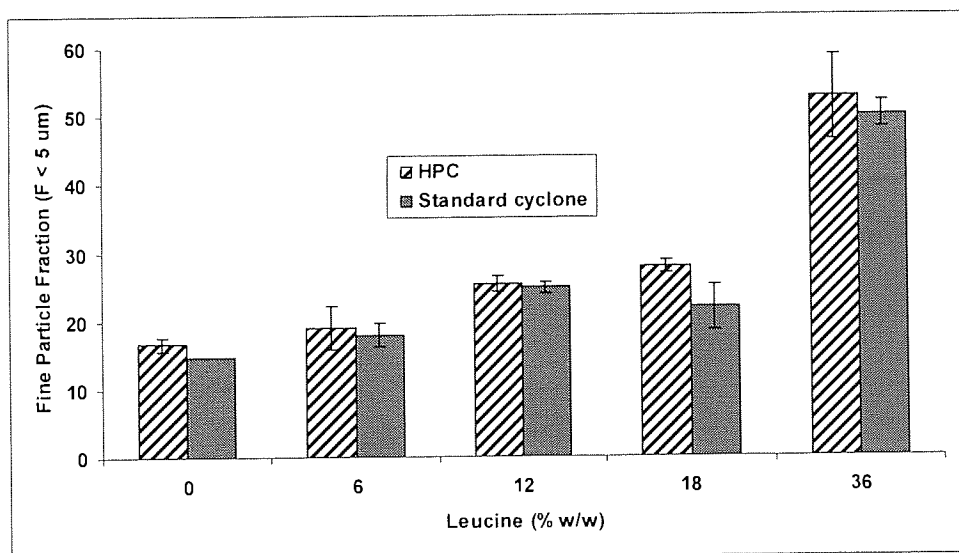
Figure 3.7: Bar chart of the fine particle fraction (FPF: F <5 μm) of LEU modified chitosan spray dried powders produced by high performance and standard Büchi B290 cyclones when inspired through an Andersen Cascade Impactor (ACI) at 60 Lmin⁻¹ (mean ± SD, n=3).

Mass median aerodynamic diameter (MMAD) showed a general decrease in particle size with increasing LEU concentration (Table 3.1). Using SEM images as a size comparison, the reduction in aerodynamic size may only partly be due to a decrease in the overall size of the powder population's theoretical primary particle diameter. Where calculated figures were brought

into question due to the wet dispersion method employed in laser diffraction sizing: a primary particle size reduction probably enhanced by the increase of pre-spray-dried formulation LEU concentration. The reduction in MMAD is more likely to be a consequence of decreased aggregation with increasing LEU concentration as previously indicated by SEM. LEU showed its ability as an aerosolisation enhancer in simple spray dried salbutamol formulations in the previous experimental chapter. The increase in LEU concentration will only increase the concentration of the simple amino acid at the microspheres surface (indicated by greater surface roughness with increasing LEU), where glidant-style properties aid capsule emptying and powder dispersion by reducing the amount of energy required to overcome surface active forces and give the dispersion of individual particles, exemplified by greater deposition in the lower stages of the ACI.

The use of cyclone also has a bearing on MMAD; the high performance cyclone gave a significant reduction in particle size compared to the standard cyclone of the Büchi B-290 mini lab spray-drier. The difference represented a shift in the median value of the poly-disperse population as a consequence of the collection of finer particulates at the lower end of the distribution (F <1 μm).

Particle deposition within the lung is governed by different methods *in vivo* depending on particle size. The required particle size for *in vivo* deposition in the bifurcations of the mid lung bronchi is 2 to 5 μm, particles within this size range are dependent on inertial impaction (3 to 5 μm) and sedimentation (0.5 to 3 μm diameter particulates), the latter enabled by the pressure differentials across the lung and the decreasing velocities of aerosolized particles (Gonda, 1988). Although

recommended *in vitro* pressure gradients of 4 bar were observed, using the implicated 60 L/min airflow for the spinhaler device, because of minimal deposition via sedimentation and anatomical differences direct *in vitro* correlation with *in vivo* behaviour can only be assumed (Srichana et al., 2000; Martin et al., 2007;Gonda, 1992).

A further variable of this research is the addition of ethanol to the pre-spray dried formulations. The benefits of the addition of ethanol to oestrogen formulations spray dried using the Büchi B-290 mini lab spray-dryer have been documented. *Rabbani & Seville* (2005) have shown how the addition of ethanol can give reduced particulate size (Gilani et al., 2005), however the researchers pointed to increased viscosity being the root cause which in fact has been documented as a cause of increasing primary particle diameter (Masters, 1991). A more probable cause of decreasing primary particle size is the effects ethanol had on the surface tension of the majority aqueous liquid feed. A co-solvent ethanol-containing liquid feed potentially causes reduced surface tension at the point of atomisation giving greater liquid feed dispersion and decrease in droplet and resultant primary particle size. Furthermore, a reduction in the thermal efficiency of the spray-dried system would be expected compared to a solely aqueous liquid feed. Spray drying with ethanol as a co-solvent lowers the boiling point of the aqueous feed, by maintaining the inlet at 180° C with the co-solvent system in-place of a solely aqueous solvent a greater wrinkled appearance to particulates would be expected due to reduced thermal efficiency of the system. With the excessive heat manifesting as kinetic energy in the liquid droplets dispersed by the atomizer into the drying chamber during spray drying, the kinetic energy giving a furrowed appearance to dry particulate surfaces when collected. In addition, the reduced surface tension of the atomized droplets caused by the ethanol co-solvent

affects water-air interface alignment, allowing further heat influx into the dispersed liquid feed giving further dry particle surface fracture. The wrinkled appearance of primary particulates gives reduced contact points for surface active forces to act up on and potentially reduces aggregation.

The addition of ethanol to the aqueous base of the formulation also aids the formulation of any water insoluble agents that may need to be added to the blend as utilized in the second section of this chapter.

In a simple comparison test the marketed Bricanyl® Turbohaler® was used to ascertain the effectiveness of the LEU modified chitosan preparations. Bricanyl Turbohaler is a formulation of pure micronised terbutaline hemisulfate residing in a high resistant device, the Turbohaler, which disperses the active agent on inspiration. The Bricanyl Turbohaler was actuated ten times into the ACI using the same gravimetric method of recovery as described earlier. In an additional test, 25 mg of the same formulation was then also loaded in to HPMC capsules and inspired through a Spinhaler in to the ACI apparatus to mimic the test conditions used to assess the chitosan preparations.

It was found that the Bricanyl formulation was largely dependent upon the high resistance Turbohaler device for dispersibility and good *in vitro* lung deposition, the FPF falling from 30.1 to 4.4% with the substitution for HPMC capsules inspired through a Spinhaler device (Amirav et al., 2005). When comparing the chitosan formulations only the 36% LEU formulation out performed the Bricanyl Turbohaler in ACI testing with an FPF of 50.1/52.7% (SC/HPC) compared to 30.1% (Figure 3.8). The comparison not only shows how a powder with potential sustained release can

out perform a marketed formulation but also highlights the role of high resistance devices in the improvement of a dry powder's aerosolisation properties (Amirav et al., 2005; Borgstrom et al., 2002).
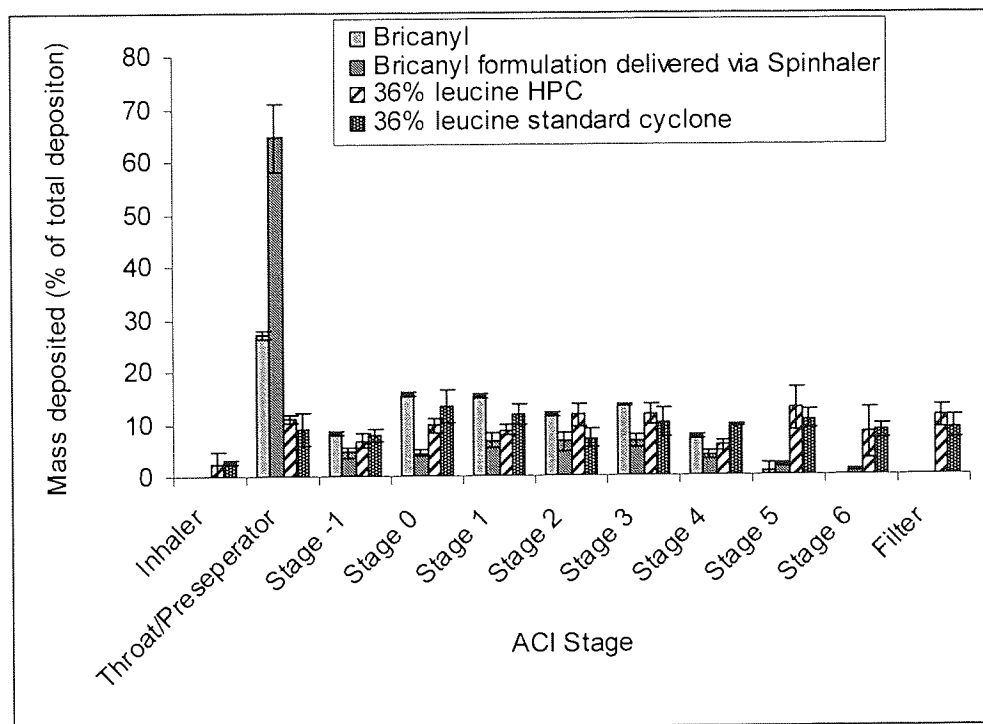
Figure 3.8: Percentage mass deposition of the marketed Bricanyl® formulation of micronised terbutaline when delivered via the intended Turbohaler® device and via 25 mg loaded HPMC capsules inspired through a Spinhaler® DPI. Compared to 36% w/w LEU modified chitosan spray dried powders produced by high performance and standard Büchi B290 cyclones and delivered by Spinhaler when inspired through an Andersen Cascade Impactor at 60 Lmin^{-1} (25 mg sample-loaded HPMC capsules; mean ± SD, n=3).

3.3.1.3   In vitro dissolution

Although several *in vitro* models exist for the prediction of respirable fraction and site of deposition in the lung following pulmonary administration (e.g. MSLI, Andersen Cascade Impactor, Next Generation Impactor, Twin Stage Impinger), there is no readily available *in vitro* model to predict the rate and extent of drug dissolution in the lung following inhalation. Literature references of dissolution models for inhalable powders are rare (Taylor et al., 2006); despite the dissolution kinetics of an inhaled active formulation being critically linked to onset and duration of

therapeutic activity. In the absence of a more appropriate model, standard *in vitro* powder dissolution testing was used to provide a comparison between the dissolution profiles of the LEU -modified chitosan spray-dried powders.
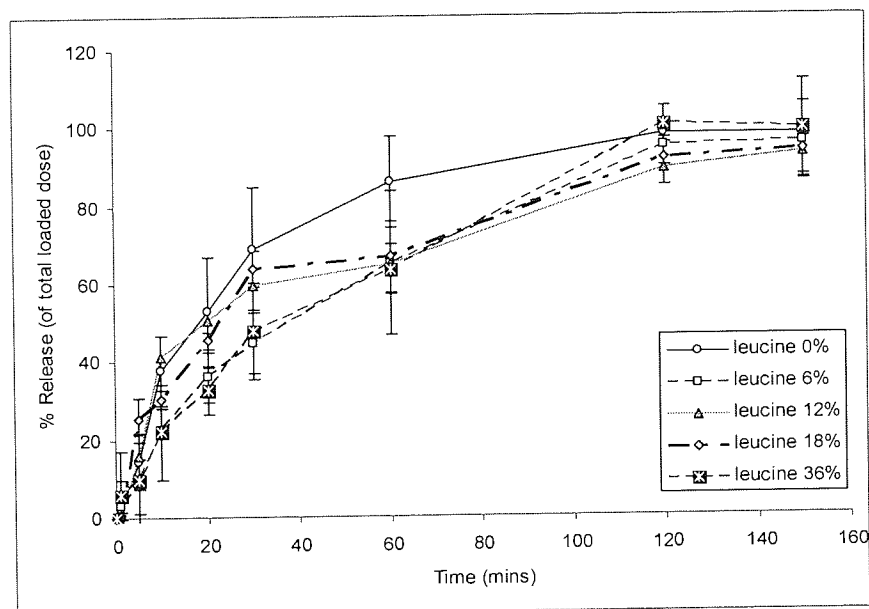
Figure 3.9: Comparison of the terbutaline dissolution profiles of the HPC LEU modified chitosan spray dried powders. Dissolution traces of 0%, 6%, 12%, 18% and 36% w/w LEU modified spray dried chitosan formulations produced using the high performance cyclone via dissolution using USPII apparatus and conditions outlined in the method (mean ± SD, n=3).

No difference was observed between the *t*max dissolution times of the ten formulations, with all powders releasing at least 80% of the expected total drug pay load of terbutaline into the phosphate buffer solution media before the 120 minute time point (Figure 3.9). Even though no visible difference could be seen in the dissolution profiles of the ten spray-dried formulations inspection of the release kinetics revealed a distinct difference (Table 3.2: Example of rate plots, Appendix 3). The release kinetics of the eight formulations all followed a similar dissolution pattern with an initial burst release relating to a zero order kinetic due to abundance of solute. However, the least squares regression method indicated a prolonged burst period following the zero order release kinetic with increasing LEU concentration. The pattern of reduced rate of terbutaline release with increasing LEU concentration could be due to the stabilisation of the

hydrophobic chitosan chains that make up the gel matrix. The stabilisation of hydrophobic chains in liposomal formulations has been noted previously by the addition of the relatively hydrophobic amino acid lysine which bears structural similarity to LEU with a defined polar and non-polar terminus (Mohammed et al., 2007). The highest r^2 values in the 6% and 12% LEU formulations were attained by regression of the origin to the t_5 time point (r^2 0.9956, 0.9986 SC, r^2 0.9935, 0.9993 HPC respectively), were as the 18% LEU formulations gave closer correlation between the origin and t_{10} points (r^2 0.9986 SC, r^2 0.9993 HPC) and the control (0% w/w LEU) and 36% LEU formulations between the origin and the t_{30} abscissa points (r^2 0.9124, 0.9852 SC, r^2 0.9578, 0.9427 HPC respectively).

The second phase from around t_{30} followed a first order release kinetic closely as would be expected when solute concentration in the depleted bolus powder becomes a limiting factor (Table 3.2). The Higuchi rate kinetic equation is designed for homogeneous matrix type formulations and a close least squares regression correlation (r^2) was observed with the dissolution of all ten powders from the origin to $t^{1/2}$ 10.95 (t_{120}), ranging from 0.8537 to 0.9901 (12% LEU SC and 0% LEU SC, respectively).

Despite a prolonged zero order kinetic with increasing LEU concentration (control aside), the overall close proximity to the release profile of the spray dried formulations indicate that a Higuchi kinetic is followed by the LEU modified chitosan spray dried formulations during dissolution under the established conditions. The Higuchi kinetic would be expected as chitosan has been previously documented as a matrix system (Illum, 1998; Philip & Pathak, 2006). However despite identical t_{max} values, the initial reduction in zero order kinetic with the addition of 6% to 18% LEU seems to suggest that the addition of the hydrophobic amino acid to the

formulation moderates initial burst release character from spray dried chitosan microspheres until the inclusion of 36% LEU, which more closely mimics the release kinetics of the control, the subtle trend is illustrated in Figure 3.9 between time points 0 and 120 minutes.

Leucine % w/w	Cyclone	zero order		1st order		Higuchi matrix	
		t (min)	r2	t (min)	r2	t (min)	r2
0	HPC	0-30	0.9578	0-120	0.9598	0-120	0.9376
0	Normal	0-30	0.9124	0-120	0.9856	0-120	0.9901
6	HPC	0-5	0.9956	0-120	0.9868	0-120	0.9824
6	Normal	0-5	0.9935	0-120	0.9510	0-120	0.9313
12	HPC	0-5	0.9986	30-150	0.9738	0-120	0.8537
12	Normal	0-5	0.9993	0-120	0.9888	0-120	0.9811
18	HPC	0-10	0.8229	0-120	0.9595	0-120	0.9218
18	Normal	0-10	0.9832	30-150	0.9920	0-120	0.9210
36	HPC	0-30	0.9852	0-60	0.9732	0-120	0.9352
36	Normal	0-30	0.9427	0-30	0.9427	0-120	0.9553

Table 3.2: Table showing the relationship of increasing LEU concentration in modified chitosan spray dried powders with dissolution release kinetics. *r2 notates correlation coefficient; HPC: Büchi high performance cyclone (sample rate plots Appendix 3).

Observation of the dissolution behaviour of the LEU-modified chitosan formulations recognized the formation of a structured gel. The gel slowly expands over time indicating a matrix release system of mechanically interlocking chitosan chains possibly assisted by the hydrogen bonding of the polar head groups of chitosan with the terbutaline active marker (Figure 3.10). Van der Waal and hydrophobic forces between adjacent chitosan hydrocarbon chains could also play a role.
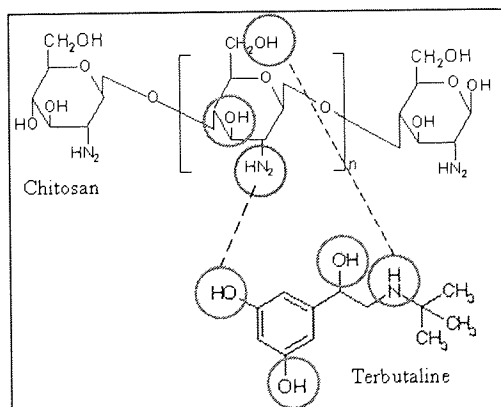
Figure 3.10: Diagram of potential sites for terbutaline and chitosan hydrogen bonding.

In vitro dissolution tests of this nature are only an indication of the capability of a formulation's behaviour *in vivo*, the comparatively large volume of dissolution media (1000 mL) compared to that of the lung's surface secretions and the lack of clearance methods such as the mucociliary escalator question validity (Gille et al., 2006). The composition of lung surfactant, of which one of the main roles is protection of the pulmonary mucosa, also deviates wildly from the PBS solution employed during *in vitro* dissolution testing (Gille et al., 2006). Particle surface area may also have an additional effect *in vivo* as finer isolated particles will have a larger surface area and demonstrate faster dissolution rates with higher diffusion coefficients compared to the bolus of spray dried powder which was allowed to gel during *in vitro* dissolution.

The similarity of all the formulations' dissolution profiles regardless of LEU content and the cyclone employed during the spray drying process show that improving the aerosolisation of the powders doesn't greatly affect the release kinetics of spray dried chitosan powders *in vitro*.

In this section it has been demonstrated that chitosan can produce respirable spray dried powders for pulmonary delivery with *in vitro* controlled release. It has also been illustrated that LEU is beneficial in the application of spray-dried chitosan microspheres for pulmonary delivery, and in addition, that the use of a high performance cyclone may improve yields and *in vitro* aerodynamic character. The major conclusion of this sub-chapter is that the highest possible concentration of LEU should be used to improve the aerodynamic capabilities of the spray dried chitosan formulations, in this case 36% w/w, and that high concentrations of the potential aerosolisation agent appear not to compromise dissolution character.

The next subsection will deal with the effect chitosan molecular weight has on the aerosolisation and dissolution of 36% w/w LEU-modified chitosan spray dried powders.

3.3.2 The Influence of varying chitosan molecular weight

3.3.2.1   Powder characterization

The 15 LEU modified chitosan spray dried powders in this section were off white in colour and free flowing in appearance compared with the three control powders of similar character but slightly paler in contrast. Out of the total of 18 formulations produced, six were loaded solely with terbutaline, six with BDP only and six spray dried powders contained both agents. Use of a high performance cyclone resulted in the collection of high yields from the terbutaline loaded spray-dried powders with a range of 59 to 79% of the anticipated recovery (Table 3.3). BDP formulated spray-drying yields varied considerably ranging from 36 to 73%, with no direct evidence of a connection between chitosan molecular weight and spray drying yields (Table 3.4). Spray drying yields of the six dual loaded formulations investigated also varied considerably (60.2 - 90%) with no evidence of a connection between formulation composition and spray drying yield (Table 3.5). The thermal efficiency of the dual loaded blends was calculated due to the requirement of having to spray dry the powders three times to complete powder characterisation, other samples spray dried only once. Thermal efficiency showed an increase with increasing chitosan chain length indicating a higher heat requirement to evaporate solvent off higher molecular weight chitosan formulations (Figure 3.11). This may indicate stronger interactions between longer chitosan chains that could increasingly deter the migration of LEU to the liberated droplet surface post atomiser preventing the greater radiance of heat energy through the droplet, the higher inlet heat workload giving reduced outlet temperatures improving thermal efficiency.
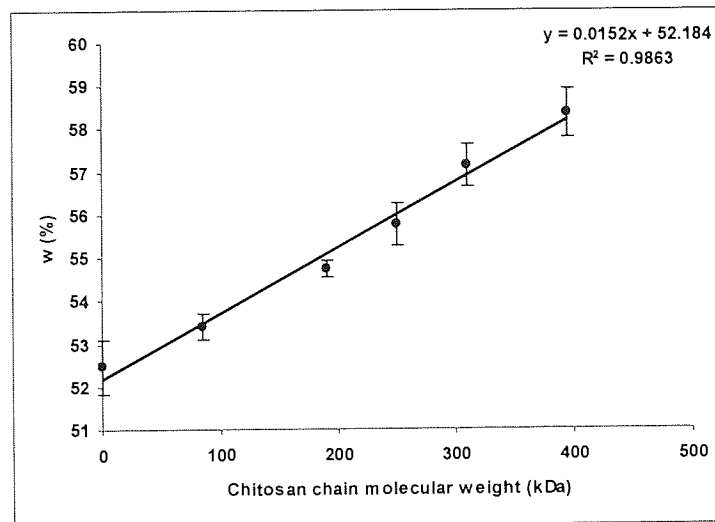
Figure 3.11: Chart showing the increase in thermal efficiency (w) with increasing chitosan molecular weight. The values for chitosan molecular weights are estimate fraction medians (Mean ± S.D, n=3).

Chitosan MW	SD Yield (%)[b]	Water Content (%)	Particle Size (μm)	Tapped Density (g cm^{-3})	Carr's Index[a] (%)	Flowability	d_{ae} (μm)	MMAD (μm)
0	73	1.22 ± 0.01	4.31 ± 0.32	0.19 ± 0.02	22.24	Poor, fluid	1.87 ± 0.17	1.66 ± 0.18
LMW	79	3.17 ± 0.16	5.65 ± 0.18	0.16 ± 0.00	30.86	Poor, cohesive	2.23 ± 0.08	2.00 ± 0.11
LMW/MMW	63	4.14 ± 0.04	6.01 ± 0.63	0.19 ± 0.01	30.71	Poor, cohesive	2.61 ± 0.35	1.76 ± 0.08
MMW	56	5.63 ± 0.20	9.93 ± 0.74	0.12 ± 0.01	26.28	Poor, cohesive	3.40 ± 0.32	1.85 ± 0.52
MMW/HMW	62	5.86 ± 0.32	6.41 ± 0.40	0.19 ± 0.01	17.45	Good	2.76 ± 0.15	1.95 ± 0.47
HMW	59	2.55 ± 0.17	7.88 ± 3.08	0.13 ± 0.01	22.58	Poor, fluid	2.85 ± 1.12	2.30 ± 0.70

Table 3.3: Table of the physical characteristics of 36% w/w LEU-modified 4% w/w terbutaline loaded spray dried chitosan powders (mean ± SD, n=3). [*a] De Villiers, 2005, [b] n=1.

Chitosan MW	SD Yield (%)[b]	Water Content (%)	Particle Size (μm)	Tapped Density (g cm^{-3})	Carr's Index[a] (%)	Flowability	d_{ae} (μm)	MMAD (μm)
0	63.5	0.61 ± 0.02	3.80 ± 0.85	0.20 ± 0.02	24.17	Poor, fluid	1.71 ± 0.44	2.22 ± 0.06
LMW	65.25	3.57 ± 0.23	4.00 ± 0.32	0.13 ± 0.02	26.54	Poor, cohesive	2.22 ± 1.46	2.46 ± 0.26
LMW/MMW	73	3.34 ± 0.14	4.94 ± 0.04	0.15 ± 0.01	33.29	Very poor	1.92 ± 0.03	2.71 ± 0.19
MMW	66.35	8.30 ± 0.05	4.02 ± 0.03	0.27 ± 0.22	30.42	Poor, cohesive	1.98 ± 0.79	2.85 ± 1.83
MMW/HMW	35.9	4.72 ± 0.74	9.25 ± 2.19	0.13 ± 0.01	25.5	Poor, cohesive	3.28 ± 0.74	2.93 ± 0.69
HMW	66.2	2.32 ± 1.98	5.28 ± 0.39	0.14 ± 0.01	32.88	Poor, cohesive	2.00 ± 0.23	3.55 ± 0.65

Table 3.4: Table of the physical characteristics of 36% w/w LEU-modified, 4% w/w BDP loaded spray dried chitosan powders (mean ± SD, n=3). [*a] De Villiers, 2005, [b] n=1.

Formulation	SD Yield [b] (%)	Water Content (%)	Particle Size (μm)	Tapped Density (g cm-3)	Carr's Index[a] (%)	Flowability	d_{ae} (μm)	MMAD (μm)
Control	63.5	0.58±0.15	3.42±0.13	0.19±0.02	23.03	Poor, fluid	1.47±0.10	2.04±0.13
LMW	68.3	3.87±0.12	3.36±0.36	0.15±0.01	37.96	Very poor	1.29±0.14	1.86±0.22
LMW/MMW	74.3	5.50±0.12	3.51±0.08	0.14±0.00	30.59	Poor, cohesive	1.30±0.04	2.75±0.12
MMW	90	3.84±0.06	4.63±0.39	0.13±0.00	31.92	Poor, cohesive	1.64±0.14	3.32±0.28
MMW/HMW	60.2	5.17±0.23	8.20±1.51	0.15±0.01	22.71	Poor, fluid	3.12±0.54	4.05±1.09
HMW	63.1	0.53±0.16	9.08±1.57	0.15±0.00	30.44	Poor, cohesive	3.61±0.52	3.35±0.18

Table 3.5: Table of the physical characteristics of 36% w/w LEU-modified spray dried chitosan powders loaded with both 4% w/w terbutaline and 4% w/w BDP (mean ± SD, n=3). [*a] De Villiers, 2005, [b] n=1.

Analysis of the drug loading of the terbutaline spray-dried powders indicated that the drug content ranged from 94% to 117% of the anticipated amount (Table 3.6) with statistical analysis indicating that the difference in drug content of each powder was non significant (one-way ANOVA/Tukey-Kramer: not significant, P> 0.05). Analysis of drug load in the BDP only spray dried products revealed a higher variance in the active content of the spray dried powders (65% - 103%), with a general upward trend of increasing drug load with increasing chitosan molecular weight. The LMW chitosan formulation giving a drug load of 65% with the HMW formulation giving a drug load of 104% (Table 3.6), once again statistical analysis indicated the variance in drug content of the series to be non significant. It is possible that the longer chitosan chain length offered greater protection to BDP during the spray drying process. In contrast, residual acetic acid may have been more in abundance in the LMW and MMW containing formulations in the presence of 30% aqueous ethanol. This due to a decrease in chitosan acetic acid sorption potentially causing BDP drug degradation independent of spray drying (Shamov et al., 2002).

For the dual loaded powders, HPLC analysis of drug loading revealed that all formulations with the exception of the LMW/MMW chitosan formulation (84.1% of expected drug load detected)

had terbutaline content within 5% of the expected total (Table 3.6). BDP loading in the six dual

loaded formulations were all within 5% of the expect total of 4% w/w.

Large variability was seen in the drug loading of the terbutaline control although this was not

seen with the sole BDP and dual loaded formulations. The variance, not seen in the terbutaline

loading of the dual loaded powder, could be down sampling errors or heterogeneous spray

drying of the drug loading from solution.

Chitosan MW	Drug loading (%)			
	Terbutaline only	BDP only	Dual	
			Terbutaline	BDP
0	116.90 ± 11.15	101.02 ± 15.88	98.20 ± 1.84	100.08 ± 5.42
LMW	107.82 ± 17.41	65.33 ± 0.31	106.17 ± 5.95	99.29 ± 14.26
LMW/MMW	93.84 ± 1.41	82.01 ± 1.11	84.11 ± 1.12	101.02 ± 17.73
MMW	103.06 ± 7.24	91.53 ± 7.79	101.54 ± 3.04	101.74 ± 10.40
MMW/HMW	99.56 ± 8.27	103.17 ± 4.72	97.54 ± 5.28	97.85 ± 3.46
HMW	101.34 ± 11.99	100.62 ± 2.61	101.74 ± 10.41	101.54 ± 3.04

Table 3.6: The drug loading of 36% w/w leucine modified chitosan spray dried powders (mean ± SD, n=3).

Scanning electron microscopy was used to visualise the particle diameter, structural and surface

morphology of the spray-dried powders (Figures 3.12 – 3.14). Interestingly, the micrograph of

the terbutaline control powder (figure 3.12A) did not show spherical particles where as the other

two control formulations did (BDP and terbutaline-BDP dual loaded: Figure 3.13 A, 3.14A); rather,

the particles appear to have undergone fusion and partial re-crystallisation. This may have

occurred either during storage at a period of high humidity or possibly at some stage during SEM

processing under either heat during gold plating or vacuum during imaging.

The micrographs of the chitosan-modified spray-dried powders (Figures 3.12 - 3.14 B-F)

indicated that the powders comprised regular spherical particles with a diameter of c. 0.5 – 10

μm highlighting poly-dispersity (σ >2), with sizes under 5 μm regarded as respirable. The chitosan particulates appear to have an undulating surface, and this is particularly apparent for the HMW chitosan powders (Figures 3.12 - 3.14F). Most striking about the SEM images of the chitosan-modified powders is the appearance of whisker-like material clinging to the surface of the particles. *Asada et al.* (2004) noted that similar artefacts developed on the surface of theophylline-chitosan spray-dried particles over time, and attributed this phenomenon to theophylline undergoing capillary condensation. It is unclear whether this phenomenon is occurring with terbutaline and BDP in these chitosan-based spray-dried powders, or whether the surface-deposited material is excess LEU or chitosan precipitated from respective formulations during spray-drying. The formation of the whiskers did not occur in the controls which indicates that chitosan plays a role in the production of such spears.

Different surface textures can be seen in some of the samples for example the dual loaded LMW/MMW formulation (Figure 3.14C) showed a variety of textures with spiked particles next to smooth particles possibly indicating separately charged particles within the population.

The SEM images suggested that the powders formed were amorphous akin to the powders produced in previous sections (Corrigan, 1995). This observation was confirmed by differential scanning calorimetry.

A yellowing of the chitosan containing powders was noticed upon storage, yellowing not seen in the three control specimens. A similar phenomenon was noticed by *Russo et al.* (2006) in the spray drying of morphine hydrochloride. The presence of the morphine acid salt hydrochloride

combined with lactose possibly gave the discoloration of spray dried products in their study, although in the report the discoloration is regarded as a consequence of the thermal conditions of spray drying. The discoloration in this study is attributed to the acid hydrolysis of lactose into glucose monosacharides by residual acetic acid left in the blend after the formation of the original chitosan gel, lactose being white where as glucose is darker in appearance. The Maillard reducing sugar reaction between amino acids (LEU) and disaccharides (lactose) can be ruled out due to the standard storage conditions, short time frame and no evidence of change in the control (Klinkesorn et al., 2005). The change in colour of the chitosan powders did not appear to change the flow properties of the powder and it is also possible that the change in colour observed in the chitosan containing formulations may be related to the gradual formation of the whisker like threads seen in the SEM imagery. An investigation into the effects of storage on aerosolisation property is investigated at a later stage in the report.
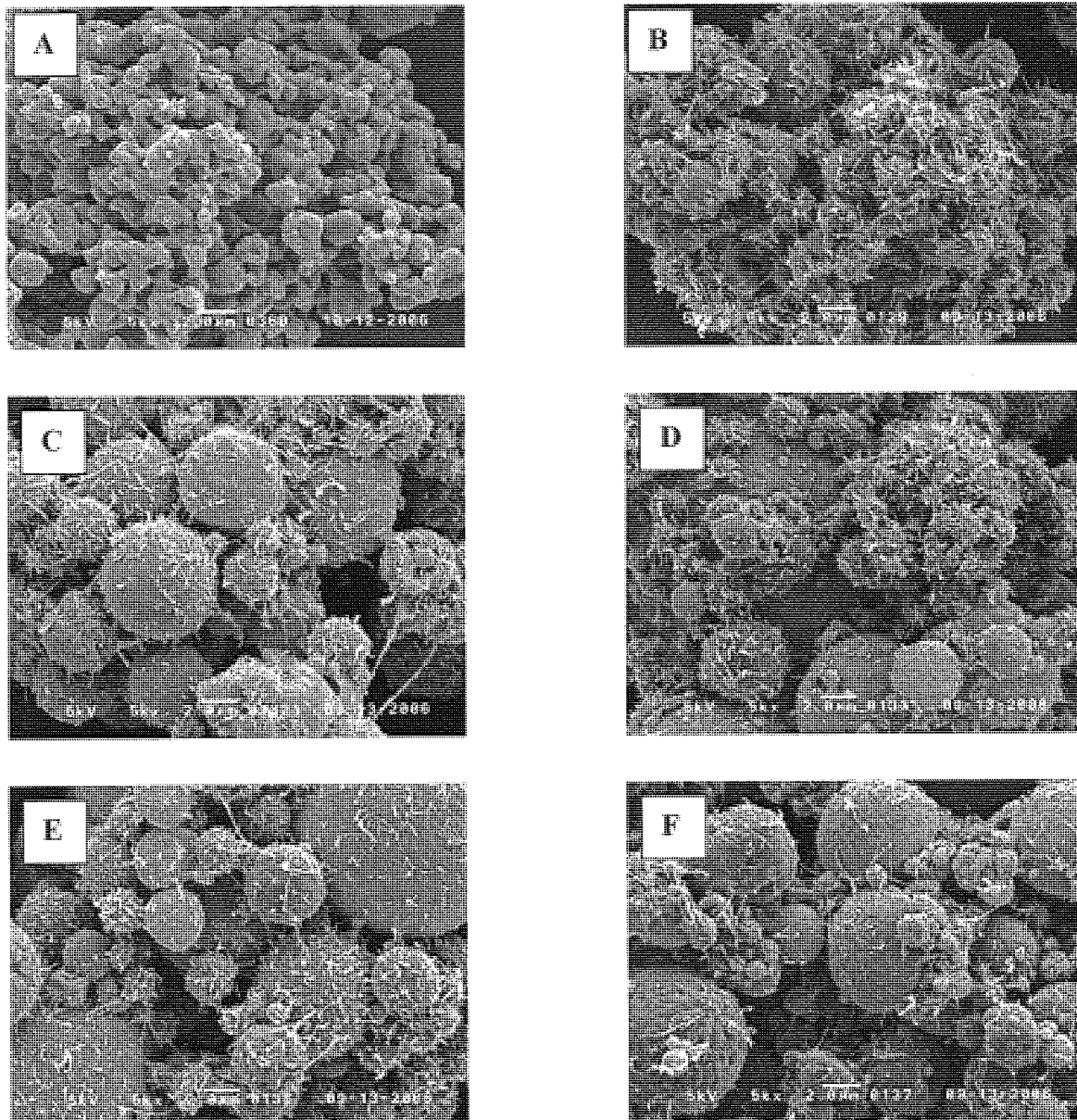
Figure 3.12: Representative scanning electron microscopy images at 5000x magnification of terbutaline loaded chitosan spray dried powders containing 36% w/w LEU: A. control; B. LMW chitosan; C. LMW/MMW chitosan; D. MMW chitosan; E. MMW/HMW chitosan; F. HMW chitosan.
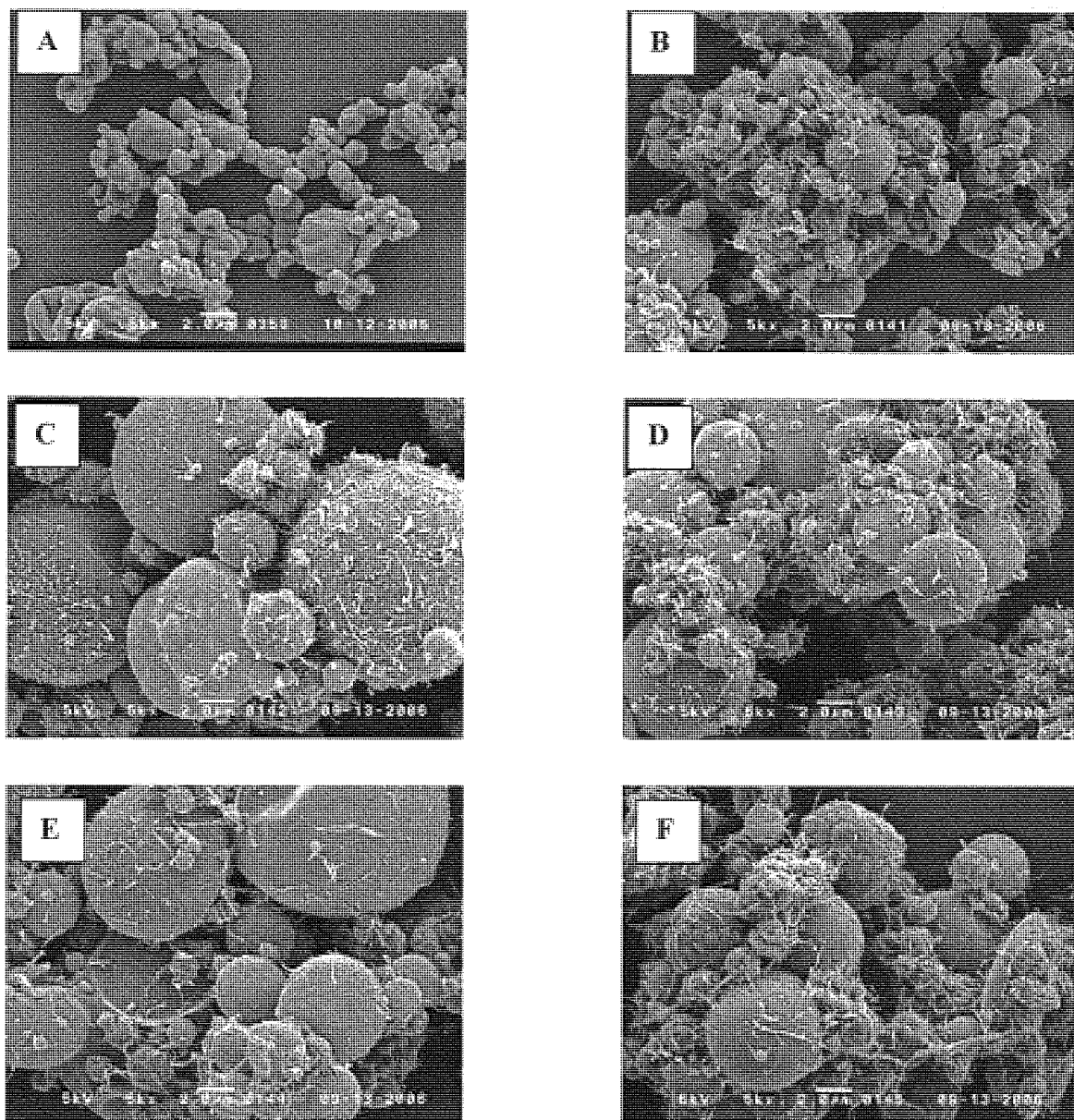
Figure 3.13: Representative scanning electron microscopy images at 5000x magnification of BDP loaded chitosan spray dried powders containing 36% w/w LEU: A. control; B. LMW chitosan; C. LMW/MMW chitosan; D. MMW chitosan; E. MMW/HMW chitosan; F. HMW chitosan.
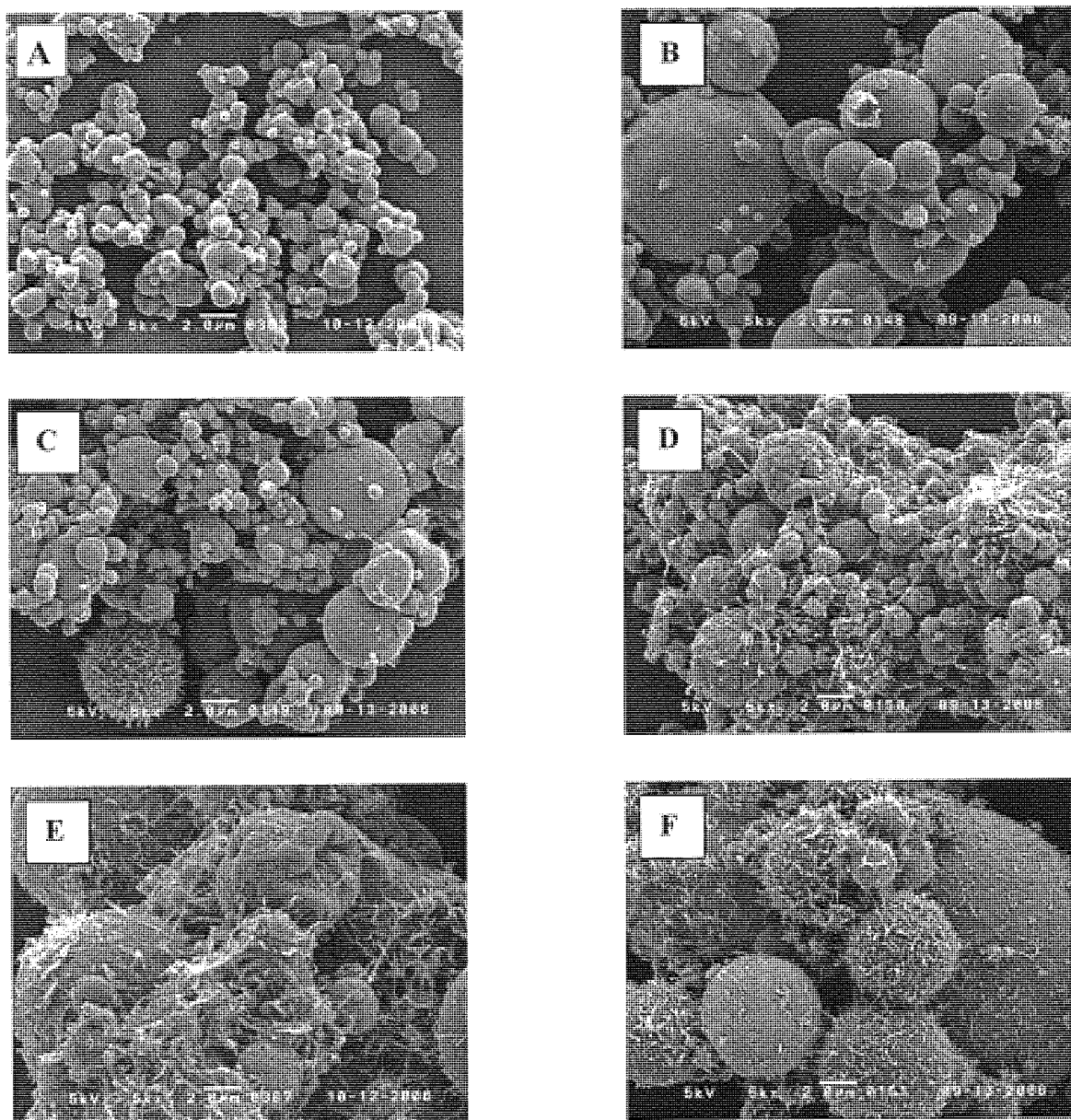
Figure 3.14: Representative Scanning electron microscopy images at 5000x magnification of dual loaded terbutaline and BDP chitosan spray dried powders containing 36% w/w LEU: A. control; B. LMW chitosan; C. LMW/MMW chitosan; D. MMW chitosan; E. MMW/HMW chitosan; F. HMW chitosan.

Differential Scanning Calorimetry (DSC) showed all eighteen spray-dried formulations to be amorphous with no crystalline endothermic fusion peaks on the respective traces (representative trace: Figure 3.15). Given the amorphous nature of the powders the samples were stored at room temperature in a dessicator immediately after spray-drying to limit the chance of

recrystallisation of the samples between powder production and aerosolisation testing. The powders showing stability in the amorphous state over a two week testing period at room temperature (Rabbani & Seville, 2005). A complication of the amorphous state is the tendency for formulations to turn crystalline over time bringing a change in physicochemical properties such as aerodynamic characteristics, although spray dried formulations have previously shown stability in the amorphous state over extended periods (Chan et al., 2005; Costantino et al., 1998: Christensen et al., 2002; Ohta & Buckton., 2004).
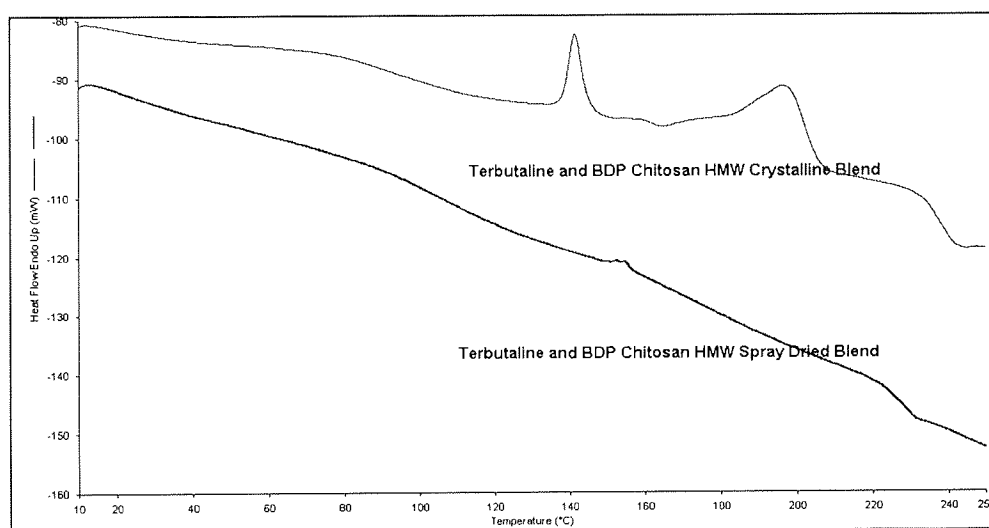
Figure 3.15: A representative comparative DSC trace of 36% leucine modified chitosan spray dried powders. Showing a crystalline and spray dried formulation containing 4% w/w terbutaline, 4% w/w BDP, 36% w/w LEU, 50% w/w high molecular weight chitosan and 6% w/w lactose. The fusion peak at c.140°C on the crystalline trace indicates lactose crystallinity/ water evaporation (Sabulal et al., 1997; Larhrib et al., 2003) with the endotherms at c. 200°C and 220°C indicating terbutaline and BDP crystallinity respectively (Murphy et al., 2005; Brodka-Pfeiffer et al., 2003), leucine decomposition at 273°C (Loo et al., 2005; Rosu et al., 2006) can not be seen on the spectra (mean ± SD, n=3).

Thermogravimetric analysis of the terbutaline contained chitosan spray-dried powders indicated that the moisture content of the powders ranged from 1.2% w/w to 5.9% w/w (Table 3.3). It was noticed that the chitosan formulations had higher water contents than the control powder. Other studies indicate moisture contents of spray-dried powders to be up to 7.5% w/w (Stahl et al., 2002; Chew et al., 2005), with higher water contents with in a spray dried formulation regarded as a disadvantage, water working by capillary action to increase aggregation and give hydrolysis

of active agents and formulants, the relatively low water contents are welcome (Chan et al., 2005).

Thermogravimetric analysis showed water contents of 3.34 - 8.30% in the BDP chitosan spray dried powders greater in comparison to the respective control (0.61%). The water contents were independent of chitosan molecular weight (Table 3.4). Water contents of 0.53 to 5.50% w/w in the powders containing both terbutaline and BDP were revealed. The water content appearing to be independent of chitosan molecular weight (Table 3.5).

Water content was indicated as being slightly higher in the BDP chitosan powders compared to the terbutaline and dual agent spray dried powders although no statistical difference was seen between the water contents of any of the formulations (ANOVA/Tukey: non significant, P> 0.05).

A consideration of the probability of hydrolysis of the lactose and subsequent yellowing of chitosan powders by any residual water should be highlighted due to higher water contents in the chitosan containing powders compared to the control (Tables 3.3 - 3.5). Higher water contents in the chitosan containing spray dried powders compared to those of the control powders are possibly due to water being retained in the chitosan matrix during spray-drying. Preventing LEU action in reducing droplet surface tension post atomiser to allow increased inlet heat radiance, increased heat radiance giving more efficient solvent evaporation from a suspended droplet.

Laser diffraction data are presented in tables 3.3 - 3.5, with example representative size distributions produced by Sympatec dry dispersion displayed in Figure 3.16. Dry powder dispersion laser diffraction (volume mean diameter: VMD) particle size analysis (performed by

WINDOX 3.4 software at 1 bar) showed each of the 18 spray-dried powders to have a mean laser diffraction (LD) size of less than 10 μm. For the terbutaline powders, the control powder and the MMW chitosan powder exhibited the smallest and largest LD size (4.3 μm and 9.9 μm, respectively; Table 3.3, Figure 3.16A). For BDP chitosan spray dried powders a volume mean diameter ranging from 3.8 to 9.25 μm with no direct correlation between chitosan molecular weight and diameter was represented (Table 3.4, Figure 3.16B). Four of the six formulations (control, LWM, LMW/MMW, MMW) would be regarded as small enough in size by dry dispersion laser diffraction not to impinge on the oropharynx, BDP HMW regarded as being statistically larger than the control (ANOVA/Tukey-Krammer: P <0.05: Atkins, 2005).

For dual terbutaline-BDP loaded spray-dried powders LD size (volume mean diameter: VMD) ranged from 3.36 to 9.08 μm (LMW and MMW/HMW respectively). The dual loaded chitosan formulations had very similar particle populations with a general trend of increasing LD size with increasing chitosan molecular weight observed (Table 3.5, Figure 3.16C). Only two of the "dual agent" formulations would be regarded as not being respirable based on volume mean diameter (VMD) laser diffraction sizes (MMW/HMW and HMW; Bosquillon et al., 2004a). The dry dispersion LD sizes at 1 bar as with spray dried lactose sizes in chapter 2 are designed to include aggregates so the increase in particle size viewed on the Sympatec® spectra are likely to be an increase in aggregate size. In addition, comparison of the SEM images (Figure 3.12-3.14) which appear to contain small primary particulates with the laser diffraction data suggests that during particle sizing the chitosan-modified powders did not behave as individual particles rather as aggregates represented by the wide or almost bimodal distributions seen in the Sympatec® spectra (Figure 3.16).
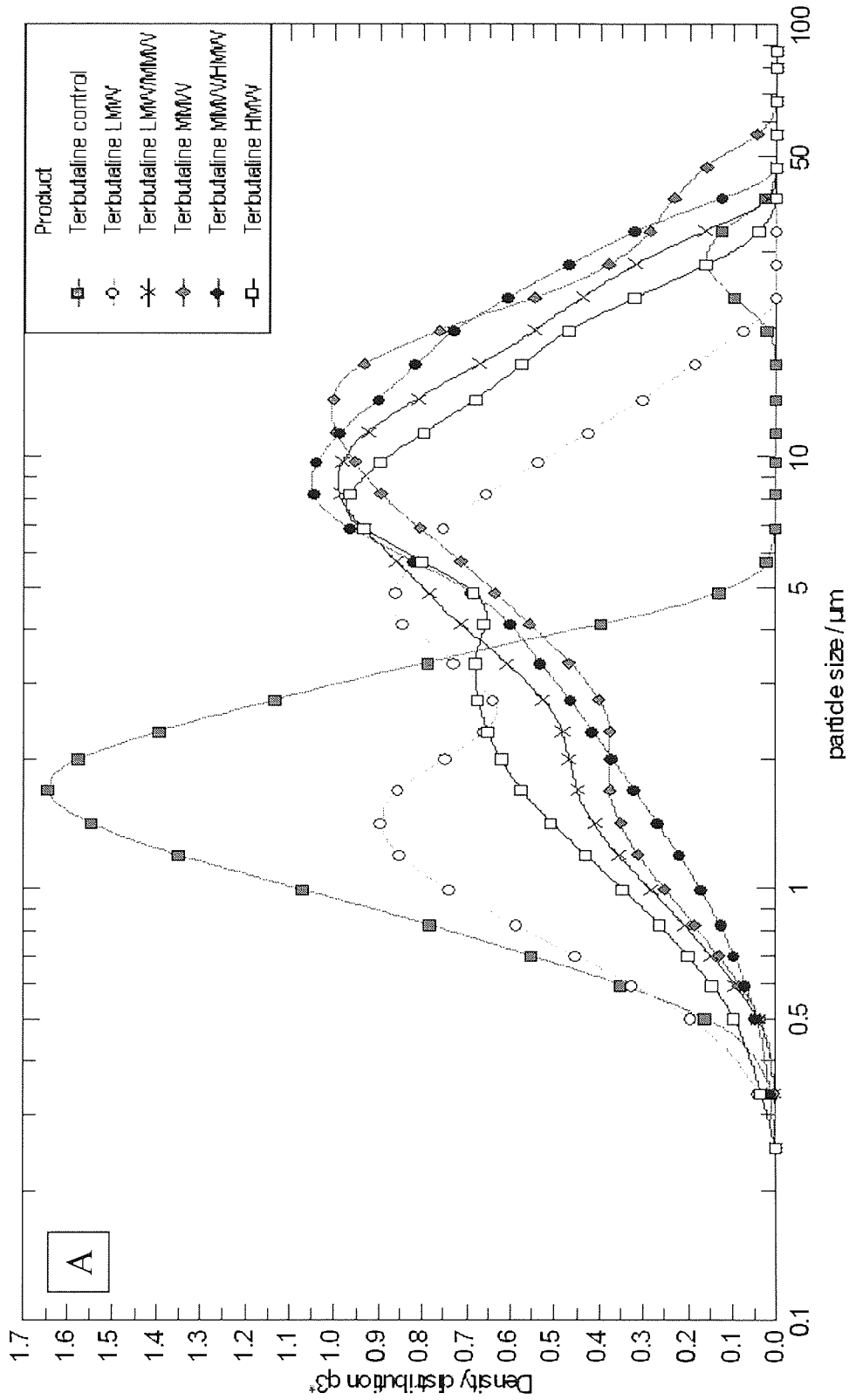
Figure 3.16A: A representative spectra of the distribution of the laser diffraction sizes produced by 36% LEU modified chitosan spray dried powders: A. terbutaline, B. BDP and C. Dual loaded chitosan samples and control. The distributions indicate poly-dispersity with the bell shaped curve indicating a normal distribution allowing theoretical primary particle diameters (d_{ae}) to be estimated.
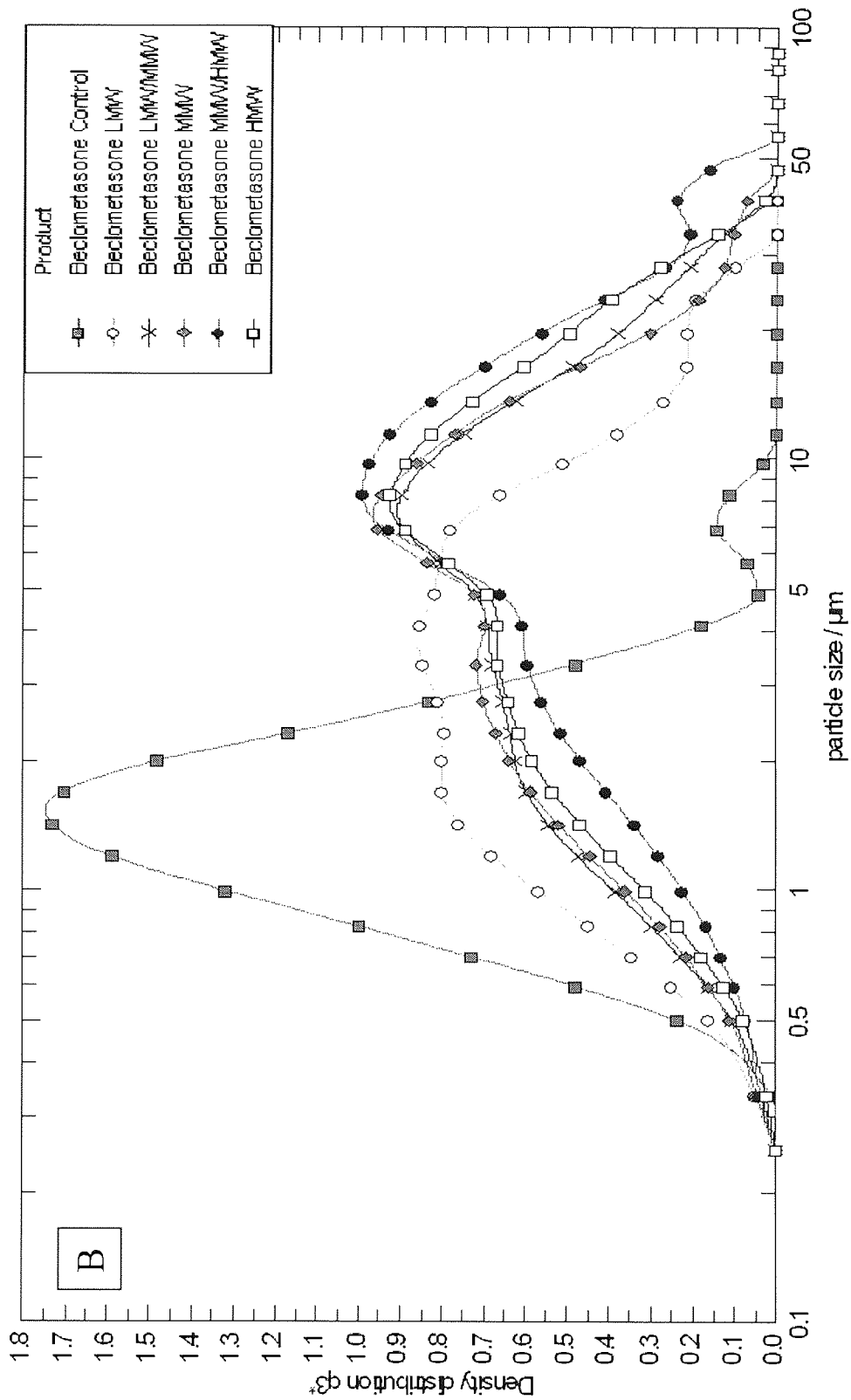
Figure 3.16B: A representative spectra of the distribution of the laser diffraction sizes produced by 36% LEU modified chitosan spray dried powders: A. terbutaline, B. BDP and C. Dual loaded chitosan samples and control. The distributions indicate poly-dispersity with the bell shaped curve indicating a normal distribution allowing theoretical primary particle diameters (d_{ae}) to be estimated.
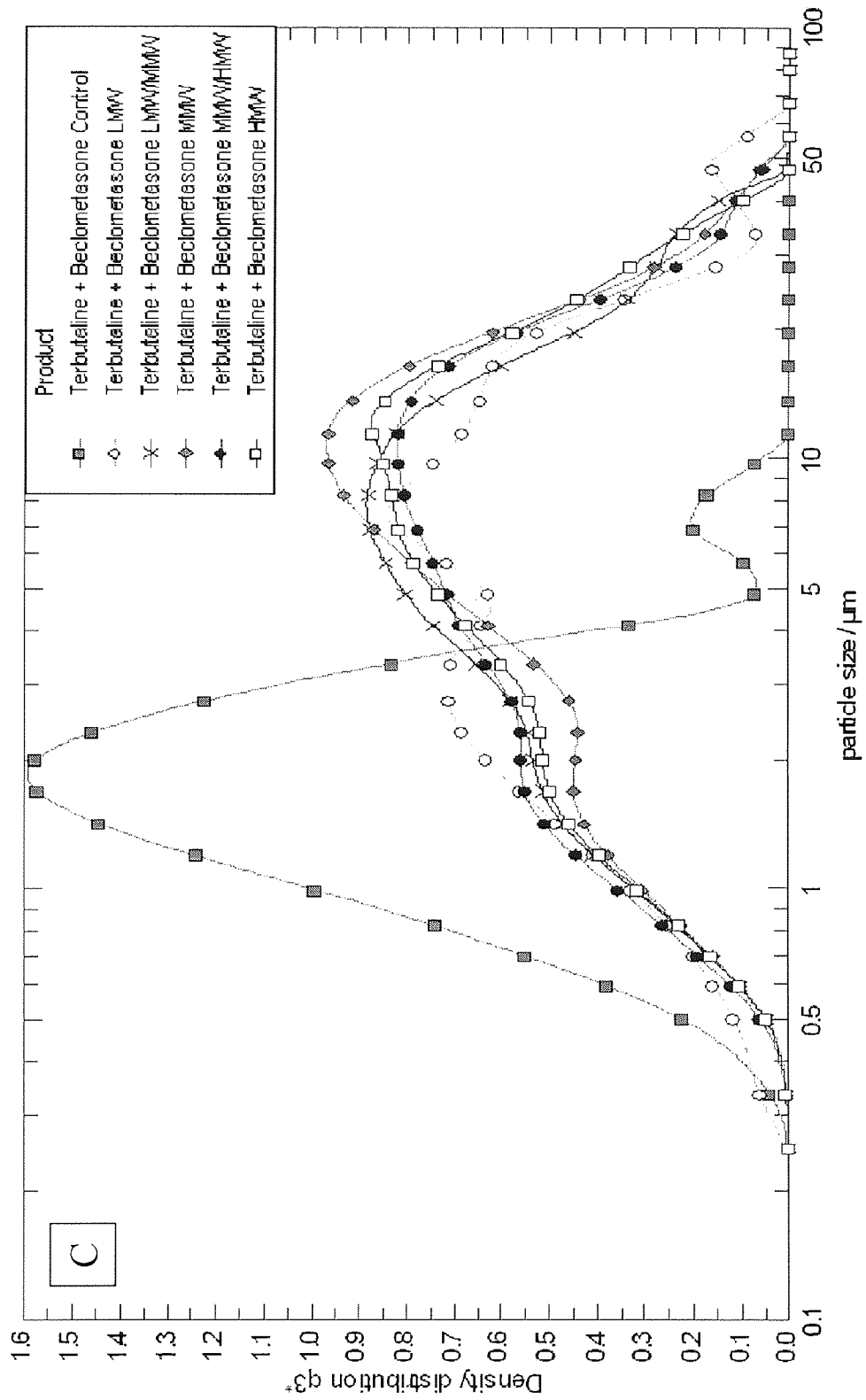
Figure 3.16C: A representative spectra of the distribution of the laser diffraction sizes produced by 36% LEU modified chitosan spray dried powders: A. terbutaline, B. BDP and C. Dual loaded chitosan samples and control. The distributions indicate poly-dispersity with the bell shaped curve indicating a normal distribution allowing theoretical primary particle diameters (d_{ae}) to be estimated.

The tapped density of the spray-dried powders was similar for all eighteen formulations. Terbutaline spray dried powders ranged from 0.12 – 0.19 g/cm^3; BDP powders ranged from 0.13 - 0.27 g/cm^3 and the dual loaded spray dried powder tapped densities ranged from 0.12 - 0.18 g/cm^3 with the controls generally giving higher values compared to the chitosan containing formulations (Table 3.3 - 3.5). These values are in line with previous investigations from within the research group (e.g. Rabbani and Seville, 2005). The tapped density of a formulation is associated with good aerosolisation; as more porous particles hold better aerodynamic property over solid particles of the same dimensions (Bosquillon et al., 2004a; Rabbani & Seville, 2005; Garcia-Arieta et al., 2001). Tapped density gives an insight into the porosity of particles, the lack of a trend in tapped density across the chitosan series points to the degree of intra-particulate airspace remaining proportional to chitosan molecular weight. This indicates that in the spray dried state longer chitosan chains are no more tightly packed than the lower molecular weight chitosan fractions but that a greater quantity of intra-particulate porosity will be observed in the higher molecular weight chitosan spray dried particles.

The theoretical primary aerodynamic diameter (d_{ae}) of each formulation was then calculated in triplicate. As shown in tables 3.3, 3.4 and 3.5, the d_{ae} values of all terbutaline chitosan spray-dried powders lay between 1.9 μm and 3.4 μm. For BDP chitosan spray-dried powders theoretical particle sizes (d_{ae}) were of a similar size range: 1.71 μm to 3.28 μm, slightly larger than the dual terbutaline-BDP containing powders: 1.29 μm to 3.61 μm in diameter. Smaller theoretical particle sizes contrasting to original laser diffraction sizes indicate that the spray dried particles were behaving as aggregates rather than individual microspheres during dry powder laser diffraction particle sizing (Bosquillon et al., 2004b).

The cohesion of individual particles to form aggregates through high surface energies leads to an overall increase in aerodynamic particle size of a formulation. With *in vivo* correlation, this means a reduction in the percentage of drug load reaching the target bronchi of the mid and deep lung. It is therefore advantageous to reduce aggregation to increase the respirable fraction and efficacy of a formulation (Bosquillon, 2001b). For all eighteen spray dried formulations the theoretical particle size is regarded as respirable ($d_{ae} < 5$ μm), with an overall increase in primary particle diameter with increasing chitosan molecular weight (Figure 3.17; Atkins 2005; Louey 2004a, 2004b). The increase in primary particles size is suspected to be a consequence of an increase in intra-particulate air-space associated with the proportional increase in chitosan chain length, molecular steric hindrance and the associated increase in particle porosity.
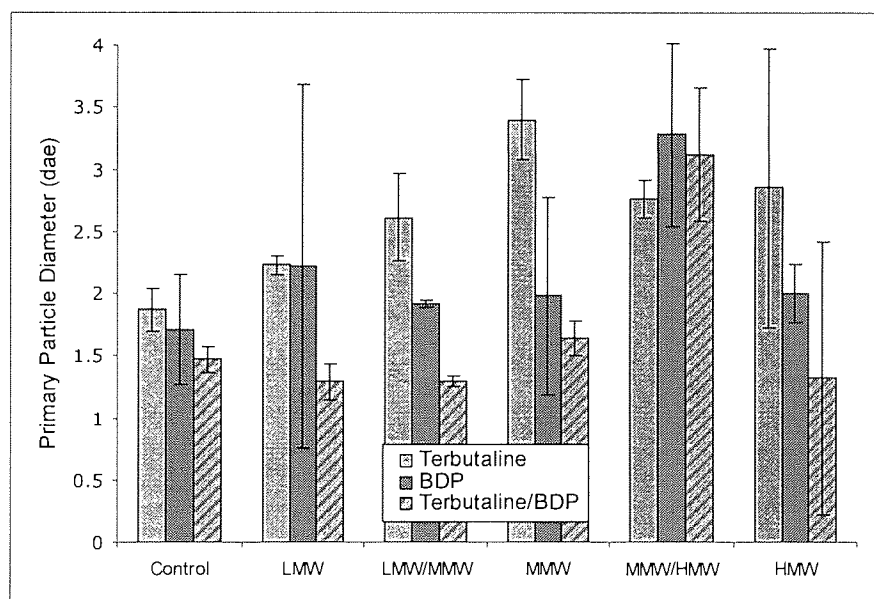
Figure 3.17: Barchart representing the approximate increase in primary particle diameter (d_{ae}) with increasing chitosan molecular weight (mean ± SD, n=3).

It is interesting to note the variation in primary particle diameter size (Figure 3.17) given that all formulations contained the same mass of solid (2% w/v) and were spray-dried using standard conditions. Aside from the presumption of increased airspaces in spray dried particles containing

higher molecular weight chitosan the effects of LEU modification should be noted. As has previously been hypothesised in the first subchapter LEU may give reduction in surface tension at the atomiser air-interface creating a finer spray of droplets which gives finer spray dried particulates, the mediation of LEU atomiser-air interface activity by increasing chitosan molecular weight may increasingly prevent this action.

It is also possible that the inclusion of chitosan gel in the formulation and increase in viscosity of the liquid being spray-dried in comparison to the control could result in a larger droplet at the spray-drying atomizer-fluid interface, which would spray-dry to give a larger particle, as previously suggested by other researchers (e.g. Bittner and Kissel, 1999).
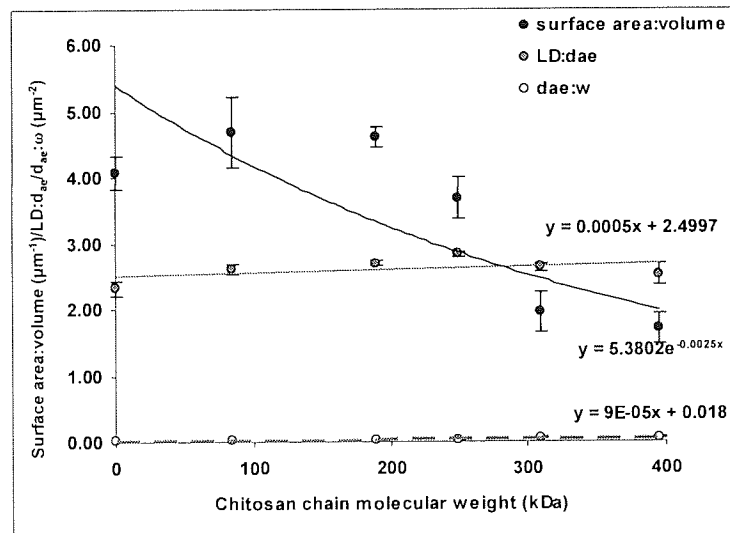
Figure 3.18: Chart illustrating the fall in surface area to volume and the increase in aggregation. Illustrated by the aggregation ratio ($LD:d_{ae}$) with increasing chitosan molecular weight of the dual loaded LEU modified chitosan spray dried powders. The chart also illustrates the proportional increase in primary particle diameter (d_{ae}) against thermal efficiency (ω: mean ± SD, n=3).

Further exploration of the effects of chitosan molecular weight on the particle size of LEU modified spray dried powders was explored with the dual loaded formulations. Figure 3.18 shows the increase in aggregation of particles associated with increasing chitosan molecular

weight. The increase in aggregation, shown by the positive gradient of LD:d_{ae}, may be due to the interaction of increasing amounts of surface chitosan or due to the increased prevention of LEU action with increasing chitosan chain length, preventing a reduction in thermal efficiency and the aerosolisation benefits that result. Figure 3.18 also shows that primary particle diameter (d_{ae}) and thermal efficiency (w) show proportional change over the different chitosan molecular weights, signifying thermal efficiency may be a contributory factor to reducing primary particle diameter in the spray drying of terbutaline-BDP dual loaded LEU-modified chitosan formulations or, that LEU is indeed responsible for both the diminution of droplet size and particle aggregation.
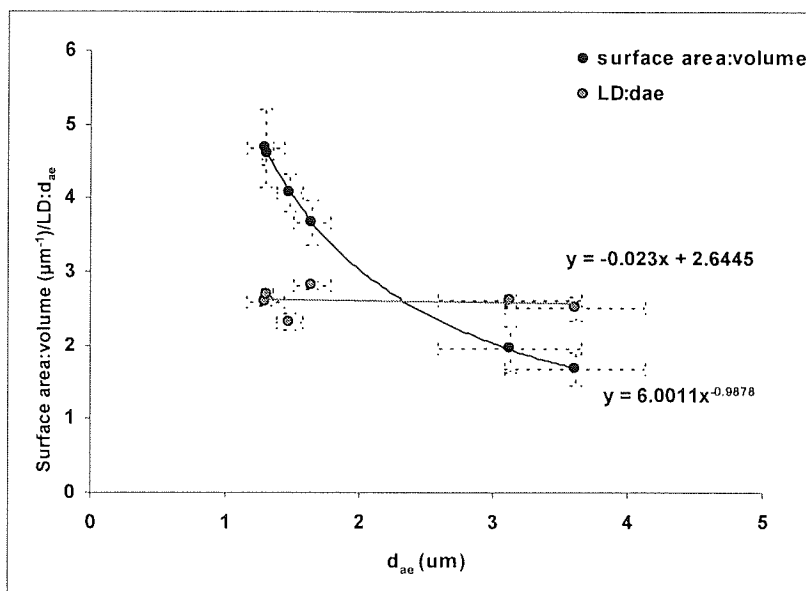
Figure 3.19: Chart showing the reduction of surface area to volume ratio with increasing primary particle diameter (d_{ae}) and the increase in aggregation with increasing size (aggregation ratio, LD:d_{ae}) (mean ± SD, n=3).

Further exploration in Figure 3.19 shows no trend of increasing aggregation (LD:d_{ae}) with decreasing primary particle diameter as seen with dry powders for inhalation. The almost level gradient (m=-0.023) accounting for poor correlation (r=0.0193) seen here with the aggregation ratio (LD:d_{ae}) is possibly due to LEU inter-particulate activity. LEU being permitted at lower

chitosan molecular weights to modify surface texture and reduce particulate contact points by reducing thermal efficiency through migration to the droplet drying-air interface. Reducing surface energy and allowing increased heat radiance through the droplet resulting in increased surface texture, such action being impeded by increasing chitosan molecular weight. The resulting increased surface texture reduces inter-particulate contact points thus reducing aggregation.

The suspected dual effects of LEU modification are thought to work together, i.e. the reduction of primary droplet size post atomizer increasing the surface folding seen on the dry particulate through the decrease in volume of droplet to be dried and the proportional increase in surface area (with reduced surface tension due to LEU surface activity) for inlet heat to radiate. Typically, reduced primary particulate diameter is associated with increased aggregation due to the increase in surface area to volume ratio increasing the total surface area available for aggregation within a blend (visualized in Figure 3.19).

Carr's Index may be used as an indication of powder flow properties; a value less than 25% indicates a fluid flowing powder, whereas a value greater than 25% indicates cohesive powder characteristics (De Villiers, 2005). The Carr's Index values of the terbutaline spray-dried powders ranged from 17.5% (MMW/HMW: good flow ability) to 30.9% (LMW: poor, cohesive flow ability: Table 3.3). As with the previous chapter observed visual flow was not represented by Carr's index evaluation, the terbuatline control powder holding a Carr's Index of 22.2%, indicating poor, fluid flow ability; suggesting that some of the chitosan formulations had better flow ability than the control powder, whereas other formulations had poorer flow ability.

For the BDP spray dried powders Carr's index values ranged from 24.2 – 33.3% representing the powder flow types as poor fluid to very poor (control and LMW/MMW respectively: Table 3.4). For the dual agent chitosan spray dried powders Carr's index values ranged from 22.7 – 38.0% representing the powder flow types as poor fluid to very poor (control and MMW/HMW chitosan formulations respectively (Table 3.5); no correlation was observed between particle size or visual flow properties and representative Carr's index values.

Carr's compressibility index, the value of density displaced expressed as a percentage of the bulk density, has previously been used to assess crystalline powders but the validity of this technique with spray dried powders remains uncertain (Wells & Aulton, 1988; Louey et al., 2004a, 2004b). In addition to any previous published research the previous chapter showed the flaws of using Carr's compressibility index in the assessment of the flow of spray-dried formulations.

3.3.2.2 *In vitro powder aerosolisation*

All powders showed high dispersibility across the eighteen formulations, with at least 90% of the capsule contents being emitted during aerosolisation testing (Figure 3.20). Statistical analysis indicated that there was no difference in the ED values of the different powders (one-way ANOVA/Tukey-Kramer; non significant, $P > 0.05$), the high dispersibility a clear reflection of the aerodynamic properties of the spray-dried powders. Properties arising from the inclusion of LEU as a dispersibility enhancer as discovered in the previous chapter and observed by other researchers (e.g. Rabbani & Seville, 2005; Chew et al., 2005b; Najafabadi et al., 2004; Li et al., 2003).
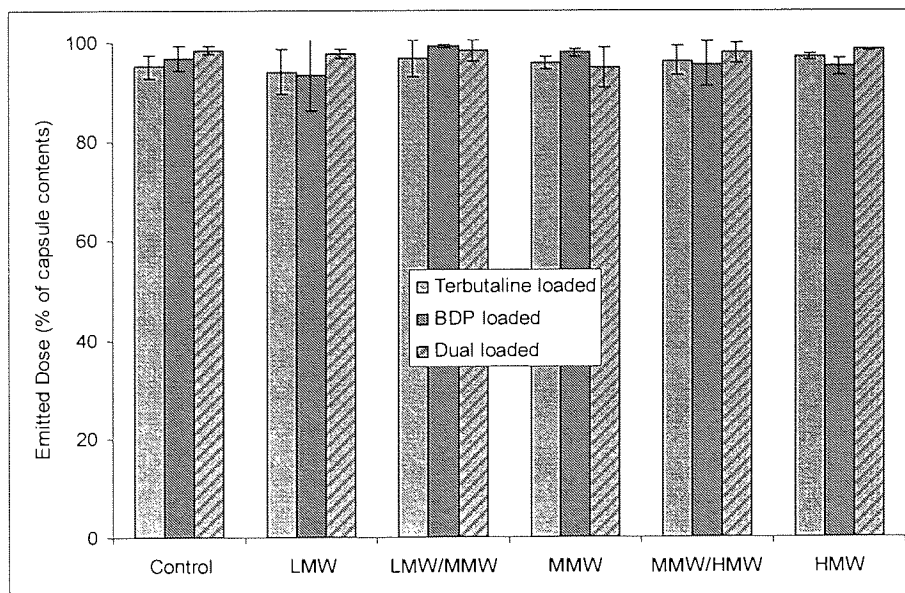
Figure 3.20: Bar chart of the percent emitted dose of 36% w/w LEU modified chitosan spray dried powders determined gravimetrically by dispersion of 25 mg aliquots from HMPC capsules via a Spinhaler at 60 Lmin⁻¹, with the emission expressed as a percentage of the loaded powder mass (mean ± SD, n=3).

The MSLI deposition patterns of the terbutaline, BDP and dual loaded spray-dried powders are shown in Figure 3.21. Low deposition in the inhaler and throat regions was recorded, again supporting the highly dispersible nature of these powders, with minimal *in vivo* oropharyngeal deposition expected. The terbutaline control powder represented a tight distribution of aerodynamic sizes with minimal deposition on Stage 1 and Stage 2 of the MSLI (Figure 3.21 A), with over 75% of the powder deposited on Stages 3 - Filter.

However, the inclusion of chitosan in to the terbutaline formulations significantly increased the deposition of powder on the earlier stages of the MSLI (one-way ANOVA/Tukey-Krammer: p <0.01), although substantial deposition was still evident at the lower stages. All terbutaline loaded chitosan spray dried powders would therefore be expected to perform well during inhalation and to deliver a large proportion of the dose to the central regions of the lung. In addition, the high proportion of powder reaching as far as Stage 4 and Filter suggest these

powders may even have the potential for peripheral lung delivery and systemic uptake of drug loads. As expected, there was no apparent correlation between the Carr's Index flowability data and the aerosolisation characteristics of the terbutaline spray dried powders. It was anticipated that those powders demonstrating lower Carr's Index values (i.e. better powder flow) would exhibit enhanced aerosolisation performance, represented by high ED and FPF. However, the aerosolisation performance of the terbutaline loaded chitosan spray-dried powders appears to be unrelated to Carr's Index. This could be due to the previously mentioned lack of application of Carr's index to spray dried powders.

For the six BDP chitosan formulations MSLI stage deposition followed a similar pattern to the terbutaline chitosan spray dried powders with minimal powder deposition in the inhaler and throat regions. A similar performance *in vivo* could mean reduced side effects such as oral candidiasis and hoarseness when compared to conventional DPI formulations containing BDP (Fukushima et al., 2005). However, deposition on stages 1 and 2 of the MSLI was greater for the BDP chitosan formulations compared to the terbutaline counterparts, the BDP formulations displaying greater poly-dispersity of aerodynamic particle sizes and possibly greater particle aggregation (Figure 3.21B).

Individual stage deposition of both terbutaline and BDP were not statistically different on any level of the MSLI for the dual loaded terbutaline-BDP formulations, showing homogeneity in the drug loading of individual microspheres and in the spray dried formulations as a whole (students paired t-test: Non significant, $P > 0.05$). The terbutaline-BDP formulations showed minimal deposition in the Spinhaler® device and the throat region of the MSLI, the control giving minimal

deposition of both terbutaline and BDP compared to the chitosan containing formulations on stage 1. Greater deposition was registered on stages three and four by the control and by the LMW formulation at the terminal filter stages of the MSLI compared to the other respective terbutaline-BDP chitosan spray dried formulations; however to no level of statistical difference (ANOVA/Tukey-Krammer: Non significant, P> 0.05: Figure 3.21C).
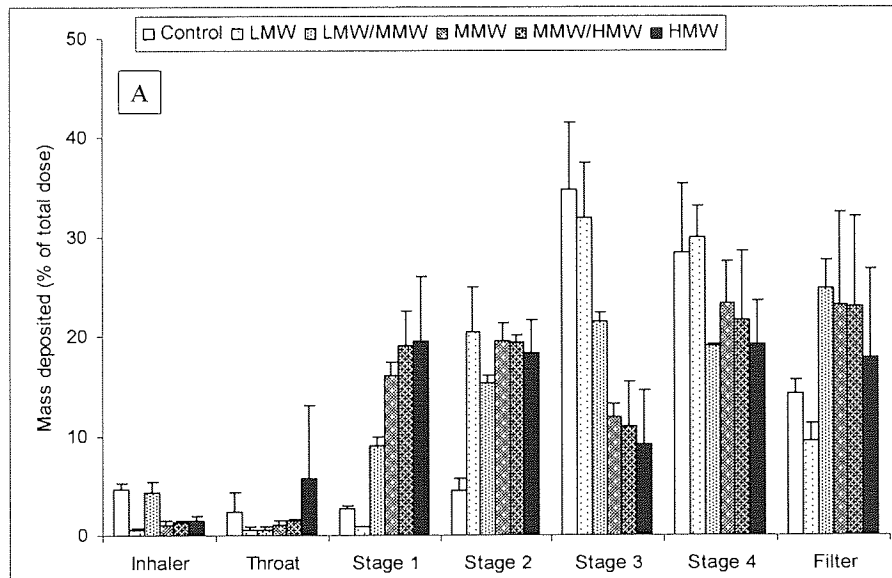
Figure 3.21A: The bar chart MSLI deposition profile of terbutaline 36% w/w LEU-modified spray dried chitosan formulations from 25 mg loaded HMPC capsules via a Spinhaler at 60 Lmin⁻¹ (mean ± SD, n=3).
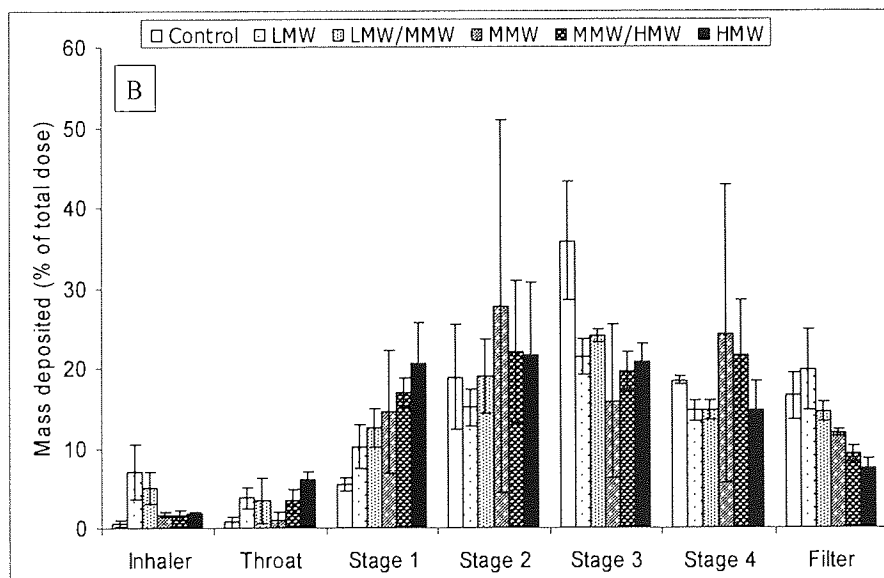
Figure 3.21B: The bar chart MSLI deposition profile of BDP 36% w/w LEU-modified spray dried chitosan formulations from 25 mg loaded HMPC capsules via a Spinhaler at 60 Lmin⁻¹ (mean ± SD, n=3).
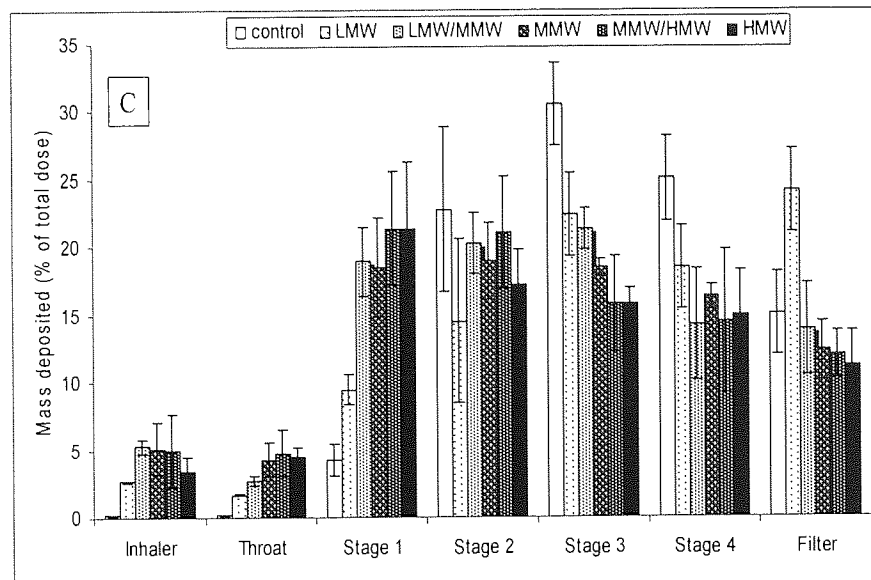
Figure 3.21C: The bar chart MSLI deposition profile of terbutaline and BDP dual loaded 36% w/w LEU-modified spray dried chitosan formulations from 25 mg loaded HMPC capsules via a Spinhaler at 60 Lmin^{-1} (mean ± SD, n=3).

All the 18 chitosan formulations were loaded with 3 mg of the respective active agent/s in each spray dried powder aerosolisation test (25 mg powder per capsule, 4% w/w drug loading, n=3). For the terbutaline loaded chitosan spray dried powders as with both the BDP and dual loaded formulations the control powder exhibited the highest FPD (terbutaline: 2.8 mg). The FPD of the terbutaline control powder was statistically higher than that of all terbutaline chitosan powders except the LMW chitosan powder (one-way ANOVA/Dunnett: p <0.05). The FPF of each terbutaline chitosan spray-dried powder, corrected for actual drug content, ranged between 56% and 82% of total capsule contents.

The FPF of the MMW/HMW and the HMW terbutaline chitosan powders (66% and 56%, respectively) proved to be significantly lower than that of the terbutaline control powder (75%, one-way ANOVA/Dunnett: p <0.05), whereas the FPF of the LMW, LMW/MMW and MMW terbutaline chitosan powders were statistically similar to the control (two-way ANOVA/Dunnett: p

>0.05). In addition, the FPF of the LMW terbutaline chitosan powder was statistically higher than that of the MMW/HMW and HMW terbutaline chitosan powders (one-way ANOVA/Dunnett: $p<0.05$) (Figure 3.22).

The fine particle fraction values for the BDP chitosan powders (FPF: F <5 μm) varied from 50.6 – 80.5% of the total dose, minor fluctuations in ED (93.6 - 99.4%) appearing to give enlarged fluctuations in % ED FPF (53.9 to 83.1% respectively). The highest FPF was given by the BDP control (80.5% of total loaded dose: Figure 3.22), the addition of chitosan of any molecular weight gave a significant decrease in FPF ($p< 0.05$: ANOVA/Tukey-Krammer) with no statistical difference between the FPF's of any of the chitosan containing formulations ($p >0.05$: ANOVA/Tukey-Krammer). Surprisingly, and in contrast with the terbutaline loaded powders, low molecular weight chitosan gave the lowest FPF (50.6%) and medium molecular weight chitosan gave the highest FPF excluding the control (66.4%). The results suggest that *in vitro* MSLI behavior of the BDP loaded chitosan powders was affected by aggregation of the primary particles; the aggregated particulates giving rise to a reduction in the fraction of the total loaded dose reaching the lower stages of the MSLI (Bosquillon et al., 2004b).

The combined effect of chitosan and BDP in the formulation may have had an effect on the lower than expected FPF values. BDP sedimentation alone in the 30% aqueous ethanol formulation can not be brought in to question due to the good performance of the control. However, it is possible that the increased particle size combined with the reduced FPF's are results of increased BDP precipitation due to the reduced solubility of BDP in 30% aqueous ethanol with the addition of chitosan (Sakagami et al., 2001).
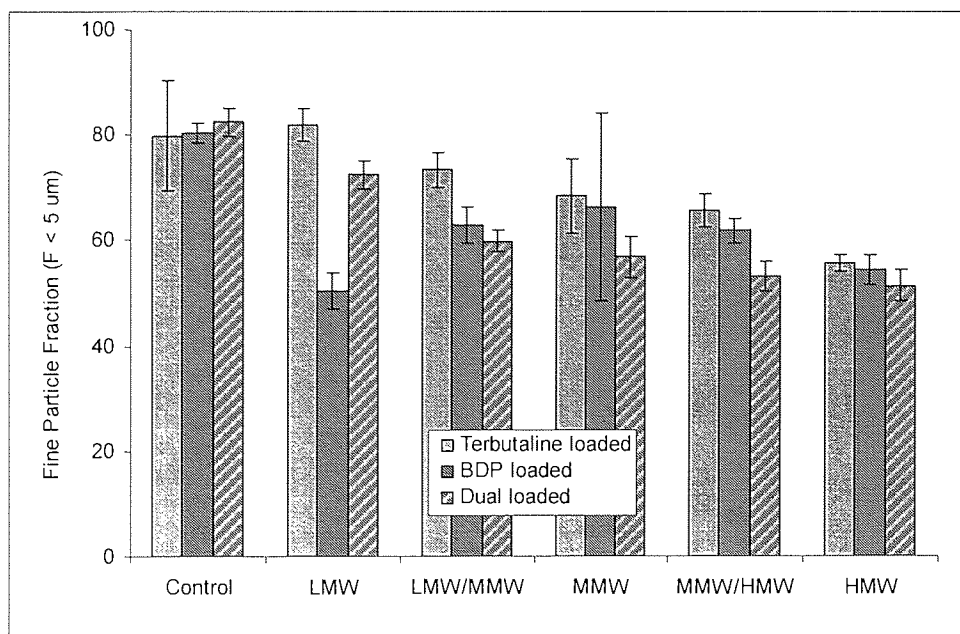
Figure 3.22: Bar chart of the fine particle fraction (F < 5 μm) of 36% LEU-modified chitosan spray dried powders extrapolated from data derived from 25 mg aliquots of spray dried material inspired from HMPC capsules by a Spinhaler at 60 Lmin⁻¹ through a multistage liquid impinger (MSLI) with analysis by HPLC (mean ± SD, n=3).

For the dual terbutaline-BDP loaded chitosan spray dried formulations no evidence of a trend between the fine particle fraction and the emitted dose was observed. A direct relationship between the chitosan molecular weight and fine particle fraction however, was observed, with a reduction in the FPF (% total dose) with increasing chitosan molecular weight (Figure 3.22). The addition of chitosan to the terbutaline-BDP formulation gave a significant decrease in FPF (control: LMW chitosan; ANOVA/Tukey-Krammer, p< 0.05), with the lowest FPF given by the HMW chitosan formulation (51.5%). Out of the dual terbutaline-BDP chitosan containing formulations, LMW chitosan gave a highly significant increase in FPF compared to the other chitosan containing formulations (ANOVA/Tukey-Krammer, p <0.001).

Overall there was a trend of improving aerosolisation with decreasing chitosan molecular weight for all 18 formulations. The reduction in FPF with increasing chitosan molecular weight may be

associated with the increasing chitosan molecular chain length giving rise to increased hydrogen bonding/ van der Waal sites for inter-particulate attraction; giving large aggregates which the force of inspiration in to the MSLI at 4 bar via the baffles of the Spinhaler DPI (60L/min) can not overcome. Furthermore the decrease in FPF across the series with increasing chitosan molecular weight could be due to the decreasing influence of LEU surface modification. Alternatively the reduction in FPF may be simply a consequence of increasing primary particle size as visualised in the SEM images (Figure 3.12 - 3.14).

However, it should be noted that although the LMW BDP chitosan powder exhibited the lowest FPF, an FPF of 50.6% is still higher than those reported by other researchers for less complex powders (e.g. Larhrib et al., 1999; Zeng et al., 1999; Corrigan et al., 2006a). All powders tested in the present study would be expected to deliver a high proportion of the total capsule contents to the conductive and peripheral airways of the lung following inhalation, with limited deposition in the device, oropharyngeal region or upper respiratory tract.

The mass median aerodynamic diameter (MMAD) is determined as the average size of the particle in the middle of a size distribution determined by the multistage liquid impinger (MSLI) when assessed on a plot of cumulative dose (y) and particle size (x) derived from the loaded dose (n=3). MMAD data revealed terbutaline chitosan spray-dried powder (range: 1.66 – 2.30 μm) aerodynamic populations were in line with the theoretical estimates of d_{ae}. Suggesting that in contrast to other investigations involving spray-dried powders (e.g. Bosquillon et al., 2004), the powders behaved as individual particles rather than particle aggregates during aerosolisation (Table 3.3). The BDP formulations ranged from 2.22 to 3.55 μm in MMAD, showing an increase

in size with increasing chitosan molecular weight (Table 3.4). The average MMAD derived from

terbutaline and BDP MMAD mean values in the terbutaline-BDP chitosan powders (range: 1.86 -

4.05 μm), also showed a defined positive correlation with the theoretical and laser diffraction

particle sizes, with a similar trend of increasing particulate size with increasing chitosan

molecular weight (Table 3.5, 3.23).
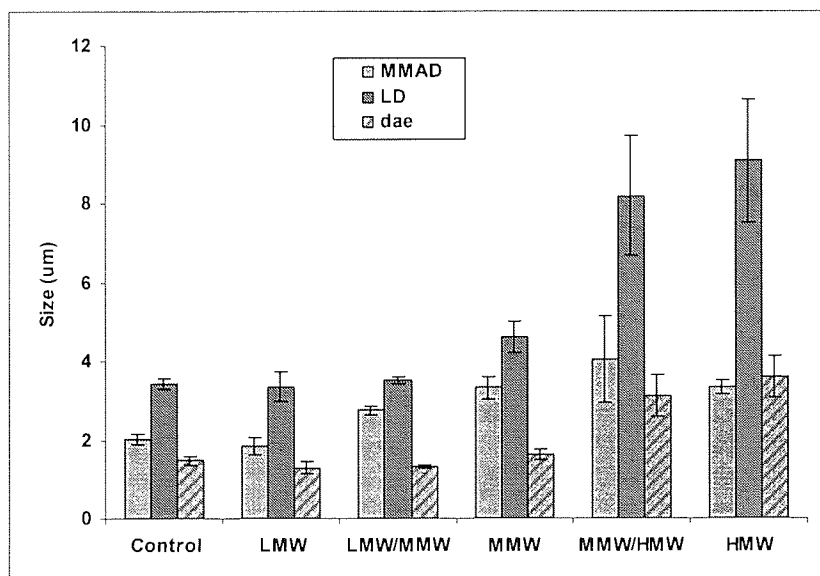
Figure 3.23: Bar chart representing the relationship in aerodynamic (MMAD), dry dispersion laser diffraction and theoretical primary particle (d_{ae}) sizes of the dual loaded terbutaline and BDP spray dried formulations (mean ± S.D, n=3).

Figure 3.23 illustrates how an increase in chitosan molecular weight gives an increase in the

particulate sizes of a leucine modified spray dried sample. The increase in size attributed to the

increased amount of chitin units (which make up chitosan) per unit area of liquid feed. As the

tapped density of the spray dried powders remained pretty much constant through out the

molecular weights it can be deduced that the degree of porosity would increase throughout the

series; with increasing chitosan molecular weight giving increased volume to individual

microspheres and the formulation as a whole. The physical entrapment of LEU by increasing

chitosan molecular weight may also be a major factor in the particle size of the dual loaded

formulations. LEU modification has been shown to give smaller primary particle size (d_{ae}) through the surfactant action at the atomiser-air spray-drying interface creating a finer spray.
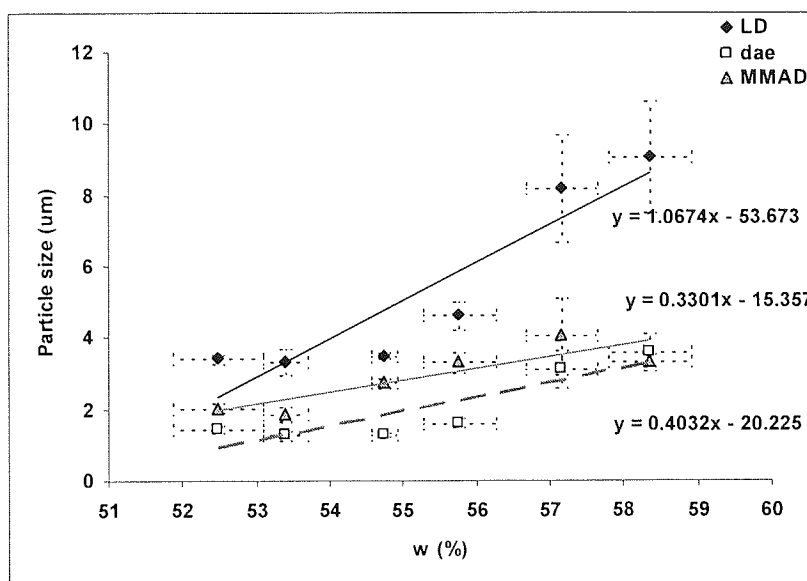
Figure 3.24: Chart illustrating the increase in Fraunhoffer dry dispersion laser diffraction diameter (LD), theoretical primary particle diameter (d_{ae}) and mass median aerodynamic diameter (MMAD) of terbutaline BDP dual loaded LEU-modified chitosan spray dried powders with increasing spray-drying thermal efficiency (w).

Figure 3.24 shows the increases seen in particle size with increased thermal efficiency (w), the relationship is based on larger droplets which produce larger primary particles (d_{ae}) requiring higher energy to dry hence reducing outlet temperatures improving unit w. The increase in dry dispersion LD size at 1 bar, due to aggregation as a consequence of a reduction in LEU modified surface texture by chitosan can also be seen. Primary particle diameters also increase in size, this thought to be due to the limitation of LEU activity at the nozzle of the spray-drying atomizer. The link to w being that the surfactant activity of LEU is critical in reducing the size of droplet, which can be dried using less energy raising outlet temperatures decreasing thermal efficiency. Furthermore the finer particulates created possess reduced surface tension through LEU surfactant action allowing greater inlet heat penetration and less energy loss at the droplet surface, giving a more fractured particle surface due to the kinetic effects of faster solvent

evaporation and a further increase to the outlet temperature. MMAD size is influenced by both d_{ae} and aggregation and is a reflection of LD size at 4 bar (Martin et al., 2007), it was also found that both d_{ae} and aggregation are effected by/affect w in leucine-modified spray dried powders in chapter 2.

The relative good performance of the chitosan powders in the MSLI is principally attributed to high LEU concentrations. LEU has previously been shown to enhance a spray-dried formulation's aerosolisation properties, the surface active action of LEU used as a reasoning for the increase in FPF as was discussed with in chapter two (Rabbani & Seville, 2005; Chew et al., 2005; Najafabadi et al., 2004; Li et al., 2005a). The use of chitosan as a potential aerosolisation enhancer has also been explored with marginal success (Li & Birchall, 2006).

The reduction in FPF with higher molecular weight chitosan formulations could involve the obstruction of LEU migration to the microsphere surface by increasingly long chitosan chains during the spray drying process, with the reduction of LEU at the surface of the powder particles resulting in an increase in aggregation and a decrease in fine particle fraction (Chew et al., 2005). Another reason for decreasing FPF could be the appearance of an increasing number of strands on the SEM images with increasing chitosan molecular weight, which could be a by-product of spray drying with chitosan; the strands may increase aggregation lowering FPF (Figures 3.12B-F, 3.13B–F, 3.14B-F).

The spray drying of BDP in a majority aqueous solvent creates solubility problems, as does the addition of other more freely soluble solutes into the formulation (Jones et al., 2006). The precipitation of BDP from solution and the use of a suspension in a spray drying process create a

lower limit on particle size dependent upon the size of the BDP crystals in the suspension (Jones et al., 2006). It is possible that such formulation problems had an effect on the particle sizes of the BDP containing formulations, LD, d_{ae} and MMAD, post spray drying.

Any changes in particle size can not be attributed to the spray drying process itself as the parameters remained constant in the production of the formulations. Differences in the particle sizes are therefore dependent upon the properties and formulation of the original suspension to be spray-dried (Maury et al., 2005).

3.3.2.3   *In vitro dissolution*

As expected, the terbutaline control powder underwent very rapid dissolution, with 100% terbutaline released after approximately 5 min (Figure 3.25), the BDP control giving 100% release after 20 minutes (Figure 3.26). The lengthy dissolution period attributed to the low water solubility of the corticosteroid and possible surface powder float reducing the surface area available for dissolution despite pre-wetting.
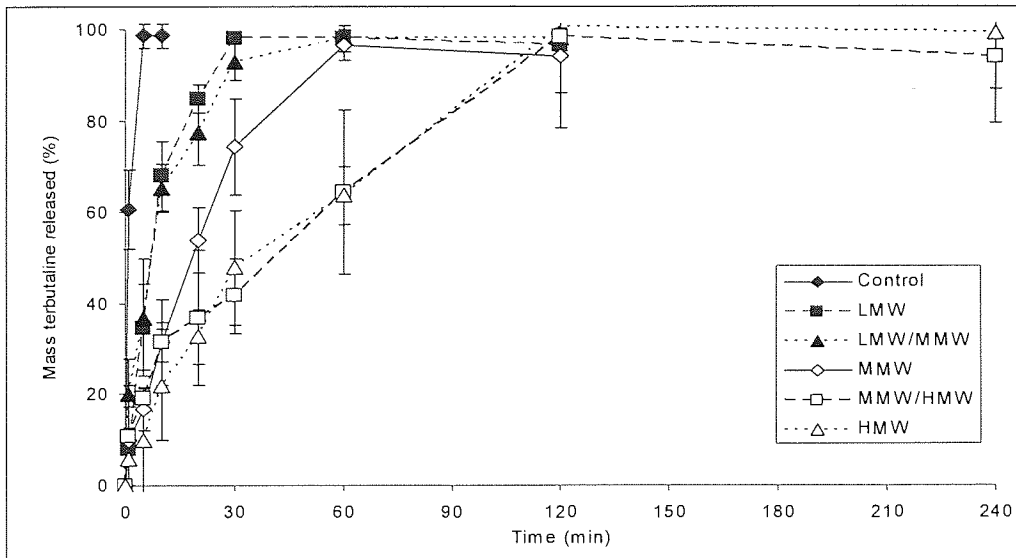
Figure 3.25: Line chart of the effect of chitosan molecular weight on terbutaline release from leucine modified spray dried powders. The terbutaline release of single loaded 200 mg 36% w/w LEU modified chitosan spray dried powder aliquots in to 1000 mL PBS from 2 cm diameter 50 rpm rotating baskets at 37°C (mean ± SD, n=3).
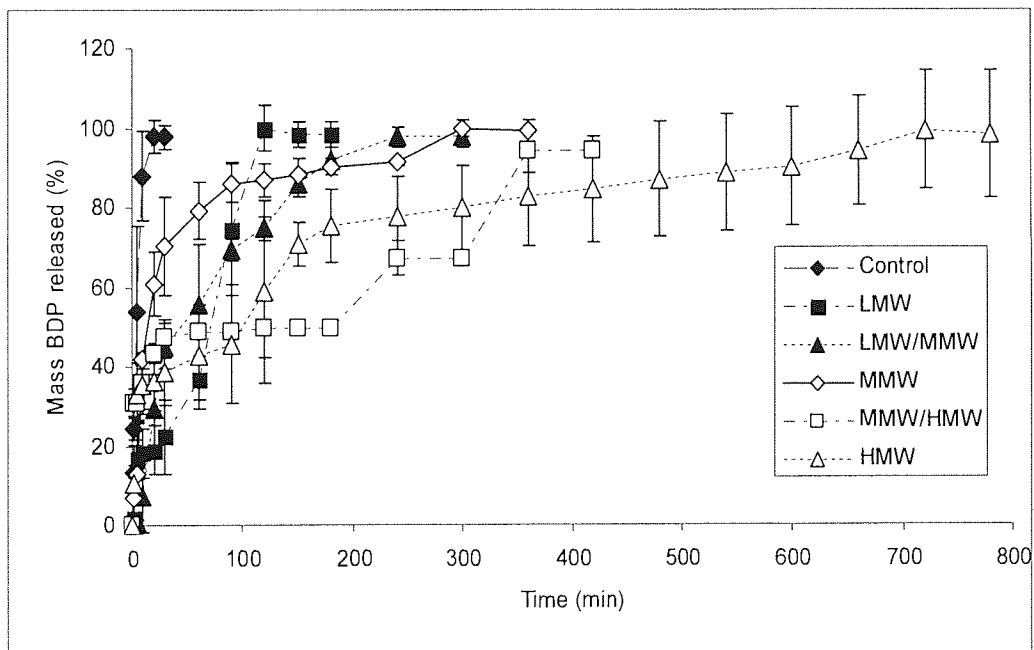
Figure 3.26: Line chart of the effect of chitosan molecular weight on BDP release from LEU modified spray dried powders. The BDP release of single loaded 200 mg 36% w/w LEU modified chitosan spray dried powder aliquots in to 1000 mL PBS from 2 cm diameter 50 rpm rotating baskets at 37°C (mean ± SD, n=3).
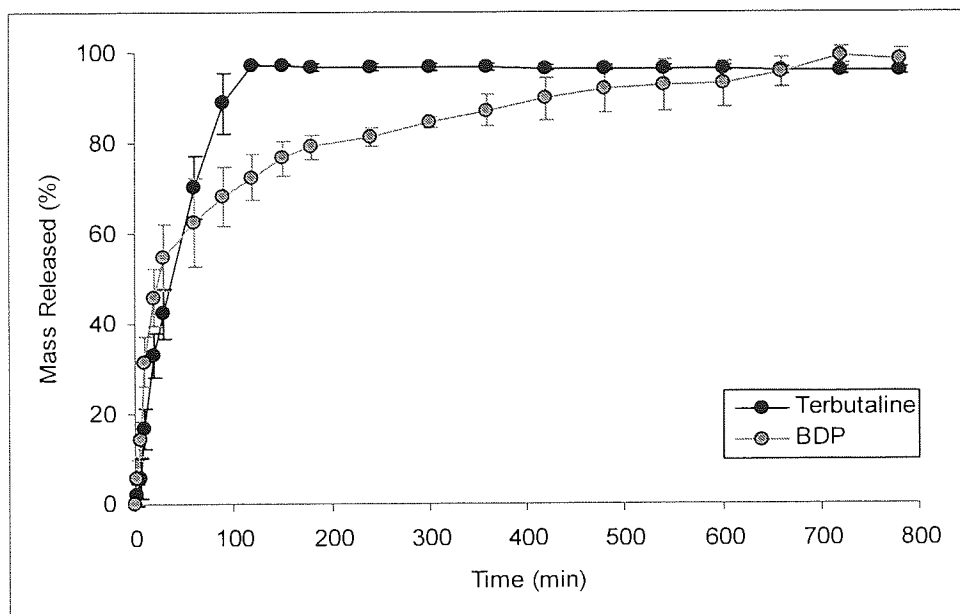
Figure 3.27: The release of terbutaline and BDP from dual loaded 200 mg 36% w/w LEU modified HMW chitosan spray dried powder aliquots in to 1000 mL PBS from 2 cm diameter 50 rpm rotating baskets at 37°C (mean ± SD, n=3).

The chitosan powders produced a viscous gel upon contact with the phosphate buffer solution (PBS) and exhibited delayed release characteristics; increasing chitosan molecular weight produced a more sustained release profile. For example, the LMW terbutaline and BDP combined chitosan powder released 100% terbutaline after 30 minutes and 100% BDP after 150 minutes (data not shown). Whereas two hours' dissolution time was necessary for the HMW chitosan powder to release its entire terbutaline drug load and 12 hours' to release the total BDP drug loading (*t*max, Figure 3.27). The MMW chitosan powder displayed an intermediate release profile, with 100% drug release of terbutaline after 60 minutes, 300 minutes for BDP.

The mixed chitosan powders (LMW/MMW and MMW/HMW) did not perform precisely as expected in all cases. It was anticipated that the LMW/MMW chitosan powder would exhibit a release profile somewhere between that of the LMW and MMW chitosan powders; in the terbutaline chitosan powders the LMW/MMW chitosan powder actually demonstrated a release

profile akin to that of the LMW chitosan powder. Likewise, the release profile of the MMW/HMW chitosan powders was not as anticipated, and was more analogous to that of the HMW chitosan powder in the case of the terbutaline chitosan powder and of the MMW powder in the case of the BDP chitosan MMW/HMW powder. However, the release of terbutaline and BDP was more consistent with regards to the hypothesis that increasing chitosan molecular weight gives prolonged dissolution from the dual loaded spray dried powders (representative curve Figure 3.27, $t$max Table 3.7).

The term "chitosan" actually refers to a series of chitosan polymers with different molecular weights (Illum, 1998); the raw chitosan used to prepare the spray dried formulations classified depending on a range of molecular weights with LMW classified as less than 190 kDa, MMW as between 190 and 310 kDa and HMW above 310 kDa. The range in molecular weights means that certain spray dried powders particularly those with mixed batches of chitosan (LMW/MMW and MMW/HMW) could portray a characteristic closer to one of the constituents compared to the other.

The reason for the difference in the dissolution times of terbutaline and BDP is the relative solubility of the two drugs in the dissolution media. Terbutaline is freely soluble in PBS so the diffusion down a concentration gradient to a more hydrophilic environment is rapid as seen in the control., the physical obstruction of chitosan and any intermolecular attraction (hydrogen bonding, van der Waals forces) retarding the release of terbutaline in the chitosan containing formulations (Chen et al., 2006). BDP is only partially soluble in PBS so the highly lipophilic corticosteroid slowly travels down the concentration gradient due to its retention in the

hydrophobic chitosan environment. The chitosan gel therefore has to swell to a greater extent to release BDP compared to the degree of swelling necessary to deliver the terbutaline fraction.

In this study, a constant amount of chitosan in the spray-drying formulation was maintained, the resultant spray-dried microspheres containing 50% w/w chitosan. A previous study into the effects of chitosan concentration on the release of a hydrophilic entity (theophylline) has already shown how increased chitosan concentration in relation to the active marker can improve sustained release character by increased physical retention (Asada et al., 2004). In this study the release characteristics were modified through the use of low, medium and high molecular weight chitosan. The hypothesis that the longer polymer chains in HMW chitosan would result in a more prolonged drug release profile compared to the lower molecular weight chitosan formulations, which comprise of shorter chains, was observed. The *in vitro* dissolution studies appear to support this theory, and demonstrate the potential to tailor the drug release profile through appropriate selection of chitosan MW. Theories behind the differences in dissolution times for each molecular weight are based on chitosan chain length as the higher the molecular weight the longer the individual fibres and the greater the hydrogen bonding, van der Waal forces and physical interlocking giving greater drug entrapment and increased dissolution time (Chen et al., 2006; Asada et al., 2004).

Comparison of the rates of release using zero and first order and the Higuchi homogeneous matrix rate equations reveal a dual release pattern (Table 3.7). An initial zero order burst phase was seen by all chitosan containing formulations, visualised as high correlation coefficients (r^2) relating the release to the zero order kinetic from t_0 to early sample time points (dependent upon formulation), possibly due to the presence of terbutaline and BDP on the surface of primary

powder particles. A secondary sustained release profile followed with the release of agents coming from terbutaline and BDP drug molecules residing with in the chitosan matrices, represented as high correlation (r^2) between zero and higher time points.

	zero order			1st order		Higuchi matrix	
	t_{max} (min)	t (min)	r^2	t (min)	r^2	t (min)	r^2
Terbutaline							
Control	2	0-2	0.9953	0-2	0.8645	0-2	0.9726
LMW	30	0-10	0.9996	0-30	0.9688	0-30	0.9729
LMW/MMW	60	0-10	0.9558	0-60	0.9912	0-60	0.9198
MMW	60	0-60	0.9188	0-60	0.9714	0-60	0.9783
MMW/HMW	90	0-90	0.9545	0-90	0.9842	0-90	0.9621
HMW	120	0-30	0.9852	0-60	0.9732	0-120	0.9352
BDP							
Control	60	0-30	0.9340	0-60	0.9678	0-60	0.9721
LMW	120	0-120	0.9610	0-90	0.8455	0-120	0.8786
LMW/MMW	240	0-30	0.9685	0-240	0.9106	0-240	0.9637
MMW	540	0-30	0.9315	0-540	0.8524	0-540	0.7317
MMW/HMW	360	0-360	0.7713	0-360	0.8384	0-360	0.8584
HMW	720	0-720	0.7692	0-540	0.9509	0-720	0.9225
Dual Release							
Control t*	5	0-5	0.9736	0-5	0.9826	0-5	0.9881
*Control b**	*30*	*0-30*	*0.8381*	*0-30*	*0.8589*	*0-30*	*0.9644*
LMW t	60	0-30	0.9485	0-30	0.7478	0-60	0.8486
LMW b	*150*	*0-150*	*0.8815*	*0-150*	*0.8913*	*0-150*	*0.9841*
LMW/MMW t	60	0-30	0.9883	0-30	0.8693	0-60	0.9009
LMW/MMW b	*180*	*0-180*	*0.8165*	*0-180*	*0.9592*	*0-180*	*0.9573*
MMW t	60	0-30	0.9937	0-30	0.9035	0-60	0.9732
MMW b	*300*	*0-30*	*0.9455*	*0-240*	*0.9533*	*0-300*	*0.9209*
MMW/HMW t	120	0-120	0.8590	0-120	0.9697	0-120	0.9694
MMW/HMW b	*360*	*0-120*	*0.7527*	*0-240*	*0.9475*	*0-360*	*0.9371*
HMW t	120	0-120	0.9475	0-120	0.9382	0-120	0.9770
HMW b	*720*	*0-20*	*0.9651*	*0-660*	*0.9445*	*0-720*	*0.8436*

Table 3.7: The t_{max} and release kinetics of 36% w/w LEU modified chitosan spray dried formulations, derived as detailed in Appendix 3, t*: terbutaline release, b*: *BDP release*. The time intervals listed are those with the highest correlation to the release kinetic.

Concerning the controls, the release of terbutaline proved zero order with the spontaneous distribution of drug load down the concentration gradient. However, the release of BDP was influenced by the relatively poor solubility in PBS and more closely related to the Higuchi matrix release pattern, an unlikely correlation due to the lack of matrix polymer in the formulation (Table 3.7). Terbutaline chitosan formulations showed close correlation with a first order release pattern; first order release shows depleting drug concentration within a system to be the rate-limiting factor (Aulton, 1988). The close relationship to first order release indicates entrapment of terbutaline within the chitosan matrix and that the sustained delivery of freely water soluble drugs from a chitosan matrix is dependent upon either a shallow concentration gradient or an initial low concentration of active agent within the formulation.

Beclometasone with the exception of the LMW and MMW formulations (zero and zero/first order release rates respectively) followed the Higuchi matrix release pattern from the solo BDP loaded chitosan spray dried powders. The close proximity to the Higuchi equation (r^2 0.8584 – 0.9637) indicates that BDP release from the chitosan formulations is limited by physical containment and not by limited solubility (Table 3.7).

The visual release patterns of the delivery of terbutaline and BDP in the single and dual loaded powders appeared to be similar but exploration of rate release kinetics show discrete discrepancies in the release patterns of the single and combined dual release formulations. All but one dissolution trace (BDP HMW) followed the Higuchi kinetic; the change in terbutaline release from a first order kinetic to a Higuchi matrix release physic represents a change in solvation properties with the addition of a hydrophobic entity (BDP). The reduction in the release rate of terbutaline was unexpected as the addition of BDP should render the chitosan matrix

more hydrophobic making the PBS dissolution media more attractive. One reason could be that the hydrophobic backbone of the corticosteroid and the chitosan polymer may interact to reduce the rate of chitosan swelling enhancing the retention of any entrapped terbutaline. Early zero order release of terbutaline in the formulations up to MMW chitosan (r^2 0.9736 - 0.9937) indicate that the phenomenon would be influenced by chitosan chain length; paradoxically, BDP showed no modification in release pattern with the addition of terbutaline.

3.3.3 Validation of technique and stability studies

The use of an alternate assay for the aerosolisation of chitosan based powders may have had an effect on the outcomes for each subsection in this chapter. To test for any statistical difference in aerosolisation behaviour and output one formulation (spray dried: 4% w/w terbutaline, 36% w/w leucine, 25% w/w chitosan MMW, 25% w/w chitosan HMW, 10% w/w lactose produced using the high performance cyclone) was tested using the ACI gravimetric and the MSLI HPLC assays. The stability of the chitosan containing spray-dried formulations was in question, with the yellowing of the particles over the months post process and the appearance of whiskers on the SEM images. The same formulation was tested using both methods after six months of storage at room temperature and humidity to determine any alteration in aerosolisation properties, dissolution was also performed to test for any change in solvation property.

The gravimetric ACI and HPLC MSLI assays showed poor correlation with statistically different FPF values when tested post spray drying (39.1 and 65.7% respectively); the MMAD also showed a marked difference (4.41 and 2.94 μm respectively, $P < 0.05$). However, after a six-

month storage period the same techniques correlated well when tested with the same sample. The FPF compared well (57.8 to 56.9%) with less discrepancy in MMAD (2.52 and 3.79μm).

The differentials in the same cohort when focused on six-month stability surprisingly show an overall improvement in the performance of the powder. A small decrease in the aerosolisation properties was seen with the MSLI HPLC assay (FPF: 65.7 to 56.9%) but a large increase was seen by the ACI gravimetric assay (FPF: 39.1 to 65.7%). It should be noted however that the ACI gravimetric assay has a comparative poor recovery compared to the MSLI assay: 78.9 and 85.0% recovery of total dose compared to 97.1 and 94.8% respectively. The poor recovery in the ACI gravimetric assay post spray drying may have also affected aerosolisation data (FPF only 39.1%). The results are not conclusive as to whether the aerosolisation of the powders alters upon storage due to only one sample being applied but for this one sample no major deterioration was noticed. The HPLC assay is dependent on the detection of the active agent, the detection after six months proves that terbutaline is stable in the LEU modified chitosan spray dried powder for that period.

Dissolution data for the powder sample appeared to be similar to the freshly prepared sample, the six month period with a *tmax* of 120 min for the elution of terbutaline from the HMW chitosan formulation (data not shown).

3.4 Conclusions

External research has highlighted the use of chitosan in spray dried formulations, often exploring the usage of chitosan cross-linkage to enhance dissolution (e.g. Gavini et al., 2005, 2006) or

mucoadhesive properties (e.g. Haupt et al., 2006; Muzzarelli et al., 2003). The use of chitosan in

nasal delivery has been extensively researched but with the reduction in particle size required for

pulmonary delivery the use of chitosan for this route is less understood (Amidi et al., 2006; Zaki

et al., 2006; Lee et al., 2006). The use of LEU in a chitosan formulation is a new concept aimed

at improving the *in vitro* deposition, the improvement in deposition seen could potentially mean

enhanced disease management and reduced side effects as a far smaller proportion of the

emitted dose is impinging in the oropharangeal region compared to typical DPI formulations (Taki

et al., 2006). The deeper understanding of LEU modification and mechanisms of action could

also aid the production of even more respirable spray dried powders or the production of

synthetic aerosolization enhancers.

The use of two active agents in a highly respirable formulation as seen in the combined

terbutaline and BDP formulation has advantages of simplified regimens and improved patient

care. An example of a marketed combination DPI formulation is the Seretide® Accuhaler®

containing 50 μg of the long acting β_2 agonist salmeterol xinafoate (FPF <5 μm: 14.2% ± 2.8)

and 100 μg of the corticosteroid fluticasone propionate (FPF <5 μm: 18.2% ± 9.7) in a typical

lactose based formulation (Taki et al., 2006). In a comparison with research undertaken by *Taki*

et al. (2006) investigating the efficacy of the Seretide discus® product all spray dried 36% LEU

modified chitosan formulations represented by the research in this subsection hold a far superior

in vitro deposition profile over the marketed counterpart (one way students t test; P<0.0001,

highly significant). In addition, the use of chitosan in the spray-dried blends potentially provides

the bonus of sustained release *in vivo* where as the Seretide® components do not.

These investigations have demonstrated that it is possible to generate highly respirable powders that exhibit sustained release. The powders would be predicted to deposit predominately in the central and peripheral regions of the lung following inhalation, with minimal oropharyngeal deposition, thereby maximizing drug targeting and decreasing the incidence of local and systemic side effects. If *in vivo* conditions proved representative of *in vitro* dissolution testing the delivery of agents to the ciliated columnar cells of the airway epithelia would be controlled rather than instantaneous.

The addition of LEU has shown great improvements in the deposition of spray-dried powders in the two preceding chapters. Cumulatively, the use of chitosan has been shown to produce controlled release in highly respirable LEU -modified spray dried powders with the potential of mucoadhesive, penetration enhancing and antifungal properties: via decreased oropharangeal deposition and the activity of chitosan itself (a concentration up to 1 mg/ml required for fungal growth inhibition: Prapagdee et al., 2007). However, the short-comings of using chitosan as a release modifier are exposed when looking for a twenty four hour 'one-a-day' release profile, HMW chitosan revealing a *t*max of only 120 minutes for the hydrophilic marker terbutaline *in vitro* (Figures 2.25, 3.27).

To achieve greater sustained release of both hydrophilic and hydrophobic markers a more robust polymer is required for incorporation into the spray dried formulations. This is to be discussed in the next chapter with the implementation of synthetic lactide-glycolide variants.

Chapter 4

The utilisation of double emulsion spray

drying in pulmonary controlled release.

4.1 *Introduction*

The use of marketed combination inhalers has enabled the single dual delivery of long acting β_2 agonists and corticosteroids from a typical dry powder formulation (Stempel et al., 2005). Despite the benefits to patient compliance and convenience drawbacks of these formulations include the need for short acting β-agonists to be added to the regimen to manage disease exacerbations, the low percentage of total dose reaching the target bronchioles and the adverse effects associated with the impaction of high doses on the back of the throat (Taki et al., 2006; Dyer et al., 2006). The use of spray drying as a potential method of producing highly respirable combination powders with sustained release has the potential of eliminating such problems. The one step process allows the addition of a polymer into a formulation for reasons such as the controlled release of either single or multiple agents from a formulation or for mucoadhesion to increase the targeting of formulated agents (Blanco et al., 2006; Schnieders et al., 2006; Cilurzo et al., 2005).

The previous chapter showed how the spray drying technique can be used to incorporate both a β_2 agonist (terbutaline sulfate) and a corticosteroid (BDP) into the same formulation which could be capable of delivering sustained release *in vivo*, when chitosan was employed as a sustained release polymer to increase the duration of *in vitro* release of the short acting agents terbutaline and BDP. However, the limited increase in duration of release, from two minutes to two hours in the case of terbutaline and from twenty minutes to 12 hours in the case of BDP (control: high molecular weight chitosan) given by the polysaccharide chitosan indicates that a more robust polymer needs to be utilised.

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In this chapter, the biocompatible polymer poly-lactic co-glycolic acid (PLGA) will be used in the ratios of 75:25 and 50:50 (lactide: glycolide) and spray dried as a controlled release entity in the oil phase of a w/o/w emulsion.

The principle emulsions formulated in this experimental chapter are termed double emulsions, consisting of a water in oil in water profile. That is the dispersion of one immiscible phase (e.g. water) as micro-droplets through a continuous phase (e.g. chloroform) enabled by the addition of a stabilising agent, or emulsifier. The primary emulsion of continuous phase containing dispersed phase then dispersed itself as micro-droplets through a secondary continuous phase, (e.g. water) which is miscible with the original dispersed phase but critically immiscible with the primary continuous phase. The result being smaller droplets dispersed inside larger droplets contained in a third and final phase (Billany, 1988). The different phases of the double emulsion will be used to locate the two agents, the short-acting β_2 agonist salbutamol sulfate (SS) and the corticosteroid BDP, to determine the different release rates of the markers from the various phases of the spray dried double emulsions.

Firstly, the use of a tried and tested formulation using the plasticizer and suspending agent polyvinyl alcohol (PVA) in the final water phase of the double emulsion will be investigated to explore the potential of spray drying the complex system. The PLGA-PVA combination has been used in a double emulsion to form microspheres using a solvent evaporation method similar to that employed by *Bouissou et al.* (2006).

Secondly the use of chitosan instead of PVA as a suspending agent in the tertiary phase of the w/o/w emulsion will be navigated; chitosan having advantages such as being mucoadhesive (Harikarnpakdee et al., 2006), antifungal (Prapagdee et al., 2006), and penetration enhancing (Gavini et al., 2006; Corrigan et al., 2006b; Rabe et al., 2006), as discussed in the previous chapter. The mucociliary escalator and mucosal secretion by the lung epithelia are a challenge to the delivery of controlled release entities to the lung as they greatly enhance foreign body clearance from the bronchi (Boitano, 2006). Therefore the addition of a mucoadhesive to counteract such clearance methods and increase contact time between the drug-polymer complex and the lungs surface is vital. Chitosan has previously been shown to be mucoadhesive and indeed a penetration enhancer of hydrophilic agents (such as SS: Chopra et al., 2006); it is also known from the research in the previous chapter that low molecular weight (LMW) chitosan gives the best aerosolisation properties *in vitro*. As a result LMW chitosan gel will be used in the second sub section of the chapter as a potentially mucoadhesive final phase, offering stabilisation of the double emulsion through the gel network of chitosan interlocking chains that of course, may lead to sustained release effects (Chopra et al., 2006).

As drug delivery to the lung is largely governed by particle size and the amino acid leucine has been shown to enhance the aerosolisation properties of previously cohesive spray dried formulations by reducing interparticulate aggregation (Bosquillon et al., 2004b; Rabbani & Seville, 2005; Li et al., 2005b; Chew NY et al., 2005b; Najafabadi et al., 2004). The amino acid will be included in the final aqueous phase of the w/o/w emulsion with either PVA or LMW chitosan to enhance the delivery of the formulation as has been demonstrated in the previous two chapters.

4.2 Materials and Methods

4.2.1 Materials

SS was kindly donated by Allchem International Ltd (Maidenhead, UK). BDP, polylactide co-glycolide 25:75 (PLGA 25:75, mw 40000-75000), polylactide co-glycolide 50:50 (PLGA 50:50, mw 40000-75000), polyvinyl alcohol (PVA, mw 10000), chitosan low molecular weight (LMW) coarse flakes 98% purity (mw <190 kDa), chitosan medium molecular weight (MMW) coarse flakes 98% purity (mw 190 -310 kDa), chitosan high molecular weight (HMW) powder (mw >310 kDa), l-leucine 98% purity, erythritol, mannitol and phosphate buffered saline tablets (PBS) were purchased from Sigma Aldrich® Chemicals (Poole, UK). HPLC grade methanol, ethanol 99%, lactose and span 80 was purchased from Fisher scientific® Ltd (Loughborough, UK). Synperonic PE/L101 was purchased from Ellis and Everard (Middlesbrough, UK).

4.2.2 Preparation of spray-dried powders

4.2.2.1   PVA w/o/w emulsion spray dried powders

Powders were formulated from 2% w/v double emulsions; the w/o/w double emulsions were prepared based on those outlined in *Hadlock et al.* (2003) by vortex-mixing 0.4 mL water (± 160 mg SS) with 0.1 mL Span 80 in a solution of 50 - 200 mg PLGA (25:75) in 12 mL chloroform (± 160 mg BDP). The initial w/o emulsion was subsequently homogenised (Heidolph® homogeniser, Heidolph-electro, Kelheim, Germany) at 1600 rpm using a flat blade with 288 mL 10% w/v PVA aqueous solution (± 160 mg SS), which was found to be enough PVA for emulsion stability, containing 720 mg leucine (aerosolisation enhancer), 600-920 mg lactose or 600 mg erythritol or 600 mg mannitol (employed as bulking agents). The mass of the bulking agent

dependent upon the inclusion or omission of salbutamol and BDP in the various stages of the double emulsion, and the concentration of PLGA 25:75 used. The utilisation of the polyols mannitol and erythritol as substitutes for lactose was to seek to understand the influence of the bulking agent on the properties of the formulation. Optionally SS was added to the final phase to form w/o/w double emulsions containing SS in the inner aqueous phase and/or BDP in the oil phase and/or SS in the outer aqueous phase to address the release from the various phases of the double emulsion. The emulsions were then spray-dried under standard operating conditions out lined in chapter 2 using a Büchi B-290 mini spray-drier (Büchi, Switzerland) fitted with a high performance cyclone.

4.2.2.2   *LMW chitosan w/o/w emulsion spray dried powders*

Following background work involving the visual assessment of the stability of the micro-emulsions using different emulsifying agents including dipalmitoyl phosphatidylcholine (DPPC), poloxamer 407, Synperonic PE/L101 (an alcohol ethoxylate co-block polymer), span 80 and tween 20. It was recognised that the greatest stability was given by the use of span 80 at the water-oil interface and Synperonic PE/L101 at the oil-water interface.

Powders were formulated from 2% w/v double emulsions. Primary emulsions were prepared by vortex-mixing 1 mL water (± 80 mg SS) with 0.02 mL Span 80 emulsifier in a solution of 200 mg PLGA 50:50 (added for controlled release, the increase in lactide portion an artefact of the research in the adjacent subchapter) in 3 mL chloroform (± 80 mg BDP). The primary emulsion was subsequently homogenised with 25 mL low molecular weight chitosan gel (4% w/v chitosan in 3% v/v glacial acetic acid) containing 520 - 680 mg leucine (dependent up on the drug loading in each layer) and optionally 160 mg SS.

The formulations were diluted to 100 mL with aqueous ethanol (30% v/v), ethanol used to decrease the thermal efficiency during spray drying to give a greater wrinkled appearance to the particulates in the hope of reducing aggregation, to form w/o/w emulsions containing SS in the inner aqueous phase and/or BDP in the oil phase and/or SS in the outer aqueous phase. The emulsions were then spray-dried using a Büchi B-290 mini spray-drier with high performance cyclone using the previously outlined parameters.

In a further study the viability of spray drying a four-phase o/w/o/w emulsion was explored where a combination of tween 20, span 80 and Synperonic PE/L101 surfactants appeared to give the greatest stability. The o/w/o/w treble emulsion was formulated from primary emulsions prepared by vortex-mixing 1 mL chloroform containing 60 mg BDP and 150 mg PLGA (50:50) with 0.1 mL tween 20 in a solution of 2 mL water containing 60 mg SS. The primary emulsion was added to an organic phase of 4 mL chloroform, 60 mg BDP, 150 mg PLGA (50:50) and 0.1 mL span 80 and vortex mixed. The stable double emulsion was then transferred to 37.5 mL of medium molecular weight chitosan acetate gel (4% w/v medium molecular weight chitosan in 3% v/v acetic acid) containing 0.1 mL of Synperonic PE/L101 and homogenised using a flat blade at 1600 rpm. A 25.5 mL aqueous mixture containing 660mg of leucine and 60 mg SS was then added to the emulsion followed by 30 mL of ethanol. The resulting 2% w/v o/w/o/w emulsion of 100 mL contained BDP in the oil phases and SS in the aqueous phases of the formulation. The emulsions were then spray-dried using a Büchi B-290 mini spray-drier (Büchi, Switzerland) with high performance cyclone using the previously outlined parameters.

4.2.3 Powder characterisation

The powders were characterised as described in Chapter 2, to determine spray-drying yield, drug loading, particle morphology, amorphous nature, water content and powder density. In addition, the primary aerodynamic diameter (d_{ae}) and Carr's Index values were derived and statistical analysis performed as previously described.

4.2.3.1   Particle size

Dry dispersion laser diffraction was used to assess particle size using a Sympatec HELOS particle size analyzer. For Sympatec parameters please refer to chapter 2.

4.2.4 In vitro powder aerosolisation

4.2.4.1   PVA w/o/w emulsion spray dried powders.

For the spray dried double emulsions using PVA as a stabiliser an Andersen Cascade Impactor ACI: Copley Scientific®) was used to assess aerosolisation properties. The aluminium ACI stages -1 to 6 were coated with 1% silicone oil to prevent particle bounce; the terminal F stage was employed with a Whatman® GF-A filter (Whatman®, UK). The flow rate through the ACI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific®). Powder aliquots (25 mg) were loaded into size 2 HPMC capsules (Shionogi Qualicaps) and placed into a Spinhaler® dry powder inhaler (DPI), attached to the ACI via a stainless steel USP throat. The capsule was pierced and the liberated powder drawn through the ACI at a flow rate of 60 L/min for 2 x 5 s aspirations using a pressure calibrator (Model TPK: Copley Scientific®). Under these conditions, the effective cut-off diameters are Stage -1: 8.6 μm;

Stage 0: 6.5 μm; Stage 1: 4.4 μm; Stage 2: 3.2 μm; Stage 3: 1.9 μm; Stage 4: 1.2 μm; Stage 5: 0.55 μm; Stage 6: 0.26 μm with a terminal filter stage. Each deposition experiment was performed in triplicate.

The inhaler, the throat region, each stage of the ACI and the terminal filter were then washed with 20 mL HPLC mobile phase; the aqueous portions then stored at room temperature in a UV protected environment for ten days to allow drug dissolution from the PLGA (25:75). HPLC was then used to quantify the fractions of salbutamol and BDP recovered from the inhaler, throat, stages -1 to 6 and filter of the ACI.

4.2.4.2   *LMW chitosan w/o/w emulsion spray dried powders.*

The aerodynamic character of the w/o/w emulsion spray dried powders containing LMW chitosan was assessed as in section 3.2.4.2 of the previous chapter, with HPLC detection of SS at 275 nm.

For both subsections the emitted dose and derived aerodynamic parameters were then calculated as outlined in the materials and methods section of chapter 2 with the exception that the FPF, FPD and MMAD indices were calculated as an average of the individual SS and BDP values (n=6).

4.2.5 HPLC analysis of salbutamol sulfate and BDP

SS and BDP concentration was determined using reverse-phase HPLC using a Dionex® AS50 autosampler, GP50 gradient pump and UVD 170 U UV lamp (Dionex® HPLC System: Dionex®,

UK) at room temperature using a 4.6 x 150 mm Phenomomex® La Luna column (Phenomomex®, USA) and 15 µl injection volume (UV detection: SS 275 nm, BDP 250 nm). The mobile phase (1 mL/min) consisted of 23% v/v aqueous methanol, with salbutamol eluting at 2.5 min and BDP eluting with a retention time of 5 min.

4.2.6 In vitro Dissolution

Dissolution testing was performed as outlined in the previous chapter with 3 mL samples assessed for SS and/or BDP content by UV spectroscopy (Jenway 6305 UVvis spectrophotometer: Dunmow, Essex, UK) at 275 nm and 250 nm respectively; the sample returned to the bath immediately after analysis (Shaw et al., 2005). Each dissolution experiment was performed in triplicate.

4.3   Results and discussion

4.3.1 PVA w/o/w emulsion spray dried powders

4.3.1.1   Powder characterisation

Spray dried double emulsions prepared using PVA were bright white and had a free flowing appearance. The spray-drying yield calculated as a percentage of the dry weight excluding the 10% w/v PVA stabiliser varied considerably from 8.4 to 63.1% (n=1). The large deviation in yield attributed to large depositions of spray dried material gathering at the base of the drying chamber of the spray-drying unit (Table 4.1): the material appearing as large aggregates with gravimetric forces too great to navigate the bends of the spray-dryer. One theory is that the PVA content

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separated at the point of atomisation and collected as the large aggregates seen at the base of the drying chamber, PVA invariably taking other excipients from the emulsion reducing the overall spray-drying yield. The variation in yield appeared independent of the location of the active agents' salbutamol and BDP (n=1, Table 4.2). Of the three diluents utilised lactose gave the lowest yield and erythritol the highest (n=1, Table 4.3).

Formulation	Orientation Phase (1/2/3)	PLGA (% w/w)	SD Yield (%)[b]	Water Content (%)	Particle Size (µm)	Tapped Density (g cm^{-3})	Carr's Index[a] (%)	Flowability	d_{ae} (µm)
A1	S/X/X	2.5	9.82	0.33 ± 0.57	2.04±0.07	0.21 ± 0.01	14.46	Good	0.95 ± 0.05
A2	S/X/X	5	10.2	0.31 ± 0.54	2.81±0.04	0.29 ± 0.00	20.63	Fair	1.51 ± 0.03
A3	S/X/X	7.5	11	0.58 ± 0.42	2.78±0.02	0.32 ± 0.01	16.58	Good	1.58 ± 0.03
A4	S/X/X	10	10.7	0.16 ± 0.22	1.98±0.01	0.24 ± 0.02	19.1	Fair	0.97 ± 0.05

Table 4.1: Table of the physical characteristics of PVA double emulsion spray dried powders with increasing PLGA concentration (mean ± S.D, n=3). [a] De Villiers, 2005, [b] n=1. S = salbutamol contained in phase, X = vacant phase, / = phase boundary. A4 = C1.

Formulation	Orientation Phase (1/2/3)	SD Yield (%)[b]	Water Content (%)	Particle Size (µm)	Tapped Density (g cm^{-3})	Carr's Index[a] (%)	Flowability	d_{ae} (µm)
B1	S/X/X	10.7	0.16 ± 0.22	1.98±0.01	0.24 ± 0.02	19.1	Fair	0.97 ± 0.05
B2	S/X/S	32.16	0.12 ± 0.13	2.03±0.06	0.34 ± 0.18	19.99	Fair	1.18 ± 0.03
B3	S/B/X	8.43	0.25 ± 0.43	1.68±0.01	0.34 ± 0.01	26.5	Poor, cohesive	0.98 ± 0.01
B4	X/X/S	63.14	0.25 ± 0.27	3.70±0.05	0.45 ± 0.02	16.62	Good	2.48 ± 0.02
B5	X/B/X	20.4	0.04 ± 0.07	7.60±1.12	0.19 ± 0.03	24.33	Poor, fluid	3.32 ± 0.31
B6	S/B/S	9.05	0.00 ± 0.00	7.22±0.14	0.19 ± 0.03	28.74	Poor, cohesive	3.16 ± 0.29
B7	X/B/S	21.48	0.34 ± 0.30	7.28±0.36	0.16 ± 0.06	25.16	Poor, cohesive	2.92 ± 0.60

Table 4.2: Table of the physical characteristics of PVA double emulsion spray dried powders with differing drug phase orientation (mean ± S.D, n=3). [a] De Villiers, 2005, [b] n=1. S = salbutamol contained in phase, B = BDP contained in phase, X = vacant phase, / = phase boundary.

Formulation	Orientation Phase (1/2/3)	Diluent (30% w/w ex. PVA)	SD Yield (%)[b]	Water Content (%)	Particle Size (µm)	Tapped Density (g cm^{-3})	Carr's Index[a] (%)	Flowability	d_{ae} (µm)
C1	S/X/X	Lactose	10.7	0.16 ± 0.22	1.98±0.01	0.24 ± 0.02	19.1	Fair	0.97 ± 0.05
C2	S/X/X	Mannitol	40.1	0.36 ± 0.54	7.27±0.38	0.17 ± 0.01	26.13	Poor, cohesive	3.03 ± 0.18
C3	S/X/X	Erythritol	44.3	0.59 ± 0.52	6.93±0.15	0.17 ± 0.00	21.11	Poor, fluid	2.83 ± 0.09

Table 4.3: Table of the physical characteristics of PVA double emulsion spray dried powders with differing diluent (mean ± S.D, n=3). [a] De Villiers, 2005, [b] n=1. S = salbutamol contained in phase, X = vacant phase, / = phase boundary. A4 = C1.

Analysis of combined drug yields revealed large variations in the amount of salbutamol (27.6 to 116.9% of the anticipated recovery) and BDP detected (50.7 to 55.9%). The large range in salbutamol dose possibly a by-product of SS leaching by the polar PVA particulates gathered at the base of the spray-drying chamber. The low recovery of BDP possibly due to the poor solubility of the corticosteroid in 23% v/v aqueous methanol during the ten day storage period. BDP was possibly retained in the more hydrophobic PLGA atmosphere rather than travelling down the concentration gradient in to the 50 mL 23% v/v aqueous methanol HPLC analysis media.

SEM imaging revealed smooth surfaced microspheres, of 0.25 to 20 μm plus in diameter, some of the particles contained in all the samples imaged had a dimpled appearance (Figure 4.1). The dimpled appearance could just be an artefact of SEM imagery due to the vacuum conditions required causing microsphere collapse, but could however also be a result of the spray drying procedure and a shift in the thermal efficiency of the system with the inclusion of PVA. PVA could migrate to air liquid interface of the droplet liberated from the atomiser during spray drying, the reduced surface tension due to the plasticiser effect of PVA allowing higher penetration of inlet heat into the droplet, resulting in "over-cooking" of the droplet and giving a dimpled or veined like appearance (Figure 4.1 C) to individual microspheres when viewed through SEM (Masters, 1991; Chow et al., 2007). The mannitol containing formulation (C2, Figure 4.1 C) showed slightly larger microspheres in comparison with a veined rough surface texture possibly indicating mannitol presence in the final formulation. The spherical shape and smooth surfaces of the microspheres viewed in the SEM images are indicative of amorphous samples as has been

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indicated previously. The objects near the centre of Figure 4.1 D are assumed debris un-associated with the addition of erythritol to the double emulsion format.
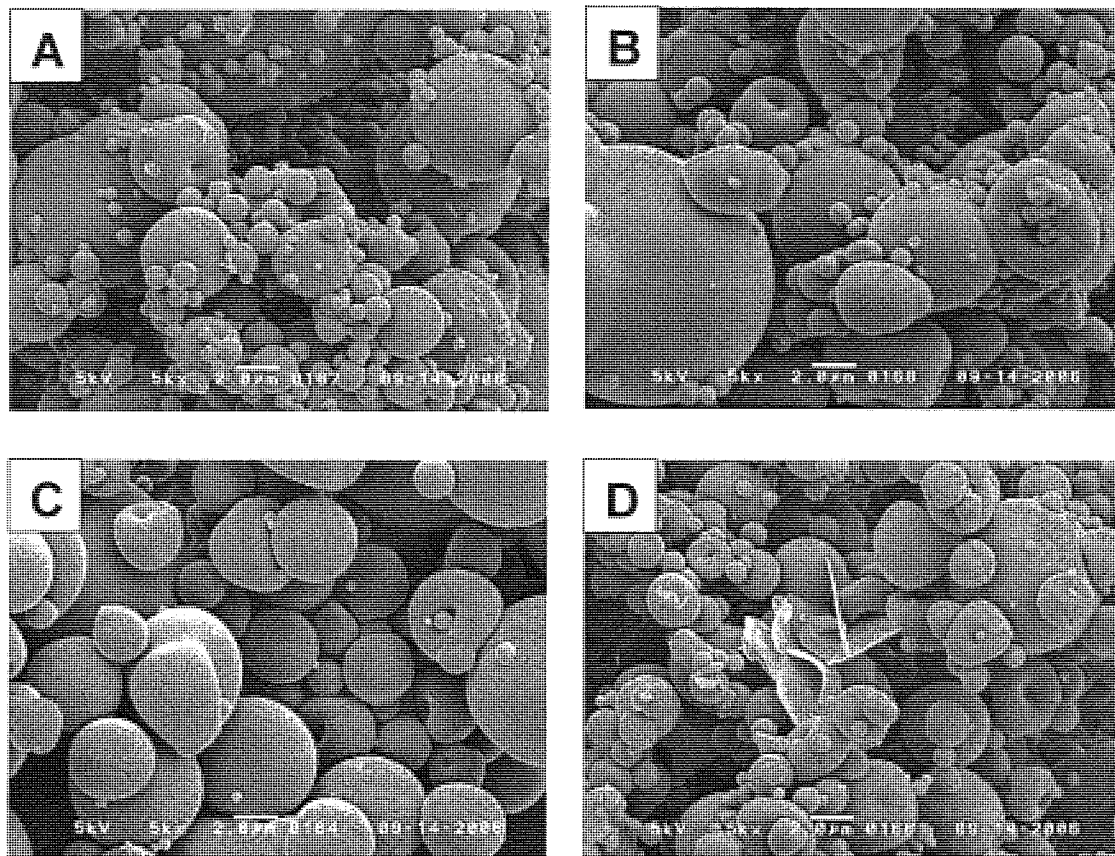
Figure 4.1: Representative scanning electron micrographs of PVA spray dried double emulsions at 5000x magnification: A. B6 (s/b/s); B. B7 (x/b/s); C. C2 (s/x/x: containing mannitol); D. C3 (s/x/x: containing erythritol).

Differential scanning calorimetery revealed all spray dried formulations produced using PVA as an emulsion stabiliser as amorphous as is expected from spray dried blends capable of molecular dispersion of various components. Only one trace (that of B1: Table 4.2) produced any peaks, the fusion peak at 215- 220°C associated with the fusion of PVA in the tertiary phase (Figure 4.2: Sanli et al., 2006). The peak was only considered a partial recrystallisation as the peak from the spray dried sample didn't have the intensity of the endotherm produced by fusion in the crystalline blend. The sample may not display molecular dispersity in the outermost part of the formulation due to the large proportion of PVA used in the original double emulsion allowing

homogeneous neighbouring particles to fuse at around 218°C and represent an endothermic event on the DSC spectra (Aulton, 1988; Sanli et al., 2006). Negligible water content within the spray dried formulations independent of mannitol and erythritol inclusion or drug phase location in the original double emulsion of 0 to 0.59% was revealed by thermogravmetric analysis (Table 4.1, 4.2 and 4.3).
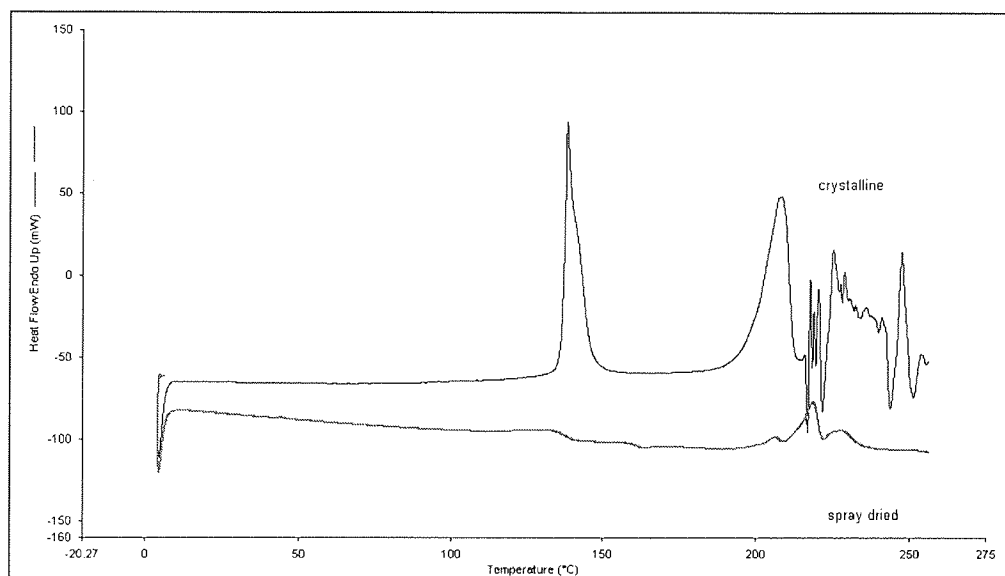
Figure 4.2: Differential scanning calorimetery trace of crystalline (upper trace) and spray dried formulation B1, containing salbutamol in the primary phase of the spray dried emulsion with vacant secondary and tertiary phases.

Laser diffraction data (LD) are presented in tables 4.1, 4.2 and 4.3; dry powder laser diffraction using Sympatec sizing apparatus portrayed the size range between the formulations as being 1.7 to 7.6 µm in diameter. No difference in LD size was noticed as a result of PLGA concentration (Table 4.1) or drug phase location in the original double emulsion (Table 4.2). However, the mannitol containing formulation (7.2 µm) and the erythritol containing formulation (6.9 µm) gave higher laser diffraction volume mean diameters compared to the lactose containing formulations (Table 4.3). The difference in diameter of the particulates of the lactose containing formulations

recorded by the Sympatec particle sizer are attributed to storage time, with B5, B6 and B7 formulations being among the first PVA spray dried formulations to be produced (Table 4.2).

The tapped densities of the spray dried PVA formulations ranged from 0.16 to 0.45 gcm^{-3}, with the mannitol and erythritol containing PVA formulations (C2 and C3; Table 4.3) having generally lower tapped densities compared to their lactose counterparts (μ= 0.17 gcm^{-3}), indicating a larger degree of porosity in the lactose blends. No trend between tapped density and drug phase location was correlated (Table 4.2).

The theoretical primary aerodynamic diameter (d_{ae}) of each formulation was calculated in triplicate, the sizes ranging from 0.95 to 3.32 μm. The reduction in particle size when theoretical and laser diffraction diameters are compared indicate that aggregation was responsible for the larger particle sizes seen in the Sympatec data. The forces of inter-particulate attraction being greater than the 1 bar pressure exerted on the formulations in the Sympatec Helos$^{®}$ device preventing adequate dispersion of the spray dried formulations to assess individual particle diameters (Table 4.1 - 4.3). If the spray dried formulations were to act as individual primary particles then the spray dried formulations should be highly respirable and reach the mid to deep lung generations (Atkins, 2005).

The utilisation of Carr's index has so far proved to be a poor indicator of a spray dried formulation's aerosolisation properties. Again there was little correlation seen between the index values and the observed visual flow through out the PVA emulsion spray dried series (Table 4.1 - 4.3). Phase location of both salbutamol and BDP, concentration of PLGA and the inclusion of

polyols in to spray dried emulsion formulations appeared to have no effect on the respective Carr's index values.

4.3.1.2　　　In vitro powder aerosolisation

Formulation	Orientation Phase (1/2/3)	PLGA (% w/w)	Emitted dose %	FPF (F < 5 μm) % av.	MMAD (μm av.)
A1	S/X/X	2.5	41.87 ± 3.58	14.73 ± 4.61	3.19 ± 0.98
A2	S/X/X	5	96.74 ± 3.28	31.94 ± 3.47	4.14 ± 0.42
A3	S/X/X	7.5	97.40 ± 0.20	30.43 ± 2.01	4.18 ± 0.43
A4	S/X/X	10	86.96 ± 3.38	35.00 ± 2.17	4.89 ± 0.59

Table 4.4: Table of the aerodynamic characteristics of PVA double emulsion spray dried powders with increasing PLGA concentration (mean ± S.D, n=3).

Formulation	Orientation Phase (1/2/3)	Emitted dose %	FPF (F < 5 μm) % av.	MMAD (μm av.)
B1	S/X/X	86.96 ± 3.38	35.00 ± 2.17	4.89 ± 0.59
B2	S/X/S	93.24 ± 2.25	39.33 ± 2.36	5.05 ± 0.40
B3	S/B/X	78.83 ± 8.11	39.31 ± 4.23	2.98 ± 0.28
B4	X/X/S	94.43 ± 4.80	42.99 ± 1.88	3.77 ± 0.03
B5	X/B/X	83.40 ± 18.66	50.15 ± 8.08	2.77 ± 0.18
B6	S/B/S	87.07 ± 13.12	48.78 ± 8.12	2.89 ± 0.43
B7	X/B/S	92.33 ± 6.99	54.58 ± 3.52	2.89 ± 0.28

Table 4.5: Table of the aerodynamic characteristics of PVA double emulsion spray dried powders with differing drug phase orientation (mean ± S.D, n=3).

Formulation	Orientation Phase (1/2/3)	Diluent (30% w/w ex. PVA)	Emitted dose %	FPF (F < 5 μm) % av.	MMAD (μm av.)
C1	S/X/X	Lactose	86.96 ± 3.38	35.00 ± 2.17	4.89 ± 0.59
C2	S/X/X	Mannitol	84.93 ± 19.80	20.44 ± 4.24	5.79 ± 0.58
C3	S/X/X	Erythritol	74.53 ± 32.42	25.15 ± 10.22	3.93 ± 1.27

Table 4.6: Table of the aerodynamic characteristics of PVA double emulsion spray dried powders with differing diluent utilised in the 3° phase (mean ± S.D, n=3).

The ED, FPF and MMAD of the PVA spray-dried powders are displayed in tables 4.4, 4.4 and 4.6. The dispersibility of the PVA based spray dried powders varied independent of salbutamol and BDP phase location and inclusion. The gravimetrically determined emitted dose ranging from 41.9 to 97.4% of total capsule contents, with PLGA concentration playing an important role;

the formulation containing only 2.5% w/w PLGA in the original double emulsion (A1) giving over a 35% reduction in FPF compared to other lactose containing formulations contained in tables 4.4 and 4.5. The large range in ED could theoretically have beeen a consequence of varying amounts of leucine either being leached from the formulation by, or masked by, the large amount of PVA included in the original formulation (amounting to 93.5% of the total dry weight of the formulation to be spray dried). It would be hopeful to insist that all the PVA would not reach the high performance cyclone collection chamber and that the correct proportions of other excipients would navigate the air current of the spray drier and be part of the recovered product. The erythritol formulation (C3) showed a relatively poor emitted dose of 74.5% compared to the lactose and mannitol formulations (Table 4.6).

The ACI deposition pattern remained constant for all formulations with a large percentage deposition on the higher stages of the ACI, stages 0 and 1 particularly (data not shown). No difference was found between the deposition patterns of salbutamol and BDP in the spray dried formulations containing both entities (students matched paired t-test; $P > 0.1$, non-significant: B3, B6, B7). The deposition patterns of salbutamol and BDP in the ACI indicative of homogeneous spray dried formulations.

The fine particle fraction (F <5 μm) of all formulations tested varied largely from 14.7 to 54.6% and was dependent up on a number of factors (Tables 4.4 - 4.6). Firstly a decrease in PLGA concentration from 10% w/w to 2.5% w/w of a formulation with vacant secondary and tertiary phases revealed a decrease in FPF from 35.0 to 14.7% (Table 4.4), although the 5 and 7.5% w/w formulations did not follow suit (Table 4.4: A2 and A3 respectively). In fact the 2.5% w/w

PLGA powders' (A1) inefficient dispersion (emitted dose: 41.9%) appears to have been the key factor in the formulations poor *in vitro* aerosolisation performance.

The location of agents proved an important factor in aerosolisation behaviour. Surprisingly relatively high FPF's were given by formulations containing salbutamol in the tertiary stage of the emulsion (Table 4.5: B2, B4, B6 and B7). It would be expected that greater polarity would be caused by the inclusion of the β-agonist in the final phase increasing inter-particulate forces through hydrogen bonding, giving increased aggregation with in the formulation. Similar particulate surfaces caused by the presence of salbutamol appear to give homo-repulsion in the case of these formulations. The addition of BDP in the organic intermediate phase of the original emulsion to be spray dried also appeared to be beneficial in increasing the FPF of the formulations, although BDP was not intended to migrate to the surface of the microspheres (Table 4.5: B3, B5, B6 and B7).

The inclusion of polyols is seen as an important step in improving the stability, shelf life and ultimately the *in vivo* deposition of typical DPI products. The utilisation of synthetic polyols, such as mannitol and erythritol, as a substitute for the diluent lactose giving an increase in the FPF of micronised actives in studies incorporating a more traditional approach to DPI manufacture (Chow et al., 2007). The effect of polyol replacement of lactose on the performance of the PVA spray dried emulsions was assessed as a sideline study. An overall decrease in the FPF of the PVA formulations from an average FPF of 35.0% for the lactose containing formulations to 20.4% and 25.2% for mannitol and erythritol respectively was observed (Table 4.6). These results were in contrast to results obtained in other research groups utilising large crystalline

polyol carrier particles to enhance micronised drug particle deposition (Chow et al., 2007). The decrease in aerosolisation property with the use of polyols over lactose is attributed to increased particle density as denser particulates have poorer aerodynamics than less dense particles of a similar size (Chew et al., 2005).

The mass median aerodynamic diameter (MMAD) of the PVA formulations ranged from 2.77 to 5.79 µm (Table 4.4 – 4.6). The values proved to be an intermediate of the smaller theoretical primary particle diameters and the larger dry dispersion laser diffraction sizes obtained previously (Table 4.1 – 4.3). This indicates that a degree of aggregation was affecting the *in vitro* ACI performance of the spray dried PVA powders and that some interparticulate forces were greater than the force of the de-aggregational actions exerted by the 60 Lmin^{-1} air flow and the baffles of the Spinhaler® propeller during aerosolisation (Figure 1.7D).

The reduction in aerosolisation performance of the PVA powders compared to those seen in the previous chapters can not be attributed to spray drying conditions which have remained constant throughout the production of all the spray dried powders. The reduction in performance would therefore point to the erratic behaviour of the high concentration PVA solution used to stabilise the original double emulsions and its effect on the aerosolisation enhancer l-leucine.

4.3.1.3   In vitro dissolution testing

Despite the disappearance of all contents from the rotation of the wire mesh baskets during the seven-day dissolution-testing period 100% of the expected drug load was never retrieved with a recovery of 17.3 to 79.2% of the salbutamol drug loading and 79.1 to 93.2% of BDP drug loading (Table 4.7, 4.8 and 4.9). The reduction in recovery during dissolution may be attributed to HPLC

dilution factor as samples are removed but is more likely to be attributed to the PVA effects during spray drying diluting active content from the expected 8% w/w per agent. The release profile was dependent upon the concentration of PLGA 75:25 with an increase in the duration of release of salbutamol from 4 to 6 days with an increase of 2.5 to 10% w/w (Table 4.7).

Phase location in the original double emulsion gave large variations in the release pattern. The addition of salbutamol into the primary aqueous phase and BDP into the secondary organic phase gave a sustained release profile of four days plus, whereas the addition of salbutamol in to the tertiary phase gave burst release characteristics. The difference in the release characteristics can be seen in formulation A4/B1; which contains only salbutamol in the primary phase with the organic secondary and the aqueous tertiary phases vacant (Figure 4.3A), formulation B5 which contains only BDP in the organic phase (Figure 4.3B) and formulation B4 which contains only salbutamol in the tertiary outer layer (Figure 4.3C).

Formulation	Orientation Phase (1/2/3)	PLGA (% w/w)	t max (days)	zero order		1st order		Higuchi matrix		Recovery %
				t (days)	r^2	t (days)	r^2	t (days)	r^2	
A1	S/x/x	2.5	4	0 - 4.00	0.9557	0 - 4	0.8375	0 - 4	0.8964	56.99
A2	S/x/x	5.0	4	0 - 4.00	0.9522	0 - 6	0.9827	0 - 4	0.8791	70.52
A3	S/x/x	7.5	6	0 - 6.00	0.9697	0 - 6	0.8077	0 - 6	0.9452	76.53
A4	S/x/x	10.0	6	0 - 0.02	0.9871	0 - 6	0.8977	0 - 6	0.8271	78.84

Table 4.7: Table showing the dissolution characteristics of SS release from PVA double emulsion spray dried powders with increasing PLGA concentration. Figures are those of closest fit, A4 = C1.

Formulation	Orientation Phase (1/2/3)	*t* max (days)	zero order *t* (days)	r²	1st order *t* (days)	r²	Higuchi matrix *t* (days)	r²	Recovery %
B1	S/x/x	6.00	0 - 0.02	0.9871	0 - 6.00	0.8977	0 - 6.00	0.8271	78.84
B2	S/x/S	6.00	0 - 5.00	0.9703	0 - 6.00	0.9778	0 - 6.00	0.9495	68.50
B3 b*	s/B/x	4.00	0 - 4.00	0.9357	0 - 4.00	0.8938	0 - 4.00	0.9479	88.89
B3 s*	S/b/x	4.00	0 - 4.00	0.9060	0 - 4.00	0.9854	0 - 4.00	0.9636	71.80
B4	x/x/S	0.02	0 - 0.02	0.9299	0 - 2.00	0.8561	0 - 1.50	0.9918	45.01
B5	x/B/x	5.00	0 - 5.00	0.9774	0 - 5.00	0.9264	0 - 5.00	0.9435	93.19
B6 b*	s/B/s	5.00	0 - 5.00	0.9531	0 - 5.00	0.9437	0 - 5.00	0.9643	79.10
B6 s*	S/b/S	5.00	0 - 5.00	0.7426	0 - 4.00	0.8912	0 - 3.00	0.8850	79.23
B7 b*	x/B/s	5.00	0 - 5.00	0.9413	0 - 4.00	0.9671	0 - 4.00	0.9642	91.47
B7 s*	x/b/S	0.01	0 - 0.01	0.9696	0 - 0.12	0.9285	0 - 0.13	0.8921	70.61

Table 4.8: Table showing the dissolution characteristics of PVA double emulsion spray dried powders with differing drug phase orientation; s* indicates the release of SS from a dual loaded formulation, b* indicates BDP release from a dual loaded spray dried formulation. Figures are those of closest fit.

Formulation	Orientation Phase (1/2/3)	*t* max (days)	zero order *t* (days)	r²	1st order *t* (days)	r²	Higuchi matrix *t* (days)	r²	Recovery %
C1	S/x/x	6	0 - 0.02	0.9871	0 - 6	0.8977	0 - 6	0.8271	78.84
C2	S/x/x	5	0 - 5.00	0.7823	0 - 5	0.7891	0 - 5	0.8983	20.13
C3	S/x/x	4	0 - 2.00	0.7053	0 - 4	0.7810	0 - 4	0.7401	17.31

Table 4.9: Table showing the dissolution characteristics of SS release from PVA double emulsion spray dried powders with differing diluent utilised in the 3° phase. Figures are those of closest fit, A4 = C1.
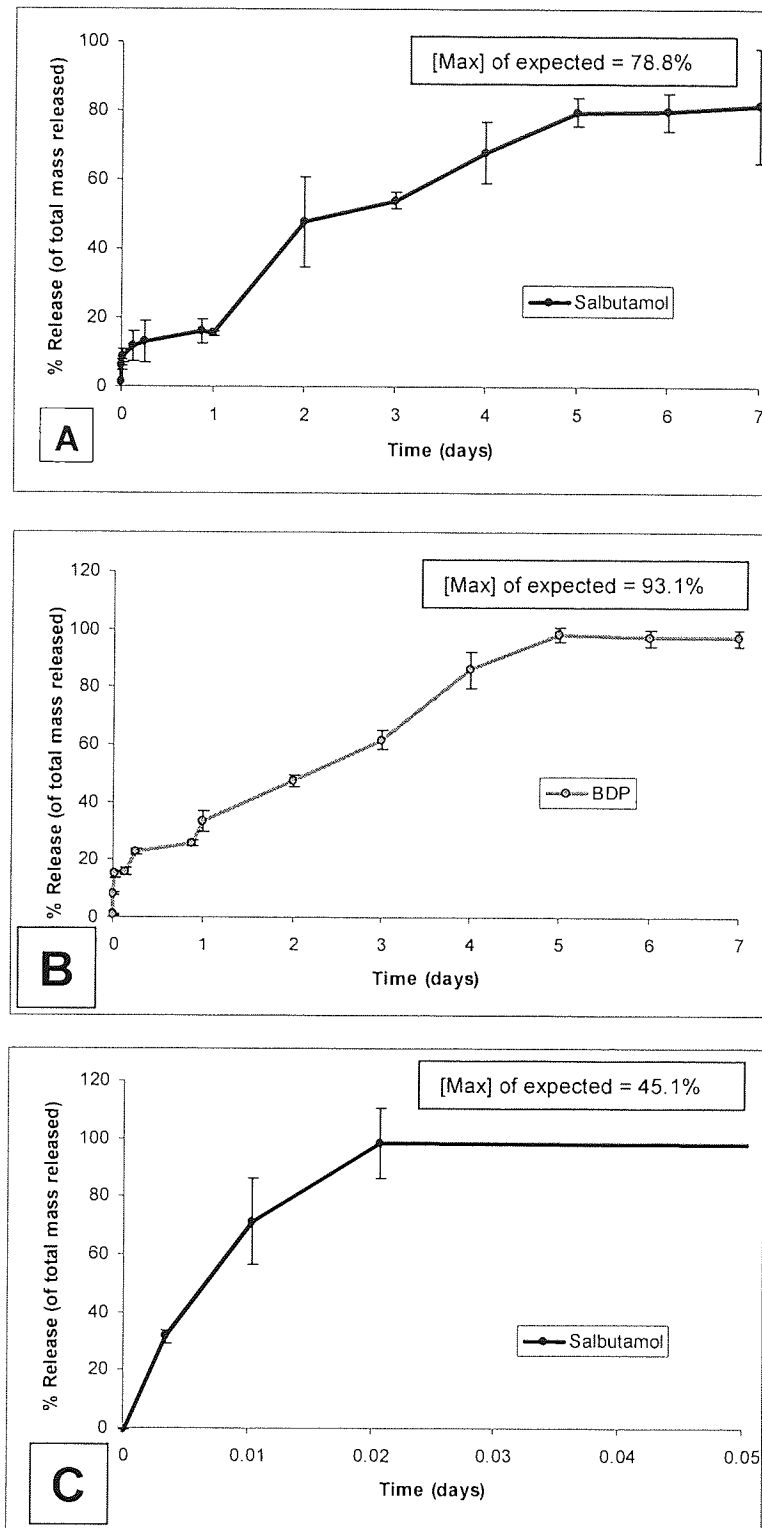
Figure 4.3: Dissolution profiles of 200mg PVA double emulsion spray dried powders theoretically containing 10% PLGA 25:75 (1). A. Formulation B1, containing SS in the primary phase of the double emulsion; B. B5, containing BDP in the secondary phase of the double emulsion; C. B4, containing SS in the tertiary layer of the double emulsion. The solute eluting into 1000 mL PBS at 37°C from a rotating basket at 50 RPM (n=3).

The dual release of agents from different phases of the original double emulsion supports the hypothesis of a possible layered structure to individual spray dried microspheres (Figure 4.4). Figure 4.5A demonstrates the sustained release of both SS from the primary phase and BDP from the secondary phase of formulation B3 (Table 4.8). The sustained release of the SS fraction indicates that the primary phase solute remains suspended inside the secondary phase PLGA 75:25 layer post spray drying. Figure 4.5B illustrates the release of B6 (Table 4.8); the burst release of SS from the tertiary layer combined with sustained release of SS and BDP from the PLGA mediated primary and secondary phases respectively. Finally, Figure 4.5C shows the burst release of salbutamol from the tertiary layer of B7 followed by the sustained release of BDP from the secondary organic layer.
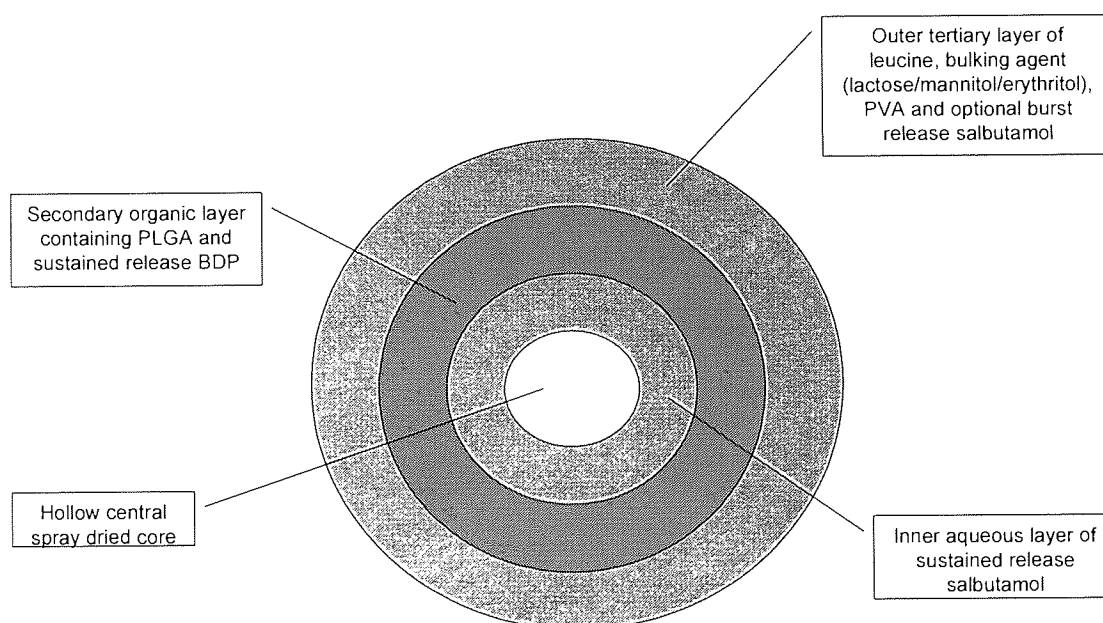
Figure 4.4: A cross-section of the hypothetical structure of a microsphere produced by spray drying a double emulsion incorporating the polymer PLGA and the suspending agent PVA.

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The sustained release of agents in the PLGA/PVA emulsion system appears to be dependent upon the location of agents either inside or in the same phase as the PLGA 75:25. The ability of a formulation to show dual release rates in the form of a burst release followed by a sustained release over the days post administration is of significance, as acute exacerbation can then be treated by the higher initial burst release followed by a sustained release period of agents giving possible prophylaxis. When the dissolution profile data of salbutamol release from spray dried formulations B2 and B6 are bisected into the initial burst and secondary sustained periods a distinct change from zero order to first order kinetics can be extrapolated (Table 4.8). The initial burst following a zero order kinetic, as the salbutamol has no impediment from following the concentration gradient into the PBS dissolution media and the secondary sustained release period following a first order release kinetic, where PLGA 75:25 polymer erosion and not drug concentration is the limiting factor.
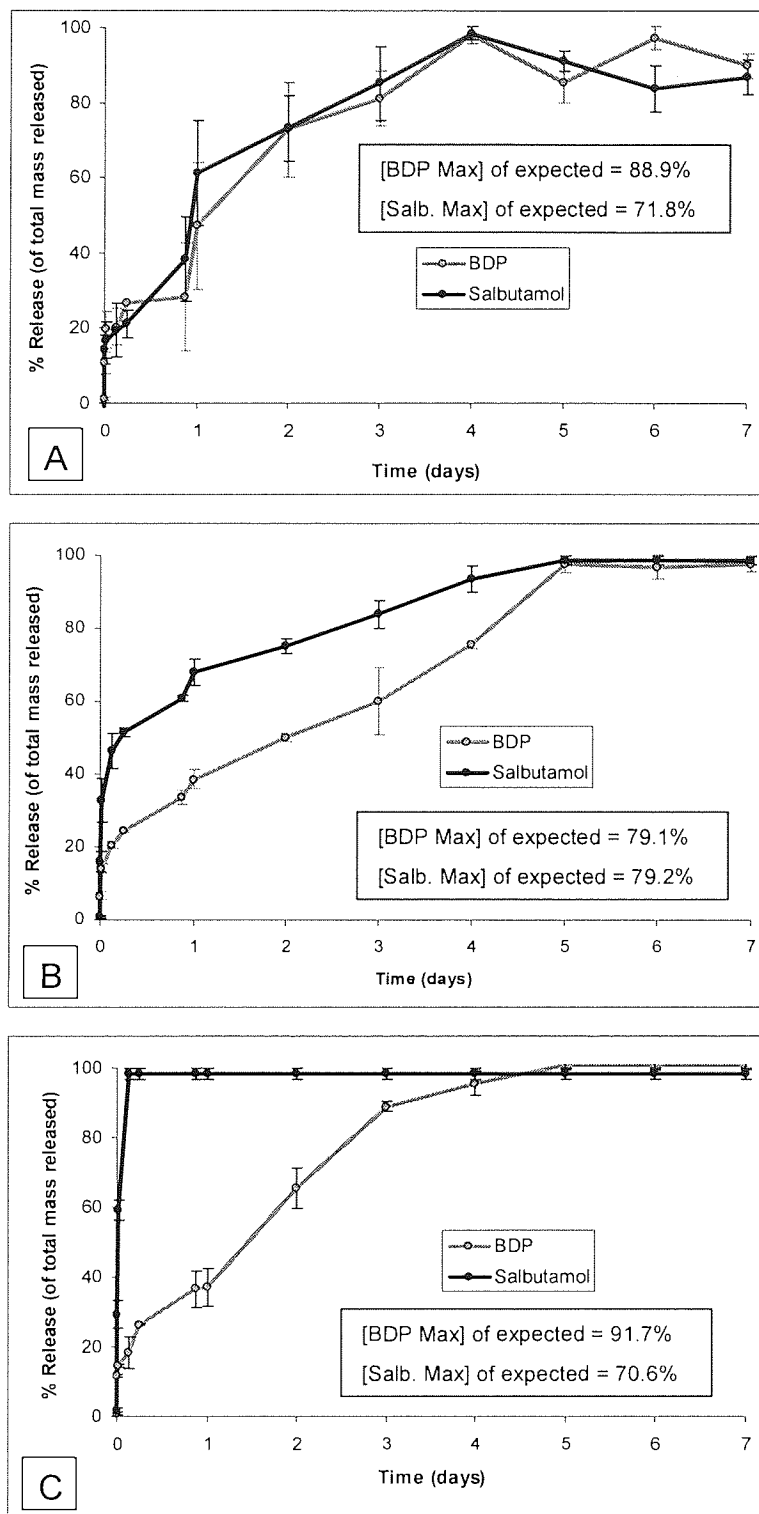
Figure 4.5: Dissolution profiles of 200mg spray dried powders theoretically containing 10% PLGA 25:75 (2). A. Formulation B3, containing SS in the primary phase of the double emulsion and BDP in the secondary phase, S/B/X; B. B6, containing SS in the primary and tertiary phases and BDP in the secondary phase of the double emulsion, S/B/S; C. B7, containing BDP in the secondary layer and SS in the tertiary layer of the double emulsion; X/B/S. The active agents eluting into 1000 mL PBS at 37°C from a rotating basket at 50 RPM (n=3).

PLGA concentration appeared to have little effect on mechanism of release from the spray dried PVA double emulsions. The only difference was a minor increase in the correlation of zero order kinetics with increasing PLGA 25:75 concentration, the increase perhaps due to better partition of the SS in the primary phase, resulting in greater definition between zero and first order kinetics (Table 4.7).

All the formulations with salbutamol and BDP residing in the primary aqueous and secondary organic phases respectively showed good correlation (r^2 0.7810 - 0.9854) with first order kinetics (Figures 4.9 -4.11); the first order kinetic indicative of sustained release from erosion systems such as those incorporating PLGA 25:75. Surprisingly, the Higuchi homogeneous matrix kinetic showed good correlation with the release profiles produced by the PLGA/PVA spray dried double emulsions (r^2 0.7401 - 0.9918), although the fit was not as exacting as those seen with the spray dried chitosan powders (r^2 0.7713 - 0.9996). The results indicating possible matrix style release despite PLGA release being erosion mediated (Blanco et al., 2006).

Evaluation of the utilisation of the polyols mannitol and erythritol in a controlled release system highlighted a reduction in the initial burst release zero order kinetic of SS from the primary phase compared to the lactose formulation (Table 4.9). The reduction in zero order release with the addition of polyols (Table 4.9), potentially related to differing solubility's compared to lactose (Sugimoto et al., 2006), or to the denser spray dried particulates they produced.

This sub chapter has dealt with the use of complex double emulsions in the production of spray-dried powders to produce respirable sustained release particulates. Although the PLGA/PVA methodology did not have the superior aerosolisation properties of the chitosan spray dried

powders seen in the previous chapter the average FPF was still comparable to marketed

products (F< 5 μm: PVA 36.1%, Seretide discus® 18.2%; Taki et al., 2006). However, the use of

PLGA 25:75 in a double emulsion method proved to give greater sustained release property to

spray dried formulations compared to the chitosan based formulations explored in the previous

chapter. With dual release profiles attainable through the locating of agents in different phases of

the double emulsion a further advantage. The use of high concentration PVA as a stabiliser for

the double emulsions proved to be the major draw back as drug loading showed great variation

and the activity of leucine was greatly reduced. To limit the variance in drug loading and

excipient ratios a set mass in volume needs to be set and low concentrations of emulsifying

agents need to be employed to limit their role in the properties of the final spray dried product.

4.3.2 LMW chitosan w/o/w emulsion spray dried powders

As the double emulsion method showed some promising results a series of double emulsions

using chitosan gel as the suspending agent in the tertiary layer were formulated. Low molecular

weight chitosan was selected due to its high performance in previous *in vitro* aerosolisation

performance testing as detailed in Chapter three, the emphasis of chitosan inclusion changing

from controlled release to potential bio-adhesion and emulsion stabilisation. A total of 7

formulations where investigated, powders appeared beige in appearance with visibly good flow

and minor aggregation. Spray drying yield varied considerably with dry yields in excess of 69%

(Table 4.10). Overall, the highest yields were generated from formulations with no salbutamol in

the tertiary layer of the emulsion, the highest yield generated by formulation D4 (phases

occupied in the original micro-emulsion: inner aqueous primary/secondary organic/outer aqueous

tertiary: salbutamol sulfate/ beclometasone dipropionate/ vacant: s/b/x) and the smallest yield by

formulation D3 (vacant/ vacant/ salbutamol: x/x/s).

4.3.2.1   Powder characterisation

Formulation	Orientation	SD Yield	Water Content	Particle Size	Tapped Density	Carr's Index[a]		d_{ae}	MMAD
		(%)[b]	(%)	(µm)	(g cm^{-3})	(%)	Flowability	(µm)	(µm)
D1	s/x/x	66.0	2.03 ± 0.62	6.48 ± 0.13	0.19 ± 0.01	12.34	Good	2.81 ± 0.09	2.23 ± 0.23
D2	x/b/x	41.9	1.21 ± 0.19	6.77 ± 0.01	0.22 ± 0.01	15.58	Good	3.21 ± 0.05	1.84 ± 0.24
D3	x/x/s	36.1	1.24 ± 0.15	6.63 ± 0.62	0.21 ± 0.03	18.84	Fair	3.04 ± 0.45	2.71 ± 0.41
D4	s/b/x	69.4	1.43 ± 0.35	6.00 ± 0.21	0.17 ± 0.00	16.67	Good	2.50 ± 0.09	2.67 ± 0.44
D5	x/b/s	51.1	1.44 ± 0.47	6.67 ± 0.84	0.17 ± 0.00	6.66	Excellent	2.77 ± 0.35	2.32 ± 0.54
D6	s/x/s	47.9	2.02 ± 0.35	7.41 ± 0.20	0.25 ± 0.00	16.67	Good	3.72 ± 0.10	2.77 ± 0.30
D7	s/b/s	42.8	1.81 ± 0.16	5.83 ± 0.69	0.18 ± 0.01	13.28	Good	2.47 ± 0.33	2.67 ± 0.37

Table 4.10: Table showing the physical characteristics of chitosan emulsion spray dried powders, i.e. spray drying yield (SD yield), water content, particle size, tapped density, Carr's index, theoretical particle size (d_{ae}) and Mass Median Aerodynamic Diameter (MMAD) (mean ± S.D, n=3). [a] De Villiers, 2005, [b] n=1.

The SS and BDP loading of the formulations were difficult to quantify due to the slow release of

active agents from within the polylactide-co-glycolide (PLGA) 50:50 GRAS (generally regarded

as safe) polymer (Figure 4.6). SS drug loading varied considerably from 30 - 97%. With the

lowest drug loading from the formulation containing SS in the innermost layer of the original

emulsion (s/x/x) and the highest percentage loaded dose (with in 5% of the expected SS loaded

dose), recovered from the formulation with BDP in the intermediate phase and SS in the

outermost layer (x/b/s). SS recovery was significantly higher in the formulations containing SS in

the external tertiary layer (ANOVA/Tukey, P <0.05: Figure 4.6). The low SS drug loading is not

thought to be attributed to drug degradation or leaching during the spray drying process (Chew et

al., 2005).

BDP drug loading varied but not with the variance of the SS drug loading, ranging between 33.1% and 55.6% of the anticipated load (Figure 4.6). The presence of BDP in a secondary layer containing PLGA 50:50 could be a reason for poor recovery, PLGA 50:50 is known to give the slow release of active agents and spray drying gives a homogeneous molecular dispersion of agents in solution (Blanco et al., 2006; Schnieders et al., 2006; Cevher et al., 2006). The slow release from PLGA 50:50 in aqueous environments suggests that the two week release period in to an aqueous environment (23% aqueous methanol) to determine drug loading was not sufficient to determine the true formulation drug loadings. With around 30% detection from the primary phase, around 60% recovery from the secondary phase and around 100% recovery from the outer tertiary phase at this time point.
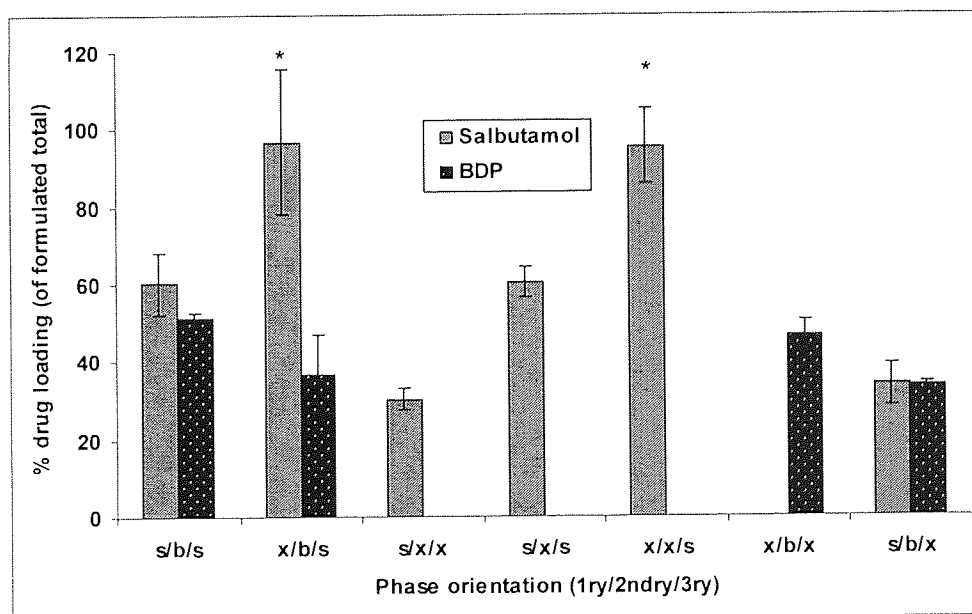
Figure 4.6: Bar chart of the drug loading of SS (grey bars) and BDP (black bars and white dots) in the chitosan emulsion spray dried powders as a percentage of the maximum expected dose based on the original liquid formulation (mean ± S.D, n=3). S = SS, B= BDP, /= phase separation. *Statistically different to the other salbutamol drug loadings (ANOVA, Tukey-kramer post hoc, P <0.05; significant).

Scanning electron microscopy (SEM) indicated that the formulations comprised of hollow microspheres 0.5 – 5 μm in diameter with differing surface textures (Figure 4.7). Some microspheres appearing to have a rough surface texture and be covered with flakes whilst others were smooth in appearance. The composition of the spray dried powders didn't appear to affect the size or surface morphology of the microspheres. The surface morphology most likely governed by the migration of leucine and any residual Synperonic® PE/L101 surfactant (from the oil/water interface) residing in the tertiary phase of the original liquid double emulsion formulation to the liberated droplet surface; potential migration taking place pre and post atomiser (Rabbani & Seville, 2005; Li et al., 2005a). As previously explained surfactant presence can alter the thermal efficiency of a spray drying system and give a pitted physical surface to the dry yield.
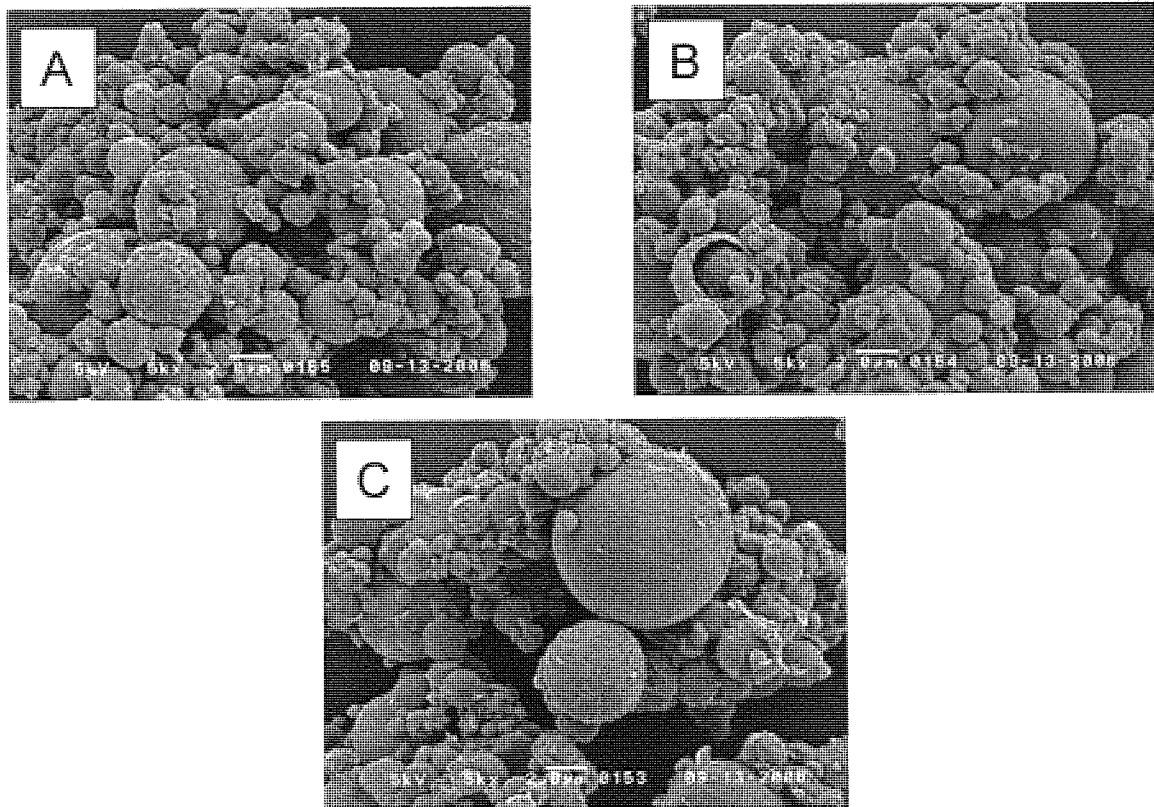
Figure 4.7: Representative scanning electron micrographs at 5000x magnification of spray dried chitosan double emulsion formulations: A. D4 (s/b/s); B. D5 (x/b/s); C. D7 (s/b/x).

Differential scanning calorimetery (DSC: Figure 4.8) gave relatively flat traces for the spray dried

formulations when compared to their crystalline counterparts. This indicates that the spray-dried

powders are amorphous in nature and are stable in this form for at least one month after spray

drying, the period of storage before examination. The exotherm at 240°C was attributed to pan

movement rather than a thermal event when compared to the crystalline trace which displayed

endothermic crystalline fusion peaks of chitosan at c.90°C, and leucine, salbutamol and BDP at

200°C and above (n=3; Aiedeh et al., 2006; Murphy et al., 2005; Brodka-Pfeiffer et al., 2003;

Hyvonen et al., 2005).
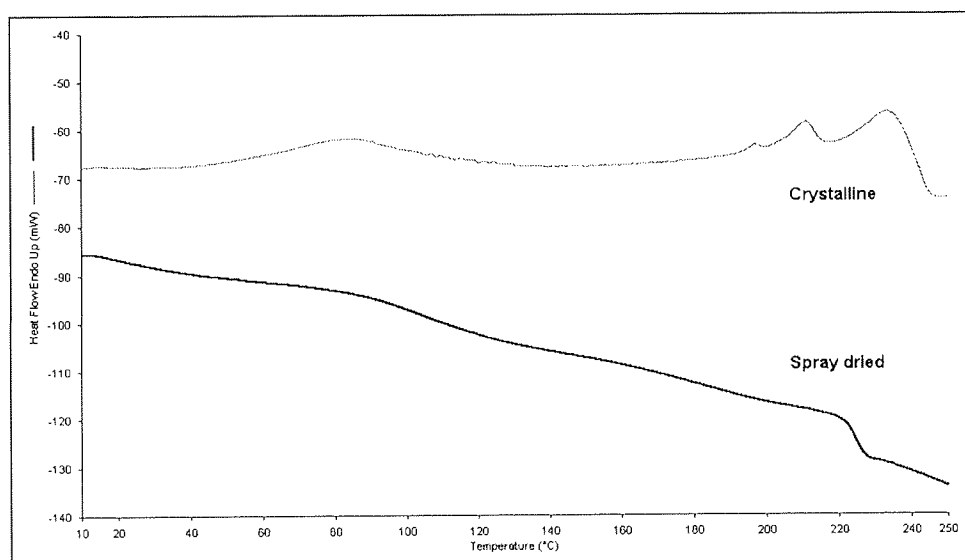
Figure 4.8: Differential scanning calorimetry trace of spray dried and crystalline blends containing 8 % w/w SS, 4% w/w BDP, 50% w/w LMW chitosan, 10% w/w PLGA 50:50 (mw 40,000 to 70,000 kDa) and 28% w/w leucine (D7).

Thermogravimeteric analysis (TGA) was used to assess the water content of the spray-dried

powders as a percentage of the dry weight. The water content ranged from 1.21 to 2.03% (Table

4.10), powders containing the highly hydrophobic drug BDP had the lowest water contents

possibly due to more water being driven from the increased hydrophobicity of the droplet/dried

particle during spray drying.

Dry powder dispersion laser diffraction evaluated by Sympatec revealed the spray dried powders to have a volume mean diameter (VMD) of 5.8 -7.4 μm at 1 bar pressure (Table 4.10). Particles of this size would be regarded as being sufficiently small as to have a reduced tendancy to impact on the oropharangeal cavity (Larhrib et al., 2003). The larger mean diameter gathered by the Sympatec system in comparison to the SEM images indicates that the 1 bar pressure used during laser diffraction sizing was not enough to overcome inter-particulate forces and break up aggregates. Particle size appeared to be independent of the location of salbutamol and presence of BDP (Table 4.10).

Theoretical particle size (d_{ae}), as described in the methods section, is derived from the laser diffraction diameter and tapped density of a formulation to give an indication of the actual size of any microspheres in a formulation and the cohesive behaviour of a formulation. Theoretical diameters ranged between 2.5 and 3.7 μm, with the size being independent of drug location with in the formulations three phases (Table 4.10). The particle sizes are regarded as small enough to reach the target deep and mid areas of the lung giving minimal impaction on the oropharynx (Larhrib et al., 2003). The small mean d_{ae} values are representational of the sizes recorded by the SEM images (Figure 4.7) and reveal that the particulates behaved as aggregates during Sympatec dry dispersion laser diffraction at 1 bar.

The tapped densities of the seven formulations ranged between 0.17 and 0.25 gcm^{-3}. Tapped density proved to be independent of drug phase location (Table 4.10). As part of the relationship with theoretical particle size and the association with flow properties a low tapped density is associated with good aerosolisation properties (Bosquillon et al., 2001a).

Carr's Compressibility Index classified the seven formulations as fair to excellent, although the validity of this test is in question as previously outlined (De Villiers, 2005). Carr's index values ranged from 6.6 to 18.8% with values independent of drug phase location (Table 4.10).

4.3.2.2   In vitro powder aerosolisation
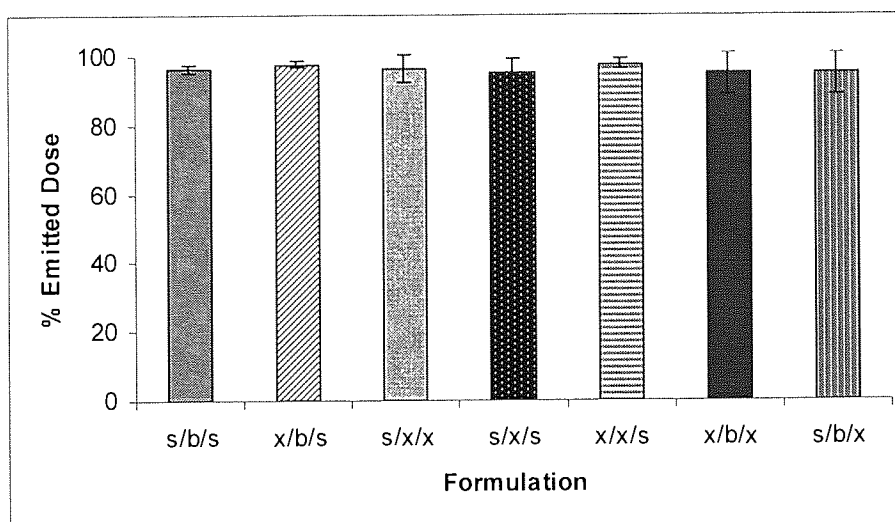
Figure 4.9: Bar chart representing the emitted dose of spray dried chitosan double emulsions (mean ± S.D, n=3); Phase order 1°/2°/3 °, x = vacant phase, s = SS occupied phase, b = BDP occupied phase.

The percent of powder mass leaving the hydroxypropyl-methylcellulose (HPMC) capsule when expelled by a Spinhaler in to the multistage liquid impinger (MSLI) at 60 Lmin[-1] was determined gravimetrically and presented as the percent emitted dose. The emitted dose of the seven formulations ranged from 95.3 to 98.0% (Figure 4.9), the high emitted doses were unaffected by the presence of salbutamol in the outer layer of the emulsion. High emitted dose is an indicator of good flow and shows relation to the collated Carr's index values (Table 4.10: Tarsin et al., 2006). No statistical difference was seen between the emitted doses of the seven formulations (ANOVA/Tukey, p >0.05).

The fine particle fraction (FPF) is commonly regarded as the percentage of drug/drug complex particles being below 5 µm in aerodynamic size (Taki et al., 2006). A high FPF *in vitro* shows the potential for a powder to show similar properties *in vivo* and reach the mid to deep lung for either local or systemic action (Ikegami et al., 2003). The MSLI fractions were stored with and standardised against the loaded dose values to give a more accurate depiction of the aerosolisation properties. The fine particle fractions of the formulations ranged from 54.6 to 60.3% of total loaded dose (D1 and D5 respectively) with the formulations containing SS in the tertiary stage of the original liquid emulsion having lower FPF's, although not statistically so (Figure 4.10: p >0.05, AVOVA/Tukey).

Similar FPF's would be expected as the formulation remained constant throughout the seven formulations with the exception of drug phase location and minor discrepancies in leucine concentration (28-36% w/w). FPF proved independent of the water content of each formulation as found by TGA, humidity can also be ruled out as a factor due to exacting storage and assessment conditions (Table 4.10, Figure 4.10).
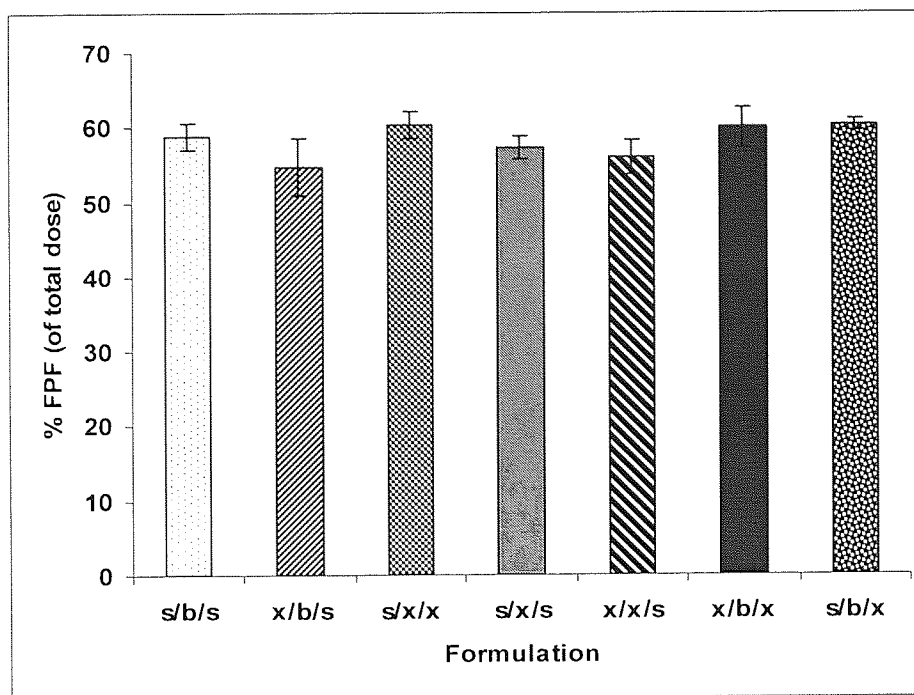
Figure 4.10: The Fine Particle Fraction (FPF: F< 5μm) of the chitosan double emulsion spray dried formulations, acquired by an MSLI at 60 L/min (mean ± S.D, n=3). S = salbutamol, B= BDP, /= phase separation.

Mass median aerodynamic diameters ranged between 1.8 and 2.8 μm (D2 and D6 respectively: Table 4.10), with the larger sizes belonging to the formulations with salbutamol in the tertiary phase indicating some aggregation during aerosolisation. All mass median diameters were of a respirable size (Bosquillon et al., 2004a).

The deposition pattern in the MSLI was similar for all powders, each giving minimal deposition in the inhaler but leaving around 10 to 15% of the total dose in the throat region of the liquid impinger. High filter deposition of around 15 to 20% of the total dose was observed with no statistical difference in the amount of either agent deposited on any MSLI stage showing a wide distribution in aerodynamic size. No statistical difference was seen between the deposition patterns of SS and BDP in the spray dried powders containing both agents indicating homogeneous powders (two way students matched paired t-test/Tukey: p >0.05: Figure 4.11).
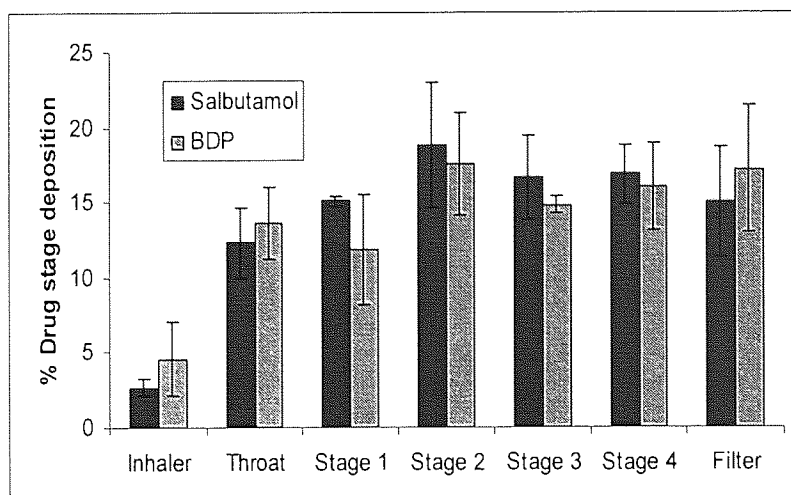
Figure 4.11: Bar chart of the MSLI deposition pattern of formulation D7, containing SS in the primary and tertiary phases of the double emulsion to be spray dried and BDP in the secondary organic layer, the deposition pattern revealing powder homogeneity (mean ± S.D, n=3).

4.3.2.3 In vitro dissolution

The dissolution time of BDP remained consistent through out the formulations containing the corticosteroid due to its replicate location in the organic section of the double emulsion. The dissolution time of salbutamol however appeared to be governed by the β_2 agonist's presence in the primary and/or tertiary aqueous phases of the original double emulsion (Figure 4.12). The longest peak dissolution times were recorded as 19 days for both entities, a dissolution peak concentration was taken as 100% release as PLGA 50:50 has previously shown its ability to retain agents indefinitely and not release 100% of total drug load (Schnieders et al., 2006; Blanco et al., 2006).

Formulation D1 utilised SS in the primary phase with vacant secondary and tertiary phases, the dissolution trace for this sample produced close proximity with the zero order (Table 4.11: r^2 0.9830) and the Higuchi kinetics (r^2 0.9623). BDP entrained with in the secondary phase showed a similar release profile to SS in the primary phase (figure 4.12 A) in formulation D2 (Figure 4.12

B), with close proximity to the zero order and Higuchi models (Table 4.11: r^2 0.9817 and 0.9488

respectively). The SS in these formulations mediated both by the PLGA 50:50 erosion polymer in

the secondary phase and the matrix style polymer chitosan in the tertiary phase, the chitosan

accounting for the close proximity to the Higuchi profile. Zero order would not be expected for

this formulation which shows a minor burst release (zero order) over the first 2 days of

dissolution (Figure 4.12 A). The results perhaps an indication that the primary phase is encased

inside the PLGA 50:50 layer and BDP is spread through out the polymer giving a similar release

pattern as the system erodes.
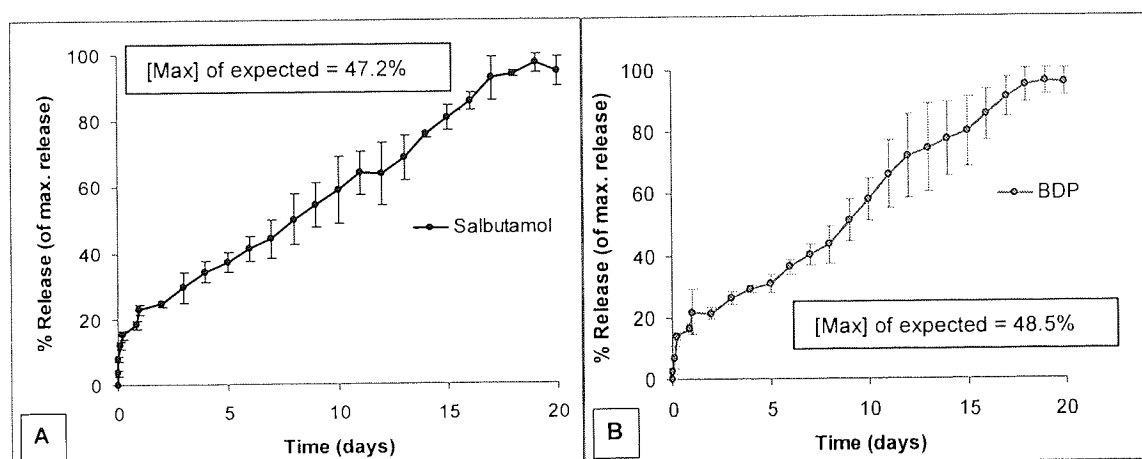
Figure 4.12: Dissolution traces of 200 mg chitosan double emulsion spray dried samples in rotating baskets at 50 RPM; media 1 L PBS 37° C, with analysis by HPLC (n=3): S = salbutamol, B= BDP, /= phase separation. A. Formulation D1 (orientation: s/x/x); B. Formulation D2 (x/b/x).

Figure 4.12: Dissolution traces of 200 mg chitosan double emulsion spray dried samples in rotating baskets at 50 RPM; media 1 L PBS 37° C, with analysis by HPLC (n=3): S = salbutamol, B= BDP, /= phase separation. C. Formulation D3 (orientation: x/x/s); D. Formulation D4 (orientation: s/b/x), E. Formulation D5 (orientation: x/b/s); F. Formulation D6 (orientation: s/x/s), G. Formulation D7 (orientation: s/b/s).

Formulation D3 showed a vastly reduced $t$max from c.18 days to only 8 days, the release profile with greater latency than expected as the active marker SS was located in the tertiary phase post PLGA mediator. The large error bars in Figure 4.12C representing formulation D3 may indicate that some entrapment of SS possibly within the micellular arrangement of the secondary and tertiary phases of the original double emulsion to be spray dried, rendering a fraction of the drug delivery during dissolution sustained release: a similar phenomenon is observed in formulation D5 (Figure 4.12E).

Addition of SS and BDP into stages one and two (D4; Figure 4.12D) showed the two agents to have an almost identical release profile as would be expected of two agents residing within the PLGA 50:50 system. Formulation D5 (Figure 4.12E) revealed sustained release BDP from the secondary phase with burst release SS form the tertiary phase.

The addition of SS in to both primary and tertiary phases (formulation D6; Figure 4.12F) revealed an intermediate dissolution profile of formulations D1 and D3 which contained solely SS in phases one and three of the original double emulsion respectively. Finally, the addition of both SS to primary and tertiary phases and BDP to the intermediate organic phase proved to be a replicate of the dissolution behaviour of formulations D2 and D6 combined as would be expected (Figure 4.12G).

Overall the placing of one agent into various phases of the double emulsion indicated that the spray-dried powder was made up of pockets of drug surrounded by polymer and highlighted potential for having differing release rates from within the same spray dried powder intended for inhalation.

Cumulative examination of the release rates showed good correlation with the Higuchi homogeneous matrix kinetic (Table 4.11: r^2 0.7013 - 0.9783), the correlation based on the inclusion of chitosan. The first order kinetic also showed good correlation (r^2 0.7430 – 0.9447), the presence of PLGA 50:50 in the organic phase mediating the *in vitro* release rate of agents in the primary and secondary phases. Zero order release was associated with the burst release from the tertiary outer layer although the formulation containing only salbutamol in the tertiary layer did not correlate well with zero order release (r^2 0.7620) possibly due to the entrapment of some SS in the micellular arrangement of the intermediate layer, despite this zero order showed high correlation with all but three dissolution traces.

Chitosan has previously been shown to increase the dissolution times (*t*max) of hydrophilic drugs: however, the effects of low molecular weight chitosan are not being attributed to the long dissolution periods, although there is a possibility that the LMW chitosan maybe working to support or prevent the erosion of the enclosed PLGA 50:50 layer (Anal et al., 2006). The difference in dissolution times for salbutamol in the primary inner and tertiary outer phase gives the opportunity to modify the release of multiple agents whether hydrophilic (SS) or hydrophobic (BDP). Previous studies have used PLGA 50:50 in the controlled release of medicinal agents; however the use of chitosan and PLGA in an emulsion system for spray drying is a novel concept with an ideology of bio-adhesiveness and sustained release (Wang et al., 2003; Blanco-Prieto et al., 2000; Lam et al., 2001). Furthermore the use of leucine in combination with both chitosan and PLGA is a novel approach to the pulmonary delivery of local agents.

Formulation	Orientation Phase (1/2/3)	t max (days)	zero order t (days)	zero order r²	1st order t (days)	1st order r²	Higuchi matrix t (days)	Higuchi matrix r²
D1	S/x/x	18	0 - 19	0.9830	0 - 17	0.8308	0 - 18	0.9623
D2	x/B/x	17	0 - 19	0.9817	0 - 17	0.9187	0 - 17	0.9488
D3	x/x/S	8	0 - 12	0.7620	0 - 8	0.9447	0 - 8	0.8019
D4 b*	s/B/x	19	0 - 19	0.9797	0 - 17	0.7790	0 - 17	0.9244
D4 s*	S/b/x	19	0 - 19	0.9811	0 - 18	0.9237	0 - 19	0.9783
D5 b*	x/B/s	19	0 - 15	0.6966	0 - 14	0.8762	0 - 15	0.8439
D5 s*	x/b/S	19	0 - 19	0.9891	0 - 19	0.8544	0 - 19	0.9574
D6	S/x/S	18	0 - 19	0.9260	0 - 18	0.9151	0 - 18	0.9713
D7 b*	s/B/s	19	0 - 19	0.9040	0 - 15	0.9091	0 - 19	0.8097
D7 s*	S/b/S	19	0 - 19	0.7242	0 - 19	0.7430	0 - 19	0.7013

Table 4.11: Table of the time of greatest recovered drug concentration ($t$max) and correlation of the release profiles of the seven spray dried chitosan emulsion formulations with zero, first and Higuchi rate kinetics (mean ± S.D, n=3). b* signifies the release of BDP from a dual loaded formulation and s* SS release from the same formulation.

4.3.3 The use of medium and high molecular weight chitosan

Further exploration of the chitosan emulsion was undertaken to explore the effect of increasing chitosan molecular weight on the aerodynamic and solvation properties of the spray dried double emulsions. Utilising the S/B/S phase orientation a decrease in FPF from 58.9% of the total dose with low molecular weight chitosan to 51.1% with medium molecular weight chitosan was shown. With high molecular weight chitosan giving the lowest FPF of 41.0%, the reduction in MSLI performance akin to the decrease in FPF with increasing chitosan molecular weight seen in the previous chapter (n=1).

The dissolution times remained unaffected by the changes in chitosan molecular weight with *t*max 19 days for all three formulations (LMW: Figure 4.12G; MMW and HMW Figure 4.13). This indicates the dissolution from the spheres is strongly mediated by the PLGA 50:50 and not the chitosan moiety, chitosan having a maximum release of up to 12 hours with a hydrophobic marker (HMW chitosan and BDP: Chapter 3). However, the release rates of the higher molecular weight chitosans showed better correlation with the Higuchi homogeneous matrix kinetic than the low molecular weight chitosan, illustrating that chitosan did have an overall effect on the dissolution release mechanism if not on the time of maximum dissolution (LMW SS r^2 0.7013, BDP r^2 0.8097: MMW SS r^2 0.9665, BDP r^2 0.9777: HMW SS r^2 0.9758, BDP r^2 0.9778). The better correlation with the Higuchi kinetic indicating an increasing degree of matrix fashioned release form the composite system with increasing chitosan molecular weight.
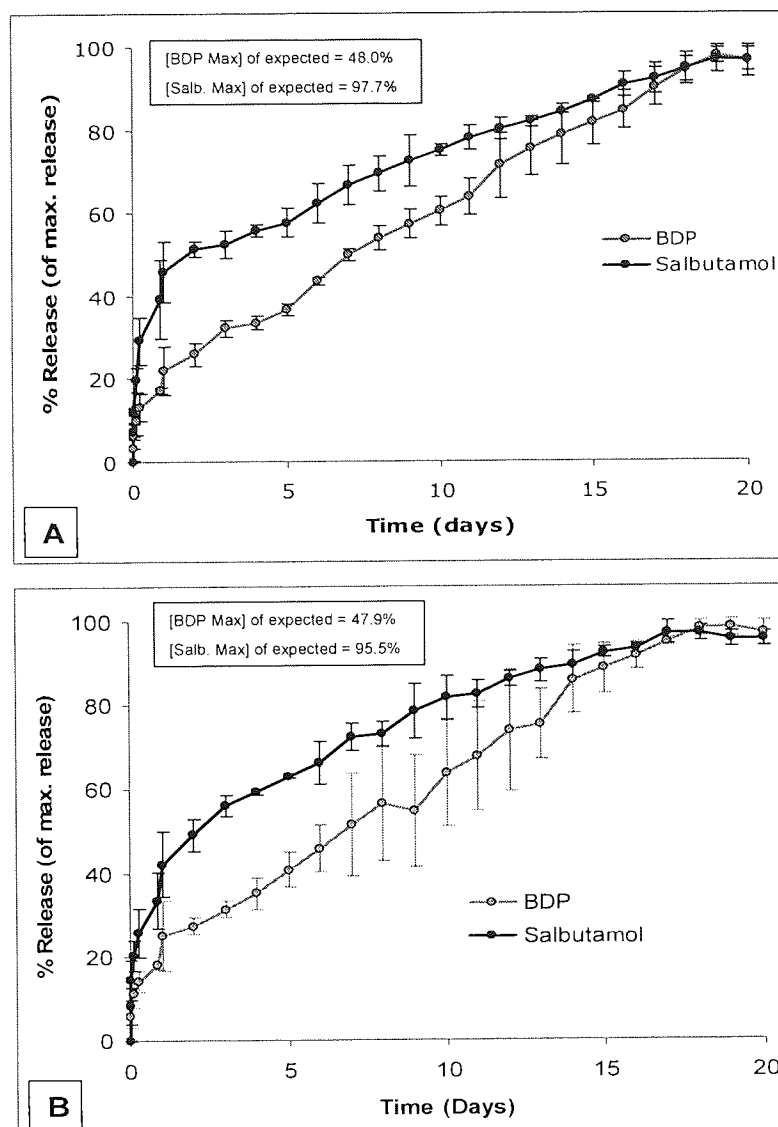
Figure 4.13: Dissolution traces of medium and high molecular weight chitosan double emulsion 200 mg spray dried samples in rotating baskets at 50 RPM; media 1 L PBS 37° C, with analysis by HPLC (mean ± SD, n=3): Salbutamol black line and points, BDP grey line and points. S = SS, B= BDP, /= phase separation. A. Formulation utilising medium molecular weight (MMW) chitosan in the final phase of the double emulsion (s/b/s); B. Formulation utilising high molecular weight chitosan (HMW) in the final phase of the double emulsion (s/b/s).

4.3.4 A four phase emulsion/system

The use of a four phase system would require inherent stability within a formulation (Pal, 2007).

One of the many advantages of using chitosan in a formulation to be spray dried is that the gel

structure can support or suspend other excipients with in the blend (Illum, 1998). The

suspension of an oil in water in oil emulsion (o/w/o) in a gel system could loosely be regarded as almost a triple emulsion (o/w/o/w), the multiple layers potentially offering a range release rates and mechanisms depending on manipulation. There is currently no literature to support the hypothesis of a triple emulsion of the oil in water in oil in water format.

The four phase system was spray dried as a 2% w/v formulation with 2% w/w BDP in both oil phases and 2% w/w SS in both aqueous phases. A high spray drying-yield of 73.3% produced a free flowing off-white powder. *In vitro* MSLI assessment of the powder gave favourable results; the gravimetrically determined emitted dose of 92.5% showed good dispersibility. An FPF of 60.4% of the total loaded dose with an MMAD of 3.74 µm indicated that the powder was highly respirable and would be capable of reaching the mid to deep lungs. Dissolution testing over a 28-day period did not render the tmax of either salbutamol or BDP moieties with a burst release of salbutamol followed by prolonged salbutamol and BDP release (Figure 4.15), the dissolution trace indicating that the treble emulsion remained stable enough to be spray dried. The simplistic view on the particle structure would be one of concentric circles of polymer and drug or of pockets of drug inside and outside of PLGA 50:50 layers. Overall the best fit for the release rate from the spray dried formulation was the first order release profile (r^2 0.9270 and 0.8294, SS and BDP respectively) which shows PLGA 50:50 not depleting drug reserves as the rate limiting factor within the polymer system.
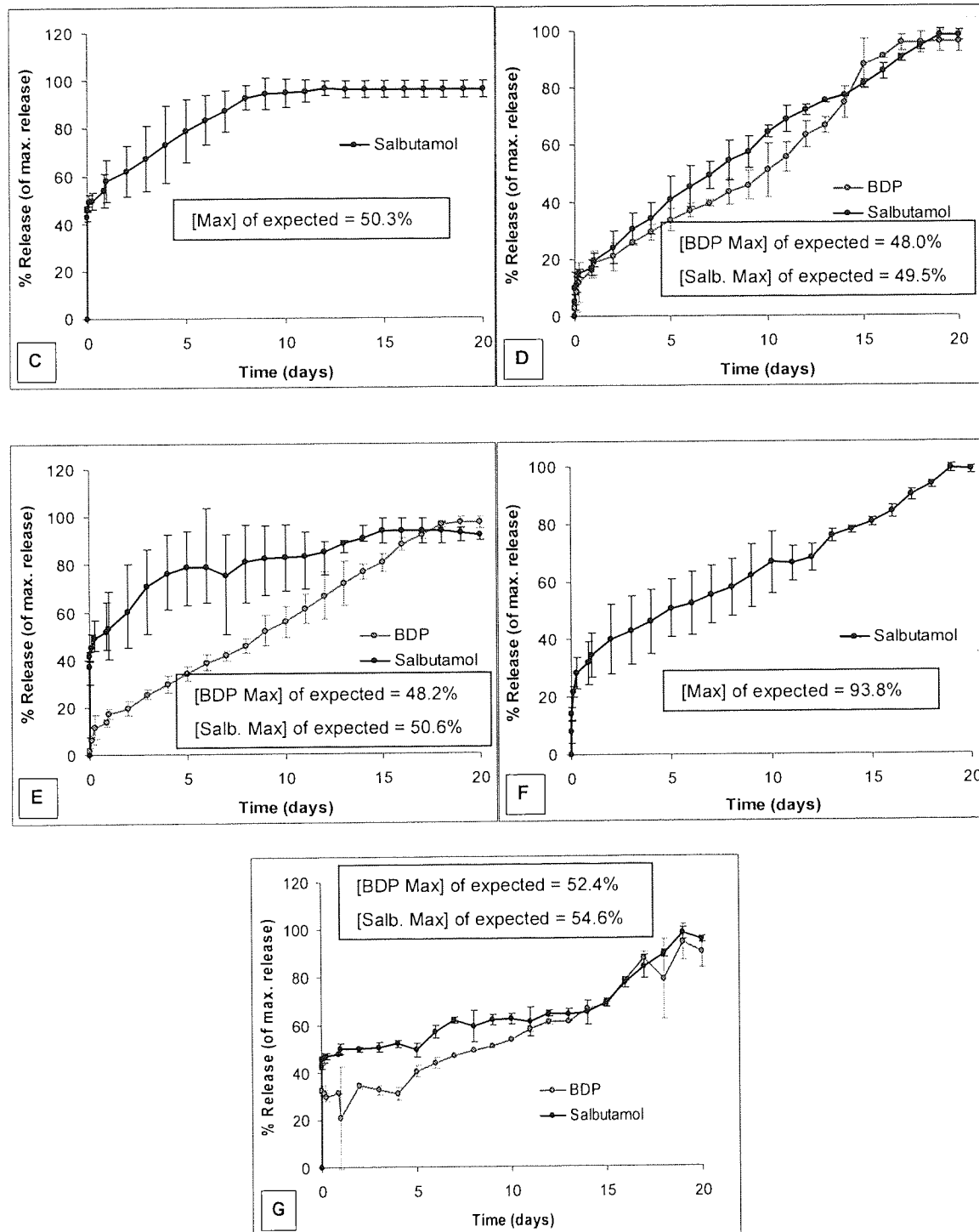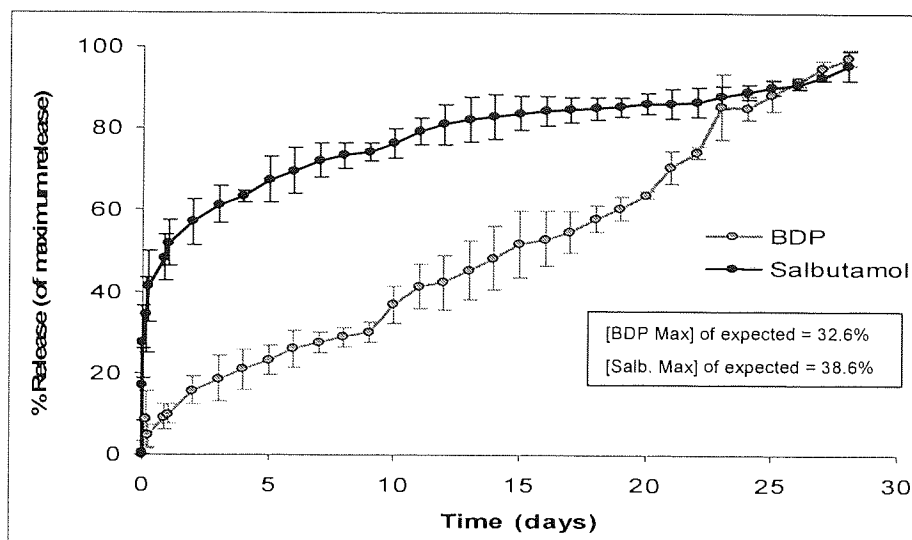
Figure 4.14: Dissolution trace of 200 mg chitosan triple emulsion spray dried samples in rotating baskets at 50 RPM; media 1 L PBS 37° C, with analysis by HPLC (mean ± SD, n=3).

4.4 Conclusions

In conclusion, the method of spray drying PLGA in a double emulsion has demonstrated the ability to produce highly respirable dry powders with sustained release up to 19 days when using the 50:50 lactide - glycolide ratio at a concentration of 10% w/w. It has been found that reducing the PLGA concentration has an undesirable effect on both aerosolisation and sustained release *in vitro* dissolution. The use of PVA as a suspending agent has also been shown to be unreliable at the research grade and concentration affecting drug loading and the action of leucine. The use of chitosan has once again proved to be of benefit in producing highly respirable particles with potential bioadhesion.

So far this thesis has researched and discussed the spray drying of aqueous solutions, suspensions and emulsions. In the final experimental chapter the use of organic suspensions in spray drying utilising an inert closed loop system will be investigated as an alternative method in the production of sustained release spray dried powders for pulmonary delivery.

Chapter 5

The utilisation of a dual-stage method of

spray drying.

5.1   Introduction

Spray drying offers the advantage of being a one step process where outcomes such as powder particle size, can be controlled by the parameters used; such as airflow and liquid feed rate (Rabbani & Seville, 2005). Another advantage is that the particles contained in a powder can be engineered to a degree as exemplified by the previous chapters.

The spray drying of the lipid soluble polylactide-co-glycolide PLGA in an organic system has the drawback of any further additives having to be hydrophobic to be encased in the polymer. Water in oil and double emulsion methods have previously been explored in this thesis as a method of capturing hydrophobic entities within the PLGA polymer to gain controlled release with varying success. In this research the viability of a novel two-stage spray-drying process to generate respirable powders that exhibit sustained drug release is explored.

An initial powder produced by an aqueous spray drying system will be suspended in an organic system in the hope that the primary powder will be encased by the organic formulation post spray drying and give the sustained release of agents contained in both systems during dissolution. For the purpose of this research as with the previous chapters two agents used in the conventional treatment of obstructive airway disease: the short acting hydrophilic β_2 agonist salbutamol sulfate and the lipophilic corticosteroid beclometasone dipropionate will be employed. Following on from the work in the previous chapter PLGA 50:50 will be employed as the sustained release polymer to attain maximum sustained release of the incorporated hydrophilic and hydrophobic markers. As the organic layer will be the outermost layer, leucine will not be employed as an aerosolisation enhancer throughout this chapter despite the improvement in

aerosolisation character, due to insolubility in organic solvents and as the limitation of crystalline

excipient suspension is the main aim of the dual spray drying process.

5.2 Materials and Methods

5.2.1 Materials

Salbutamol sulfate (SS) was kindly donated by Allchem International Ltd (Maidenhead, UK).

Beclometasone dipropionate (BDP), Polylactide co-glycolide 50:50 (PLGA 50:50: mw 40000-

75000) and phosphate buffered saline tablets (PBS) were purchased from Sigma Aldrich®

Chemicals (Poole, UK). With chloroform, dichloromethane, lactose and Span 80 purchased from

Fisher scientific® Ltd (Loughborough, UK).

5.2.2 Preparation of spray-dried powders

Primary phase spray drying was performed as outlined in chapter 2 using varying amounts of

lactose (0-76% w/w) and SS (±2-4% w/w) and/or BDP (± 2-4% w/w) in 100 mL 30% v/v

aqueous ethanol.

The primary spray dried powder was then suspended in 100 mL dichloromethane in chloroform

(20:80). The combination of organic solvents used to reduce thermal efficiency when spray

drying and reduce aggregation in the final dry product as explained for the 30% v/v aqueous

ethanol liquid feed in chapter three. The organic phase contained 20% w/w PLGA and SS (± 2-

4% w/w) and/or BDP (± 2-4% w/w) depending on the required location of the active agent/s and

lactose in the two phases. The suspension was then sonicated for ten minutes. The organic

suspension was then spray dried using the Büchi B290 mini lab spray-drier in an enclosed

system using nitrogen and the inert loop Büchi B295 with standard glassware for the enclosed Büchi B-290 system (Figure 5.1: spray flow rate 600 L/h, inlet temperature 120°C, pump 3.2 mL/min). The final powders theoretically containing 0-4% w/w SS, 0-4% w/w BDP, 20% w/w PLGA and 72-76% w/w lactose.
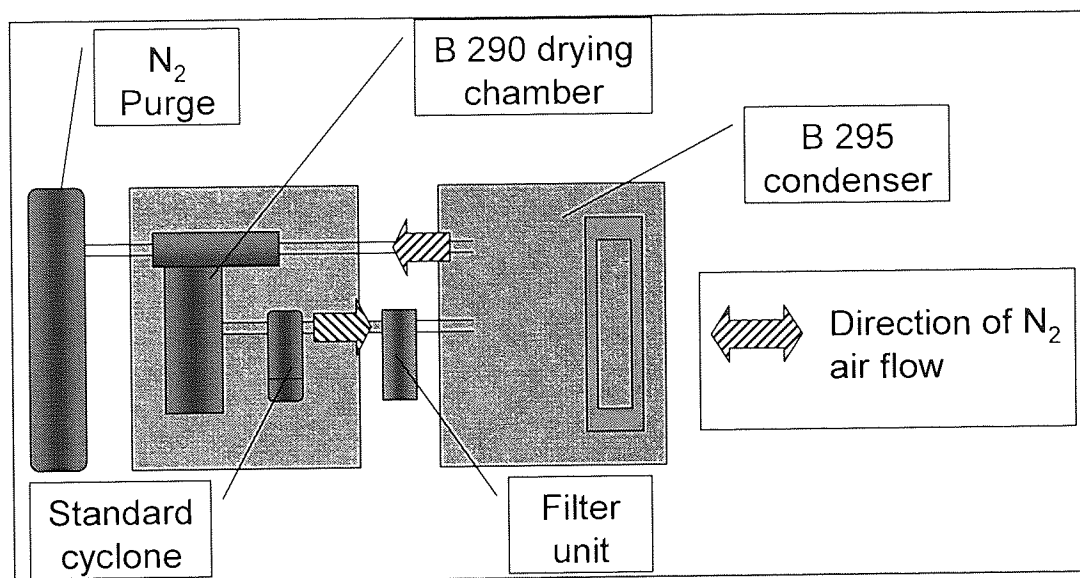
Figure 5.1: Simplified diagram of the Büchi B290 mini lab spray drier closed loop system incorporating the B295 condenser for spray drying flammable organic systems.

5.2.3 Powder characterisation

The powders were characterised as described in Chapter 2, determining spray-drying yield, particle morphology, amorphous nature, water content and powder density. In addition, the primary aerodynamic diameter (d_{ae}) and Carr's Index values were derived and statistical analysis was performed as previously described.

5.2.3.1 Drug content

The SS and BDP content of each yield was assessed in triplicate; 25 mg aliquots of spray dried powder were stored in 50 mL of HPLC mobile phase for 10 days (the same storage period as

MSLI media extractions) and then assessed using HPLC (UV detection: BDP 250 nm, salbutamol 276 nm). The mass of each drug was then expressed as a percentage of expected drug content.

5.2.3.2   *In vitro powder aerosolisation*

The *in vitro* aerosolisation assessment of the dual spray dried samples was performed as outlined in chapter two with the exception that the 20 mL extractions were stored for ten days at room temperature in darkness before HPLC quantification (wavelength: BDP 250 nm, SS 276 nm).

In vitro dissolution testing, HPLC and statistical analysis were performed as described in chapter three.

5.3   Results and Discussion

5.3.1 Powder characterisation

A total of eleven powders were investigated, the powders were off white in appearance with visibly poor flow and large aggregates. The spray drying yields from the primary aqueous spray drying run varied little (57.1 to 69.2%) but after the secondary organic spray drying process varied dramatically (20.5 to 66.0%), the highest yield generated by formulation S10 and the lowest from S1 (Table 5.1). The larger variation perhaps an indication that the organic system is less reproducible than that of the open loop possibly due to the unpredictable nature of aspiration which can not be quantified when using the Büchi B295 condenser. The higher yields from the aqueous spray drying operations over the closed loop organic cycles are attributed to the high

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performance cyclone (HPC), which was used in the open loop but can not be incorporated into the closed loop system. The HPC has been shown to improve yields in chapter 3 through the alteration in gravimetric forces acting on particle sizes at the lower end of a polydisperse powder population (Maury et al., 2005).

Formulation	Aqueous		Organic		SD Yield	Water Content	Tapped Density	Carr's Index[a]	
	Salb %	BDP %	Salb %	BDP %	(%)[b]	(%)	(g cm^{-3})	(%)	Flowability
S1	4			4	20.45	0	0.16 ± 0.01	33.81	Very poor
S2	4	4			39.41	0	0.19 ± 0.01	19.99	Fair
S3			4	4	30.16	0	0.23 ± 0.01	19.10	Fair
S4	2		2	4	34.70	0.11 ± 0.19	0.19 ± 0.01	16.58	Good
S5	2	4	2		33.52	0.87 ± 0.75	0.20 ± 0.01	20.63	Fair
S6	2	2	2	2	44.73	0	0.17 ± 0.00	14.46	Good
S7	4	2		2	43.93	0	0.19 ± 0.01	26.50	Poor, cohesive
S8	4				45.48	0	0.19 ± 0.01	16.62	Good
S9		4			38.82	0	0.20 ± 0.01	15.20	Good
S10			4		66.02	0	0.23 ± 0.02	26.13	Poor, cohesive
S11				4	54.00	2.54 ± 1.05	0.21 ± 0.01	21.11	Poor, fluid

Table 5.1: Representation of the physical characteristics of the dual spray dried powders (mean ± S.D, n=3), [a]De Villiers, 2005, [b]n=1.

The salbutamol sulfate (SS) and beclometasone dipropionate (BDP) loaded doses varied greatly. SS loaded doses varied from 49.0 to 64.6% of the expected content (4% w/w: Table 5.2). The difference was independent of which spray drying run, either aqueous or organic, the SS was added to. The spread in drug load between the 11 formulations proved to be from the same population (no statistical difference, ANOVA/Tukey, p> 0.1). The relatively low recovery in SS drug loads was attributed to PLGA retention and possible re-crystallisation, which could enhance the retention properties (Blanco et al., 2006; Schnieders et al., 2006; Cevher et al., 2006). The BDP contents had a marked variation of 21.4 to 68.9%, the relatively large variation possibly due to a combination of factors such as PLGA retention, PLGA re-crystallisation and low affinity for the 77% v/v aqueous mobile phase. The spread in BPD drug load across the formulations was independent of phase inclusion and was found to be statistically significant indicating that the drug loadings were not of the same population (ANOVA/Tukey, p <0.05).

Formulation	Aqueous		Organic		Drug Loading	
	Salb %	BDP %	Salb %	BDP %	Salb (% of anticipated)	BDP (% of anticipated)
S1	4			4	62.60 ± 3.06	21.38 ± 4.02
S2	4	4			64.28 ± 8.14	60.30 ± 3.67
S3			4	4	57.06 ± 5.12	56.60 ± 31.29
S4	2		2	4	54.11 ± 19.81	21.45 ± 6.57
S5	2	4	2		54.48 ± 8.10	65.71 ± 20.23
S6	2	2	2	2	52.05 ± 3.52	68.55 ± 21.49
S7	4	2		2	64.57 ± 10.71	65.91 ± 31.56
S8	4				63.10 ± 2.91	
S9		4				68.91 ± 8.16
S10			4		48.96 ± 7.13	
S11				4		59.66 ± 4.72

Table 5.2: Table of the respective drug loadings of the dual spray dried powders (mean ± S.D, n=3).

Scanning electron microscopy images taken two months post spray drying revealed structures around 2 μm in diameter with many fused particles (Figure 5.2). The particles appeared as rhombohedra or rectangles with smooth surfaces, defined edges and distinct corners indicating that the spray dried particles were either crystalline post spray drying or had turned crystalline on storage (Zeng et al., 2000b; Larhrib et al., 2003a). Previous studies have shown spray drying to produce microspheres with the change in the physical appearance over the weeks following organic spray drying indicating a change from the amorphous state to a relatively more stable crystalline state (Li HY et al., 2005a; Rabbani & Seville, 2005). Alternatively the addition of BDP, a lipophilic drug, into the aqueous phase and of salbutamol, a hydrophilic drug into the organic phase will have created a crystalline suspension which may have avoided solvation at the relatively high inlet temperature of 180°C during aqueous spray drying and of 120°C during organic spray drying respectively.
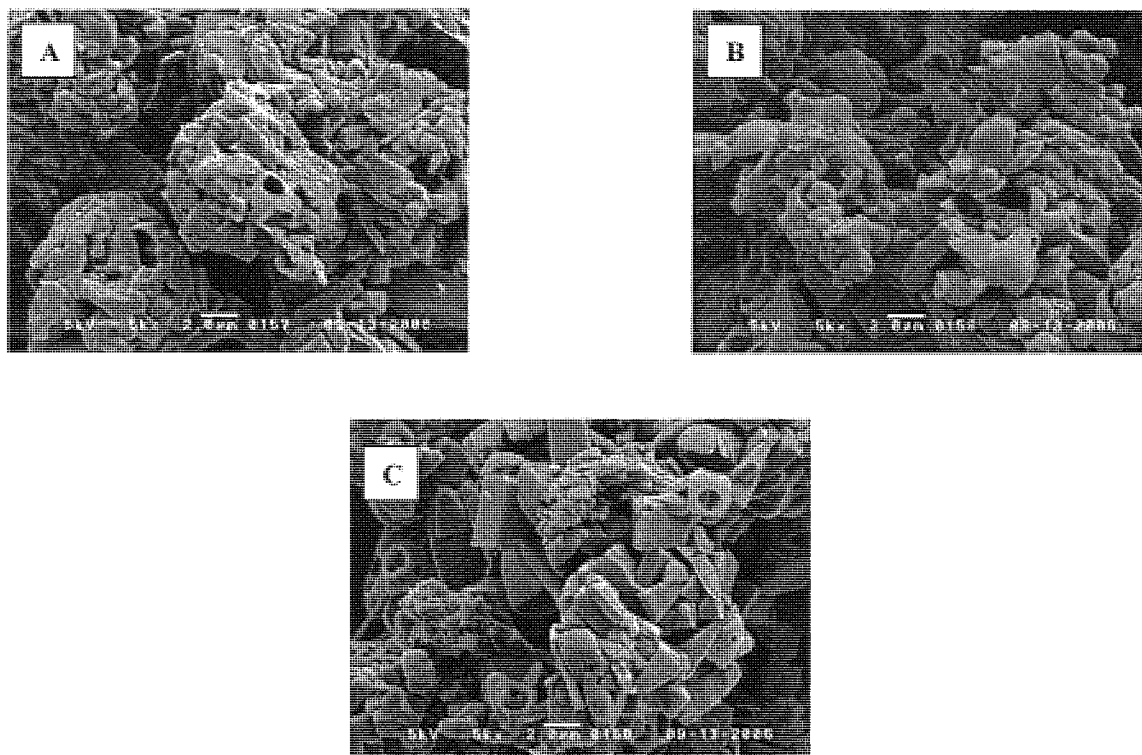
Figure 5.2: Representative scanning electron micrographs of double spray dried formulations: A. S4, containing 2% w/w salbutamol in the aqueous phase with 2% w/w BDP and 4% w/w salbutamol in the organic phase; B. S5, containing 2% w/w salbutamol and 4% w/w BDP in the aqueous phase with 2% w/w BDP in the organic phase; C. S6, containing 2% w/w salbutamol and BDP in the aqueous phase and 2% w/w salbutamol and BDP in the organic phase.

Differential scanning calorimetery (DSC) gave partially peaked traces for all spray-dried formulations similar in comparison with their crystalline blend counterparts (Figure 5.3). This indicates that the spray-dried powders are either unstable at room temperature and humidity and re-crystallise to a more stable form post spray drying or that crystalline entities suspended pre-process remain post spray drying. BDP proved to be the component most likely to present as crystalline, whether added to the aqueous or organic phases, seen as a peak at 190-200°C on the DSC spectra (Loo et al., 2005; Rosu et al., 2006). The re-crystallisation of lactose (peak 130°C) occurred in all spray dried formulations to different degrees (Figure 5.3: Sarisuta et al., 2006). SS (exotherm and endotherm at 240°C: Liu et al., 2005) and PLGA 50:50 (endotherm at

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50°C: Mu et al., 2005) proved to be the most stable components with a partial crystalline peak seen for SS only in formulations were the drug had not been added in the aqueous spray drying run (with the exception of S2: Figure 5.3B).

PLGA 50:50 failed to give a crystalline peak using standard nitrogen purge DSC in crystalline and spray-dried blends, as was expected of the spray dried blends as the polymer fully dissolved in the organic continuous phase of DCM:CH₃Cl (20:80). For BDP the smallest crystalline peaks were seen in the formulations containing dissolved agent during the organic spray drying operation. Spray drying a solution gives a homogeneous blend that prevents the re-crystallisation of components through molecular dispersion preventing neighbouring particles from realigning to an energy efficient state and reverting to a crystalline formation (Mosen et al., 2006). In formulations where suspended particles were present crystallisation peaks of such formulants could be seen, this is because suspended particles are less likely to yield the molecular dispersion required to prevent the re-crystallisation of components post spray drying (Figure 5.3; Mosen et al., 2006). The change from a glassy or rubbery amorphous state to a crystalline state can have profound physicochemical effects on a particulate system; effects such as changes in flow, surface morphology and shape, aerosolisation properties and solvation (Loo et al., 2005).
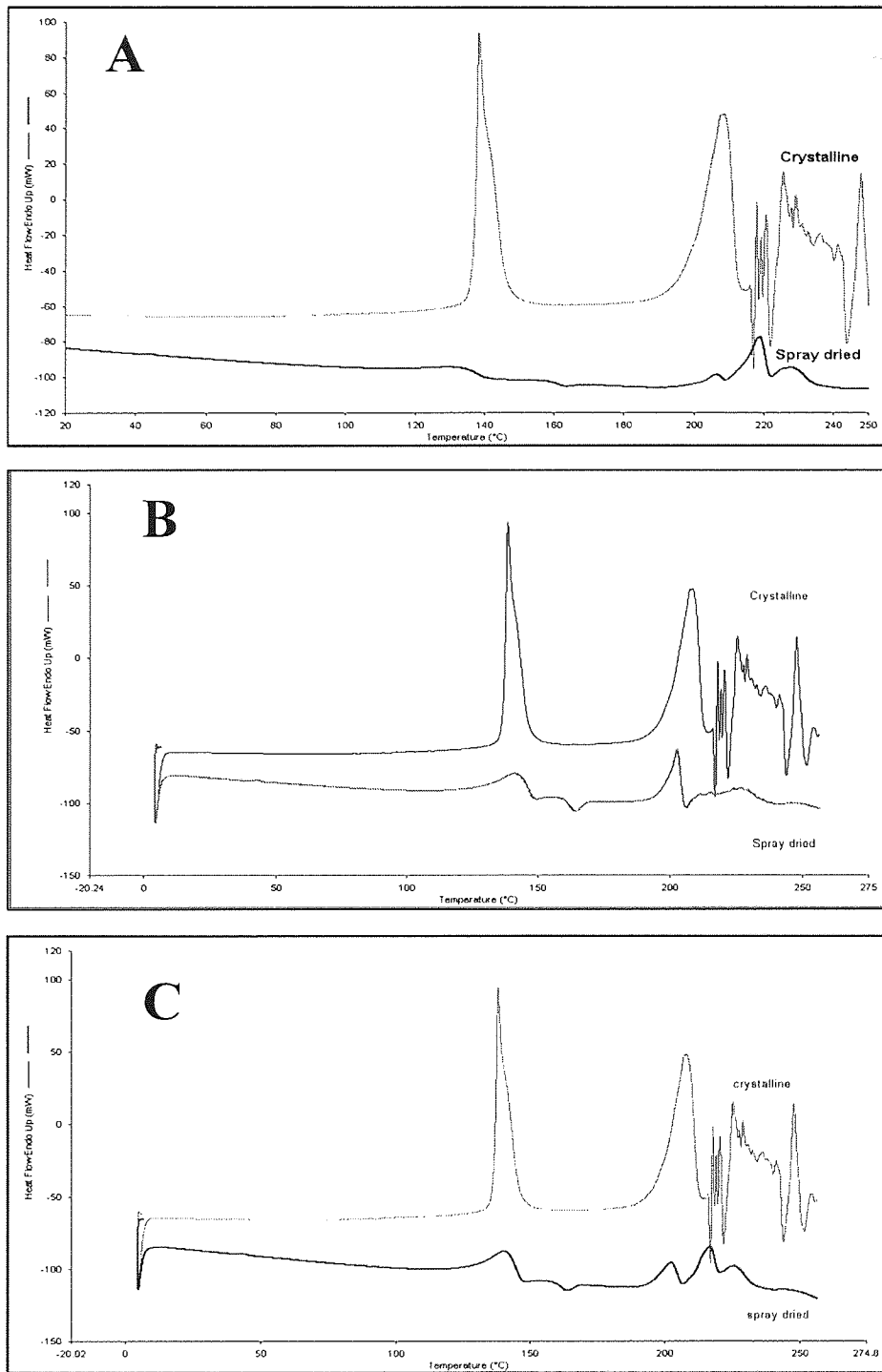
Figure 5.3: Representative DSC traces of dual spray dried formulations, Peaks identified as: lactose 140°C, BDP 200 °C, salbutamol/ pan movement c.240 °C: A. S1: Spray dried formulation containing 4% w/w SS, 72% w/w lactose suspended in an organic phase of 4% w/w BDP and 20% w/w PLGA 50:50 and its crystalline blend comparative. B. S2: Spray dried formulation containing spray dried 4% w/w SS, 4% w/w BDP and 72% w/w lactose suspended in an organic phase with 20% w/w PLGA 50:50 and its crystalline blend comparative. S6: Spray dried formulation containing spray dried 2% w/w SS, 2% w/w BDP and 72% w/w lactose suspended in an organic phase of 2% salbutamol, 2% w/w BDP and 20% w/w PLGA 50:50 and its crystalline blend comparative.

Thermogravimeteric analysis (TGA) was used to assess the water content of the spray-dried powders as a percentage of the sample dry weight. The water content of the eleven formulations was negligible with only S4, S5 and S11 having a mass of water that could be detected by the Perkin Elmer Pyris 1 TGA instrument (Table 5.1).

Dry powder dispersion laser diffraction evaluated by a Sympatec system revealed the spray dried powders to have a volume mean diameter (VMD) of 8.2 -12.2 μm at 1 bar pressure. Particles of this size would be regarded as being too large to reach the mid to deep pulmonary regions (Larhrib et al., 1999, 2003). The larger mean diameter gathered by the Sympatec system in comparison to the SEM images indicates that the 1 bar pressure used during laser diffraction sizing was not enough to over come inter-particulate forces and break up aggregates. VMD appears to be independent of the location of salbutamol and BDP moieties (Table 5.3). As Sympatec particle size analysis was performed immediately after the organic spray drying process it is unlikely that any re-crystallisation of individual particles will have taken place to affect the size distribution. Although the suspension and subsequent presence of crystalline material in the spray dried product may have had a size-limiting factor.

Theoretical particle size (d_{ae}), as described in the methods section, is derived from the laser diffraction diameter and tapped density of a formulation to give an indication of the actual size of any microspheres in a formulation and the cohesive behaviour of a formulation. Theoretical diameters ranged between 3.3 and 5.8 μm (formulations S1 and S10 respectively), with particle size being independent of SS and BDP location (Table 5.3). In all formulations (with the exception of S2 and S10) the theoretical primary particle sizes are regarded as small enough to reach the target deep and mid areas of the lung giving minimal impaction on the oropharynx

(Larhrib et al., 1999). The comparatively smaller mean d_{ae} values reveal that the particulates behaved as aggregates during Sympatec dry dispersion laser diffraction at 1 bar.

Formulation	Aqueous		Organic		Particle Size (μm)	d_{ae} (μm)	MMAD (μm)
	Salb %	BDP %	Salb %	BDP %			
S1	4			4	8.22 ± 0.00	3.28 ± 0.07	3.30 ± 2.34
S2	4	4			12.20 ± 1.29	5.26 ± 0.46	4.04 ± 0.90
S3			4	4	8.73 ± 0.85	4.15 ± 0.44	2.54 ± 1.19
S4	2		2	4	8.21 ± 0.00	3.59 ± 0.05	3.09 ± 0.78
S5	2	4	2		10.30 ± 1.00	4.59 ± 0.05	0.99 ± 2.38
S6	2	2	2	2	8.21 ± 0.00	3.37 ± 0.05	4.99 ± 3.32
S7	4	2		2	8.22 ± 0.00	3.63 ± 0.05	2.19 ± 0.77
S8	4				11.46 ± 0.00	4.96 ± 0.08	0.31 ± 3.36
S9		4			8.22 ± 0.00	3.71 ± 0.05	1.72 ± 0.31
S10			4		11.46 ± 0.00	5.58 ± 0.08	5.55 ± 0.24
S11				4	9.71 ± 0.00	4.43 ± 0.05	1.56 ± 0.46

Table 5.3: Table of the representation of the particulate size of the dual spray dried powders: Fraunhoffer dry dispersion laser diffraction particle size derived from the Sympatec system, theoretical primary particle size (d_{ae}) and Median Mass Aerodynamic diameter (MMAD) (mean ± S.D, n=3).

The tapped densities of the seven formulations ranged between 0.16 and 0.23 gcm^{-3} (S1 and S10 respectively). Tapped density proved to be independent of drug phase location (Table 5.1). As part of the relationship with theoretical particle size the formulations with lower tapped density values were those with the smaller theoretical particle sizes. The tapped density of a formulation is an indication of flow properties with a low tapped density being associated with good aerosolisation properties (Bosquillon et al., 2001b).

Carr's Compressibility Index uses bulk and tapped density values to classify the flow type of a powder, the seven formulations were graded as very poor to good (De Villiers, 2005). Carr's index values ranging from 14.5 to 33.8% (S6 and S1 respectively) with values independent of drug phase location (Table 5.1). The largest Carr's index value and therefore the powder

regarded as having the poorest flow was S1 which gave the lowest d_{ae} diameter, revealing

greater cohesion between the smaller particles contained within this population compared to the

other ten formulations.

5.3.2 In vitro powder aerosolisation

The percent of powder mass leaving the hydroxy-propyl methylcellulose (HPMC) capsule when

expelled by a Spinhaler in to the multistage liquid impinger at 60 Lmin^{-1} was determined by

gravimetric assessment of the HMPC size 2 capsules and presented as the percent emitted

dose. The emitted dose of the seven formulations ranged from 89.3 to 99.7% (Table 5.4: S3 to

S10 respectively), a general trend of lower emitted dose associated with increasing organic

phase content (either solute or suspended) was noted. High emitted dose is an indicator of good

flow but shows no relation to the collated Carr's index values (Table 5.1, 5.4; Tarsin et al., 2006).

In addition no statistical difference was seen between the emitted doses of the eleven

formulations (ANOVA/Tukey, p >0.05).

Formulation	Aqueous		Organic		Emitted dose	FPF
	Salb %	BDP %	Salb %	BDP %	%	F< 5 um
S1	4			4	96.70 ± 3.14	43.88 ± 15.23
S2	4	4			98.15 ± 0.41	45.27 ± 6.40
S3			4	4	89.30 ± 4.16	55.98 ± 10.35
S4	2		2	4	92.10 ± 3.68	44.74 ± 6.95
S5	2	4	2		90.41 ± 6.54	52.91 ± 9.52
S6	2	2	2	2	97.63 ± 1.44	35.98 ± 10.13
S7	4	2		2	86.81 ± 6.18	52.91 ± 12.49
S8	4				93.32 ± 1.60	54.80 ± 6.53
S9		4			91.32 ± 3.87	55.70 ± 14.22
S10			4		99.68 ± 0.55	39.91 ± 4.43
S11				4	97.36 ± 1.21	42.97 ± 5.40

Table 5.4: Table showing the gravimetrically determined emitted dose and the Fine Particle Fraction (FPF: F <5 μm) of the dual spray dried formulations, acquired by 25 mg sample loaded capsules exited via a Spinhaler DPI through an MSLI at 60 L/min (mean ± S.D, n=3).

Fine particle fraction (FPF) is commonly regarded as the percentage of drug/drug complex particles below 5 µm in aerodynamic size (Taki et al., 2006). A high FPF *in vitro* shows the potential for a powder to show similar properties *in vivo* and reach the mid to deep lung for either local or systemic action (Ikegami et al., 2003). The FPF's were standardised against the loaded dose values determined after assay post ten days dark storage (Table 5.4) to give a more accurate depiction of the aerosolisation properties. The fine particle fractions of the formulations ranged from 36.0 to 56.0% TD (formulations S6 to S3 respectively) with the formulations containing suspended particles in the organic phase generally having lower FPF's (non significant $p > 0.05$, AVOVA/Tukey). The reduction in ED was mirrored by reduction in FPF perhaps due to static interactions caused by suspended particles appearing at the surface of the spray dried particulates rendering more cohesive forces, thus creating larger aggregates reducing formulation deposition on the terminal stages of the multi stage liquid impinger (MSLI). The negligible water content of each formulation (disclosed by TGA) and a humidity controlled environment rule out moisture factors that may affect aerosolisation.

Mass median aerodynamic diameters ranged between 0.3 and 5.6 µm (formulations 8 and 10 respectively) with the larger sizes generally belonging to the formulations with suspended particles in the organic phase (Table 5.4). All mass median diameters (except formulation 10) were of a respirable size (Bosquillon et al., 2001a).

The trapezoidal shapes revealed by SEM and the crystallinity unveiled by DSC may have had an effect on the formulations aerosolisation performance (Najafabadi et al., 2004). The rectangular

shaped particles may align during aerosolisation to give higher FPF values and lower MMAD values than predicted by laser diffraction and d_{ae} particle sizing. Large porous particles produced by spray drying are recognised as having relatively low aerodynamic size compared to laser diffraction size partly due to high porosity and partly due to their high shape factor described by the following relationships (Formula 5.1: Chow et al., 2007; Formula 5.2: Zeng et al., 2000b). Due to the presence of aggregates during laser diffraction sizing primary particle perimeters could not be isolated and therefore shape factors could not be calculated. Alternatively the varying degrees of crystallinity may affect inter-particulate forces and affect aerosolisation although no correlation was found between DSC crystallisation peak intensity and fine particle fraction.

$$\delta_A = \delta_V \sqrt{\frac{\rho}{\alpha \rho_o}}$$

Formula 5.1: Formula for the relationship of Aerodynamic volume (δ_A) Fraunhoffer dry dispersion laser diffraction particle size (δ_V), tapped density (ρ), density of a sphere of the dimensions of the actual particulate (ρ_o) and shape factor (α) (Chow et al., 2007). Therefore an increase in shape factor as seen with spear shaped particles will see a reduction in aerodynamic size (δ_A) compared to Fraunhoffer size (δ_V).

$$\alpha = 4\pi \left(\frac{A}{P^2} \right)$$

Formula 5.2: Formula for relating the shape factor (α) of a particulate to the projected image of primary particle 2-dimensional area (A) and perimeter (P).

No statistical difference was found between the deposition patterns of the powders on any stage of the MSLI (both SS and BDP), indicating a wide distribution of aerodynamic particle sizes and varying degrees of aggregation. No difference was found between the deposition patterns of SS

and BDP in all formulations containing both agents except formulation S1 (p <0.05: ANOVA/Tukey) indicating homogeneous powders in all but one formulation. A highlighted problem with the double spray drying method is the possibility of two particle populations; one made up of a suspended aqueous core surrounded by PLGA and the other made solely of PLGA and any active incorporated into the secondary organic continuous phase. If the particles were of a slightly different size or density sedimentation or separation could have occurred within the sample to reduce homogeneity, this phenomenon possibly seen with formulation S1.

5.3.3 In vitro dissolution

All formulations showed sustained release of both SS and BDP indicating that the suspended particles from the aqueous spray drying run were encapsulated within the PLGA 50:50 from the secondary organic spray drying run (Figure 5.4). All eleven formulations showed sustained release of SS and BDP beyond the 28 day dissolution period with dual rates of release of both agents: an initial burst release followed by a more sustained release rate (Figure 5.4).

PLGA 50:50 is known to release agents over a long period by a method of erosion and can withhold a percentage of the loaded dose indefinitely as has been previously mentioned. The total percentage release of SS over the series ranged from 57.0 to 84.6% after dissolution period of 28 days when expressed as a percentage of the anticipated loaded dose (4% w/w: S2 and S10 respectively) with BDP release ranging from 48.2 to 80.5% (S1 and S11 respectively). The highest release totals coming from the formulations with agents incorporated in to the secondary organic phase. The release profiles of SS and BDP showed no difference. The results possibly indicating pockets of previously suspended particles near or at the surface of the dry particulates,

or that perhaps some suspended particles were not encapsulated or were encapsulated by a very thin layer of PLGA (Figure 5.4). The burst release may also suggest that BDP is residing at the surface of some dry particulates. The large particle distribution as determined by the MSLI may be a reason for the dual release profile with the smaller particles having a larger surface area releasing drug loads at a faster rate compared to any larger PLGA particulates, which will erode slower and give a more sustained release profile. In addition it is also possible that any PLGA 50:50 re-crystallisation may have had an effect on dissolution times (Loo et al., 2005).

Bisecting the release profiles using the zero, first and Higuchi rate order kinetics and linear regression indicated strong positive correlation with first order kinetics for both salbutamol and BDP (r^2 0.8896 – 0.9670); PLGA 50:50 being the rate limiting factor preventing zero order release. Good correlation was also seen with the Higuchi kinetic (r^2 0.6420 -0.9759), a surprising comparison as PLGA 50:50 does not represent a matrix style of release as previously discussed. Zero order correlation would not be expected as PLGA should enclose the formulation and prevent any burst release of either salbutamol or BDP (r^2 0.5419 – 0.9612).
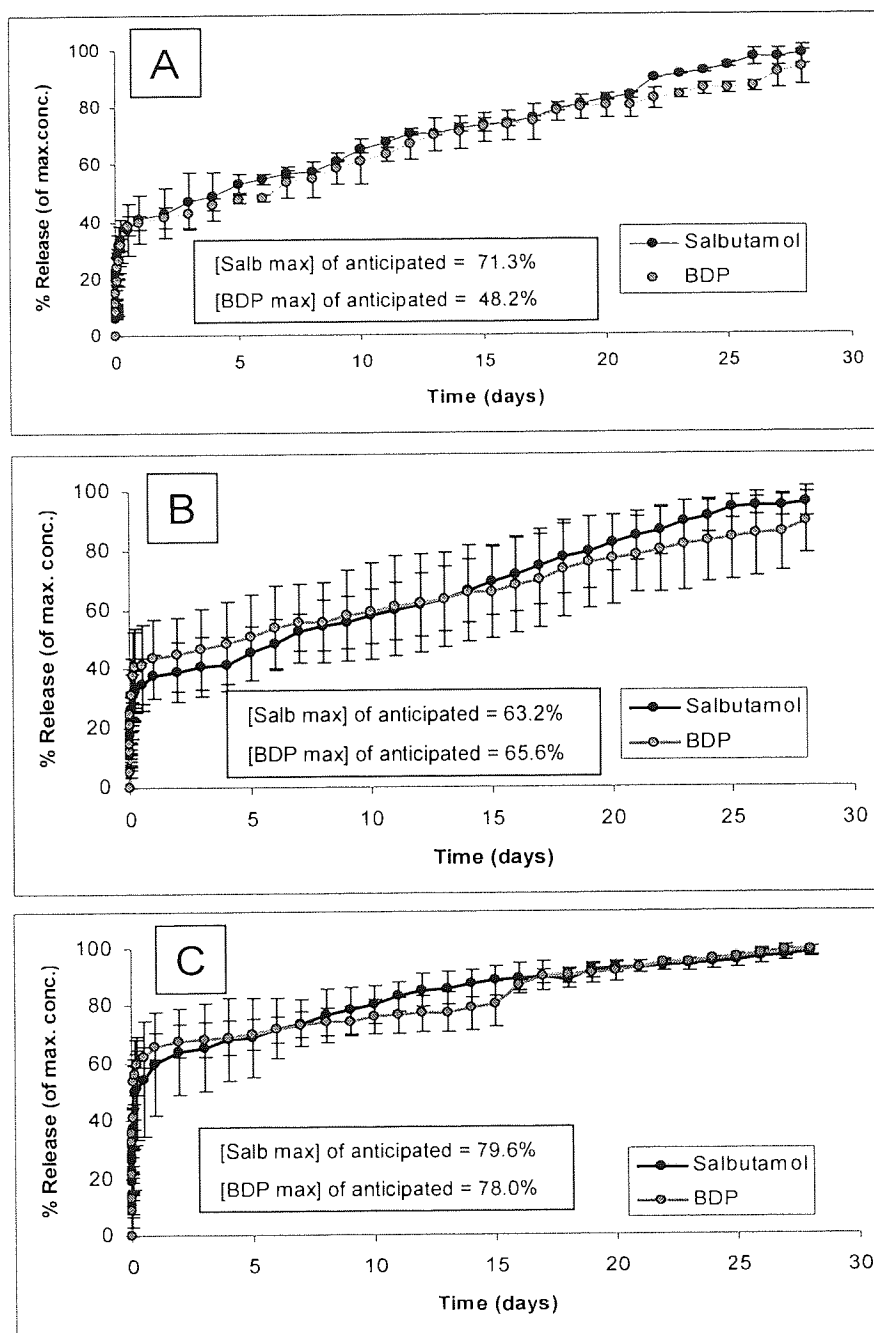
Figure 5.4: Dissolution traces illustrating the similarity of the release profiles of both salbutamol and BDP in the double spray dried formulations independent of stage inclusion either primary aqueous spray drying/ secondary organic spray drying. A. S1: 4% w/w salbutamol in the primary aqueous phase and 4% w/w BDP in the organic phase. B. S3: The addition of 4% w/w salbutamol and 4% w/w BDP in the primary aqueous phase. C. S6: The addition of 4% w/w salbutamol and 4% w/w BDP in the secondary organic spray drying phase (mean ± S.D, n=3).

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5.4   Conclusions

The aspect of two individual spray-drying runs: an aqueous spray-drying run followed by an organic run for controlled release is largely governed by the particle size of the primary population produced by the aqueous spray drying session. Any attempts at creating a highly respirable powder may need to involve higher air flow rates than the ones employed in this research. The addition of leucine and ethanol in producing primary particles with a smaller diameter in a less cohesive formulation may be of benefit in producing a more manageable aqueous based powder, which could then be easily dispersed in to the suspension for organic spray drying (Rabbani & Seville, 2005; Li et al., 2005a; Chew et al., 2005b; Najafabadi et al., 2004).

When the dual spray drying method is compared to the double emulsion methods explored in the previous chapters an extension of *in vitro* dissolution is seen, *t*max increasing from 19 to 28 days. However the increase in duration of release was to the detriment of aerosolisation character with a decrease in the average FPF of 58.2% obtained with the low molecular weight chitosan preparations to 47.7% for the dual spray dried formulations. With the potential of *in vivo* bioadhesion removed with the voidance of chitosan from the formulation.

In the four experimental chapters the use of spray drying in the production of sustained release powders for pulmonary delivery has been researched in depth. Using a range of techniques involving open and closed loop spray drying and various types of formulation including solutions, aqueous and organic suspensions and emulsions. In the final chapter a general discussion will attempt to bring together all the research undertaken, highlighting the shortcomings and the

significance of the research undertaken and highlight the implication of the findings on the research area and upon the broader area of disease management.

Chapter 6

General discussion.

Spray drying is widely regarded as a fringe method of manufacture in the process of developing aerosols for drug delivery to the lungs despite the production of narrower size distributions compared to other methods of formulation manufacture such as micronisation because of disadvantages such as high installation costs (Sacchetti & van Oort, 1996, Masters, 1991). The more traditional approach of blending micronised drug with large carrier particle (typically lactose) being the stalwart in the production of dry powder inhaler (DPI) formulations (Timsina et al., 1994), with the key limitations to the implementation of spray drying into the production of DPI formulations being the highly cohesive nature of the powders produced and the high temperatures used giving thermo-degradation of heat labile formulants (Sacchetti & van Oort, 1996; Adjei & Gupta, 1997).

In the first experimental chapter the use of amino acids to counteract the high surface energies of particles produced by spray drying was investigated. Spray dried formulations have previously been documented to give spherical particles but the particles produced had a tendency to 'stack' or aggregate. The use of sucrose, trehalose and other excipients have been shown to reduce the 'stacking' or aggregation of spray-dried particles possibly by reducing spray drying thermal efficiency and giving the surface fracture of micro-particulates which in turn reduced inter-particulate contact points and reduced aggregation (Sacchetti & van Oort, 1996, 1996; Liao et al, 2003; Alexander et al., 1980; Masters, 1991).

Some highlighted research articles on dispersibilty enhancers in typical DPI formulations have utilised erythritol in standard lactose DPI glucagon formulations to achieve higher FPF's, and the use of alternative carriers to lactose such as mannitol (Endo et al., 2005; Steckel & Bolzen, 2004).

Within the spray drying remit dispersibility enhancers such as chitosan, silica and carbopol have been utilised to counteract aggregation to limited success (Li & Birchall, 2006; Takeuchi et al., 2005; Pringels et al., 2005).

Detailed examination of the results showed leucine to produce significantly improved *in vitro* aerosolisation over the control when used at 10, 15 and 20% w/w in a 4% w/w salbutamol in lactose blend, these statistical improvements were not seen in the other four amino acid series assessed in the chapter (arginine, aspartic acid, phenylalanine and threonine). The improvement in aerosolisation enhancement mirrored results from other research groups highlighting an increase in the aerosolisation property of their respective spray dried formulations when incorporating leucine (LEU), however such researchers have failed to produce a mechanism of action for such an improvement in aerosolisation (Chen et al., 2005b; Li et al., 2005a; Najafabadi et al., 2004). The proposed improvement in the aerosolisation of spray dried powders in chapter 2 was attributed to the surfactant like nature of the LEU molecule that conforms to give a polar region consisting of the zwitterions and a non-polar region consisting of the hydrocarbon side-chain. The conformation proved unique to LEU in the five amino acid series of chapter one. And allows LEU to display a degree of surface activity lowering surface tension at air-liquid interfaces (Black & Mould,1991).

$$H_3N^+ \text{----} CH \text{----} COO^-$$
$$|$$
$$CH_2$$
$$|$$
$$CH \text{----} CH_3$$
$$|$$
$$CH_3$$
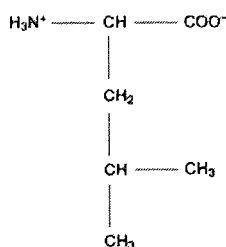
Figure 6.1: The chemical structure of leucine (LEU).

Such action proves to be beneficial in spray drying as it hypothetically reduces the surface tension of the liquid feed upon atomisation allowing the production of a finer spray, the finer spray producing particulates with reduced primary particle size. In addition to the action at the atomiser-air interface LEU also proved to have an extensive effect in reducing spray drying thermal efficiency. The reduction in thermal efficiency through the reduction in suspended droplet surface tension post atomiser whilst residing in the drying chamber of the spray dryer allows greater radiation of inlet heat into the dispersed liquid phase. The dual effect of less surface tension combined with the reduced liquid load of the droplet produces higher outlet temperatures reducing thermal efficiency. The artefact of increased heat permeation into the suspended droplets showcased as a more wrinkled/corrugated particle surface due to the increased evaporative kinetic energy passing through the droplet. The increased surface texture of the LEU modified particles presents less inter-particulate surface contact points for surface forces such as van der Waal forces to act reducing aggregation. It was shown in chapter two that reduced primary particle size gave reduced aggregation with LEU modified powders by using aggregate ratios (LD:d_{ae}), indicating smaller droplets had a lower number of inter-particulate contact points for surface forces to act on and give aggregation. The trend of decreasing size decreasing aggregation going against convention which shows that reducing particle size increases the surface area to volume ratio hence increasing aggregation.

Detailed exploration of the forces underpinning aggregation in dry powder formulations of respirable size is currently being investigated by research groups using a range of techniques including scanning electron microscopy (SEM: as used in this research), ultra-violet fluorescence microscopy, specific sieve techniques, vibration techniques, and centrifugation (Bernard et al., 2002). Of the available techniques used to assess particulate surface forces Atomic force

microscopy (AFM), which involves the use of a nanometer size probe with optical laser recognition of the movement applied by surface forces, is being increasingly used to assess the adhesive and cohesive forces within dry powders for inhalation in addition to the high detailed surface imagery the apparatus was originally intended for (Bernard et al., 2002; Heinz et al., 1999; Louey et al., 2001; Young et al., 2003).

The high *in vitro* deposition shown by leucine powders, especially the 20% w/w leucine formulation (fine particle fraction, FPF >80% of total dose: F <5 μm), would be expected to give high pulmonary deposition with increased higher generation lung targeting therefore allowing the administration of lower drug concentrations to achieve a pharmacological response saving drug costs. The *in vitro* conditions used for testing aerosolisation involved the use of a Spinhaler® DPI device despite the previous relative poor performance of the device that was invented in the 1960's for the pulmonary delivery of a sodium chromoglycate lactose blend; the Spinhaler shown to deliver only 5.5% of the loaded dose to the lungs (Intal: Borgstrom et al., 2002). Even taking into account the discrepancies seen between the use of *in vitro* aerosolisation and *in vivo* animal model tests, which range from a positive correlation (r²) of 0.67 to 1 due to patient factors such as throat geometry (Clark & Borgstrom, 2002; Srichana et al., 2000), the device performs relatively poorly compared to other higher resistance DPI's. Although, it should also be noted that DPI devices do show better *in vitro / in vivo* correlation than pMDI's (pressurised metered dose inhaler; Borgstrom et al., 2002). Therefore, comparison of the aerosolisation data produced by the higher LEU concentration formulation within the second chapter of the thesis can be regarded in high esteem compared to other research undertaken using higher resistance devices. The dependency upon device of a conventional formulation was seen in chapter three

with the reduction from an FPF of 30.1% total dose (TD) of a micronised terbutaline formulation delivered from a Turbohaler® (formulation: Bricanyl®) to an FPF of only 4.53% TD, when the respective formulation was delivered at the same flow rate of 60 L/min but via a hydroxy-propyl methylcellulose capsule loaded into a Spinhaler.

As previously mentioned correlation between *in vitro* aerosolisation and *in vivo* animal model tests range from (r^2) 0.67 to 1 (Clark & Borgstrom, 2002). *In vitro* deposition patterns and aerosolisation data produced by the multistage liquid impinger (MSLI) and the Andersen cascade impactor (ACI) rely up on the impingement of aerosolised material whereas pulmonary deposition differs relying on various methods of deposition. Pulmonary deposition and particle stopping distance in the lungs can be approximated using Stokes' Law which considers the gravitational forces exerted on a falling sphere (Formula 6.1: Richards, 1988).

$$v = \frac{2a^2 g(\sigma - \rho)}{9\eta}$$

Formula 6.1: Stokes law, where a spherical particle of radius a, and density σ, falls through a liquid of density ρ, and viscosity η, at the velocity of sedimentation v, where g, is the acceleration due to gravity.

Depending up on the size of airborne particulates the degree of impaction varies proportionally in the first ten generations of lung bronchi, larger particles impacting higher in the airways (Altiere & Thompson, 1996). Inertial impaction is largely governed by centrifugal forces and is affected by the calibre of the airway (e.g. narrowing of the airways by disease) and the thickness of fluid lining including any mucus plugging of the bronchi (Stocks & Hislop, 2002). In obstructive diseases such as asthma, where the airways narrow, the degree of impaction in the higher part of the lung increases which could affect the delivery of high performing *in vitro* formulations.

Sedimentation is the method of deposition for particulates usually around 3 μm in size from generations 10 plus in the lung and is largely dependent upon residence time, hence the instruction of breath holding delivered to asthmatic patients (Nannini et al., 2006). Diffusion usually occurs with particulates which undergo Brownian motion towards the lower end of a poly-disperse distribution (sub. 1 μm in size) and gives particle deposition between generations 5 to 20 plus in the lung, once again dependent upon particle size, respiratory tract geometry (dependent upon age and disease) and air-stream velocity distribution (Srichana et al., 2000; Martin et al., 2007). The velocity and distribution of the incoming air are determined by the tidal volume and breathing frequency (Nannini et al., 2006). Another discrepancy between lung deposition and the *in vitro* aerosolisation tests is the hygroscopic growth of any airborne particulates as they increase in size with the acquisition of moisture in the increasingly humid environment of the lungs' structure, comparable growth not seen *in vitro* and determined by the equation of *Groom et al.* (Formula 6.2: Groom, et al., 1980).

$$D_e = D \sqrt{\frac{P}{P_e}} \left[1 + \frac{1000}{M.m_e} \right]$$

Formula 6.2: Formulae for the determination of hygroscopic growth: Where D_e is the diameter of the droplet containing the isotonic drug solution up on saturation in the lung, D is the diameter of the original dry particle, P and P_e are the densities of the dry particle and isotonic drug solution, M is the molecular weight of the drug and m_e represents the drugs isotonic molarity (Groom et al., 1980; Gonda, 1988).

Determination of *in vivo* deposition of inhaled particulates can be achieved using the following non-invasive methods (Everard & Dolovich, 2002):

- Planar gamma scintigraphy, using labelled technetium;

- Single photon emission computed tomography (SPECT) utilising gamma camera imaging;

- Positron Emission Tomography – 3D sliced imaging using a radioactive source

  of labelled nitrogen, oxygen, fluorine, carbon or neon.

In vitro aerosolisation tests can also show variation between each other depending on the method employed. Andersen Cascade Impactor (ACI) performance based on the application of viscous materials (e.g. silicone oil) or fibrous filters to the stages of an ACI can affect the slip factor of individual stages, i.e. the particles of sufficiently small size not to impact on a particular stage (Dunbar et al., 2005; Kamiya et al., 2004). Such modifications can alter the correlation of the ACI with the multi-stage liquid impinger (MSLI). The method of ACI plate lining and of aerosolisation as a whole is therefore crucial. Plate overloading of the ACI and particle bounce are also problems that are not as commonly associated with the MSLI (Dunbar et al., 2005). Humidity and sampling are other variables that need to be assumed constant.

The use of chitosan in spray drying to deliver the sustained release of active agents has previously been documented in research into tablet binders (Nunthanid et al., 2004), into implants (Weidenauer et al., 2004), and into a dry powder for nasal administration (Witschi & Mrsny, 1999). Chitosan has also been researched for the lung delivery of spray dried theophylline with reasonable success (estimated FPF F< 5 μm; c. 30%: Asada et al., 2004).

This work showed how chitosan could be used in conjunction with leucine to create highly respirable powders, with the potential of the sustained release of active agents *in vivo*. It was found that an increase in chitosan molecular weight from less than 190 kDa to over 310 kDa could increase the duration of release of the hydrophilic marker terbutaline from 30 to 120 minutes and increase the duration of release of the hydrophobic marker BDP from 60 to 720

minutes: Sustained release is advantageous as the less frequent the dosing the better the compliance with improved therapeutic efficacy akin to more frequent dosing. It was also found that a similar increase in chitosan molecular weight gave deterioration of *in vitro* aerosolisation performance, for instance dual loaded terbutaline and BDP chitosan powders recorded a decrease in FPF from 82.0 to 51.5% of the total dose when chitosan molecular weight was increased from under 190 kDa to over 310 kDa. As detailed in chapter three an FPF of around 50% still classifies the powder as highly respirable compared to marketed counterparts (e.g. Larhrib et al., 1999; Zeng et al., 1999; Taki et al., 2006) and research conducted in the spray drying field (Corrigan et al., 2006a). The results of chapter 3 also show a resounding improvement on previously researched spray dried chitosan formulations designed for lung delivery (Li & Birchall, 2006; Asada et al., 2004).

Upon reaching the human lung the chitosan powders would be expected to undergo very different methods of dissolution to those represented by the *in vitro* tests. The rate of drug dissolution from the chitosan microspheres would originally be dependent on the size of the particle, the smaller the particle diameter the higher the relative surface area, and as smaller particles tend to deposit in the higher numbered generations of the lower lung, higher rates of absorption would be expected to be seen in the smaller respiratory bronchioles. Clearance from the lower lungs is also less efficient compared to the larger airways higher in the lung due to the slower clearance by the mucociliary escalator in these airways as less ciliated columnar epithelia are present, it is therefore advantageous to target such airways as lung residency time can be up to 48 hours (Altiere & Thompson, 1996). The lower lungs also possess less viscous fluid linings of the airways compared to the higher lung bifurcations as the lining is more serous based compared to the goblet cell mucus based secretions in the early generations of lung airway post

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trachea (Kim & Folinsbee, 1997); serous fluid being easier to penetrate and being more hospitable potentially allowing bioadhesive interaction between chitosan and the epithelia cells. One possible disadvantage to targeting the lower airways is the large presence of macrophage (95% of total cell recovery from lower bronchiole sputum), which can reduce the amount of drug diffusing into the epithelia due to the phagocytosis of agents (Altiere & Thompson, 1996).

The delivery of a potentially low mass of formulation to the large surface area of the human lung (130 m^2) may also affect the dissolution time of a chitosan powder loaded with terbutaline and/or BDP. Particulates could potentially be isolated and unable to form the gel seen during *in vitro* dissolution possibly shortening the duration of release (Zambito, 2005).

To predict the efficacy of the presentation of terbutaline and BDP from inhaled chitosan loaded spray-dried microspheres to the lung epithelia and the degree of sustained release *in vivo* the absorption, distribution, metabolism and excretion from the local pulmonary airway has to be considered. Terbutaline is hydrophilic and freely dissolves in the aqueous fluid linings of the lungs whereas BDP has only limited solubility despite the presence of lung surfactants such as phosphatidylcholine derivatives (Hickey & Thompson, 1992). The slow dissolution of BDP could also be potentially enhanced by the presence of the chitosan matrix that could saturate into a gel (if adjacent spheres are present) on the surface of the airways following inspiration.

Regarding pulmonary drug uptake the absorption of terbutaline and BDP via the transcellular route will differ, BDP's high lipophilicity aiding additional diffusion through the phospholipid membrane (Stocks and Hislop, 2002). The vesicular transport/distribution of terbutaline to the

basement interstitum and surface of the smooth muscle cell (the location of β_2 receptors) will also produce a delay in response or an increase in extent of duration (Kim & Folinsbee, 1997). The local action of the corticosteroid BDP in the inhibition of cycloxygenase and lipoxygenase via the membrane bound phospholipase A_2 enzyme and the subsequent duration of smooth muscle relaxation produced by the elimination of prostaglandin and leukotriene mediators should only be enhanced by the slow diffusion of BDP from the chitosan matrices (Hickey & Thompson, 1992). The paracellular route of absorption for both terbutaline and BDP may also be enhanced; chitosan is known to open the tight cell junctions exposing the interstitum (Ciu et al., 2005), which is an advantage in the delivery of terbutaline to the surface of the underlying smooth muscle cells. The absorption from the chitosan matrix of both terbutaline and BDP is dependent upon the close proximity of the vector to the ciliated columnar epithelia cells and the residency time of the formulation, chitosan a known bioadhesive was employed for these reasons (Giannantoni et al., 2006).

Chitosan is a cationic polymer that has been shown to form hydrogen bonds with the anionic cilia membrane of the airway lumen ciliated columnar cells (Grabovac et al., 2005). The use of chitosan has reported to result in a 7-8 h period of mucoadhesion, temporarily resisting the pulmonary mucus velocity of 0.5 to 2.1 cm/min in non-smoking adults (Hickey & Thompson, 1992). Particles delivered to the terminal end of the mucociliary escalator are usually cleared from the respiratory tract with in 24 to 48 h so the addition of a mucoadhesive such as chitosan can prolong the residency and contact time of a sustained release system with a particular site on the airway lumen wall (Kim & Folinsbee, 1997).

The intra-cellular metabolism of agents could potentially be limited by the delivery vector, with extracellular macrophage metabolism of active agents in the peripheral airways potentially retarded or even prevented by the protection of agents in the chitosan vector (if over 2 μm in size).

The aim of delivering a sustained release formulation to the lung is to produce a steady state profile of active agent at the site of action so that periods of toxicity, which are associated which receptor overload and side effects, and areas of sub-therapeutic doses associated with symptom breakthrough can be avoided. If the chitosan formulations show good correlation between *in vitro* and *in vivo* performance and are shown to be biocompatible the consequences would be a far higher dose (dose for dose) being delivered to further reaching parts of the lungs for longer in comparison with other researched and marketed formulations with the bonus of more than one active agent being delivered simultaneously (Larhrib et al., 1999; Zeng et al., 1999; Taki et al., 2006; Corrigan et al., 2006a).

The limitations of using chitosan in a DPI formulation include possible increased hypersensitivity of inflamed airways by the potential interaction of the excipients with hyper-responsive irritant receptors on the surface of the ciliated columnar cells as seen occasionally in asthmatic patients administered BDP (Hyland & Rand. 2002). Chitosan itself although regarded as biocompatible when administered orally may do damage to the lung anatomy with the forcing open of epithelial tight junctions by the polymers ionic charge (Issa et al., 2006). The lung clearance of chitosan would either be via mucociliary transport with particle sizes less than 1 μm that have reached the alveoli and respiratory bronchioles being removed from the serous fluid via macrophage activity

(Soane et al., 1998). Chitosan is relatively resistant to degradation *in vivo* with the human body unable to process the β-orientation of the chitin subunits and is subsequently excreted intact (Saito et al., 2006), showing improved biocompatibility over other synthetic polymers such as vinyl-based compounds which are suspect carcinogens (Mastrangelo et al., 2005).

A further limitation may be that LEU modified respirable powders may be so respirable that mass particulates accumulate in the alveoli, where debris can take over two years to be metabolised by the alveolar macrophages (Stocks & Hislop, 2002), and thus may prevent or deter gaseous exchange. Alternatively plugging may result as a consequence of chitosan gelling in the narrower higher generations of bronchi at the base of the lung. Finally the relatively low pH of the chitosan formulations (≈pH 5.5) due to the use of acetic acid to solubilise the amino-polysaccharide is an issue *in vivo* as the delicate cilia lining and lung fluid balance could be disturbed by an adjustment in pH from the near neutral pH of the lung secretions (≈pH 7.4; Kim & Folinsbee, 1997).

The use of spray dried double emulsions was explored. The double emulsions prepared using polyvinyl alcohol (PVA) showed the importance of limiting emulsion stabiliser/ surfactant concentration with regard to accurate drug loading. The concentration of polylactide co-glycolide (PLGA) also appeared to have an important role in aerosolisation with a decrease in FPF of the PVA spray dried double emulsions from 39.3% TD when 200 mg PLGA 75:25 was contained in the formulation to be spray dried to 14.7% (50 mg PLGA 75:25 utilised). PLGA is a synthetic polymer comprised of two chief constituents lactic and glycolic acid polymerised sub units where

increasing the lactide ratio increases the release time of an encapsulated agent (Figure 6.2:

Blanco et al., 2006).
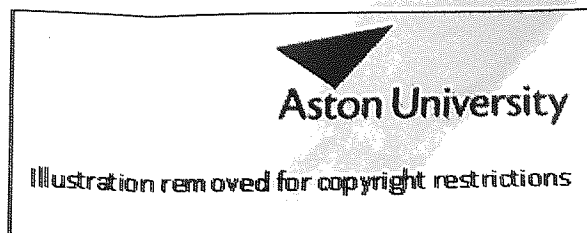
Figure 6.2: The chemical structure of Polylactide co-glycolide (Blanco et al., 2006).

The increase in concentration of PLGA 75:25 in the original double emulsion lead to an increase

in dissolution time (4 - 6 days) as the micro-encapsulation of the primary aqueous and secondary

organic phases probably proved more efficient at the higher concentration.

The use of PVA at high concentrations (93.5% w/v) and the lack of any clear bioadhesive

property limit the possibility of the application of the PVA double emulsions for sustained

pulmonary drug delivery. The substitution of PVA for a far lower concentration of LMW chitosan

and the replacement of PLGA 75:25 by PLGA 50:50 proved to be a major advance in terms of

loaded dose prediction and dissolution with a more measured release of agents.

The chitosan double emulsion spray dried powders gave FPF's of around 60%, which as

explained earlier, indicates that the formulations are highly respirable. As with the chitosan

formulations of chapter 3, LEU modification is attributed for the good aerosolisation

characteristics of the spray-dried chitosan containing emulsions in chapter 4. The powders can

be seen as an improvement to the chitosan spray dried powders seen in chapter 3 with the major

modification being the placing of agents in different phases of an emulsion either side of a PLGA 50:50 organic layer to give greater control over a longer release profile of up to 19 days. The formulation once deposited on the lung epithelia would be expected to deliver agents in a similar mechanism to the chitosan spray dried formulations with one key difference. If and when the bioadhesive attractive forces anchor the chitosan shell of the particulates to the epithelial walls of the lower and mid airways, the erosion of PLGA 50:50 pockets and subsequent diffusion of any hydrophilic drug molecules from within the primary aqueous phase and hydrophobic drug molecules from the organic secondary phase would have to occur (Figure 6.3). A further advantage of the chitosan spray dried double emulsions (chapter 4) over the chitosan spray dried formulations (chapter 3) is the encapsulation of BDP inside an organic PLGA 50:50 layer, as undisguised inhaled corticosteroid powder is known to give aggravation of any underlying bronchial hyper-responsiveness, a major problem when the focus of a formulation is to treat such a condition, the encapsulation of the drug can be perceived as beneficial (Smith & Bernstein, 1996).
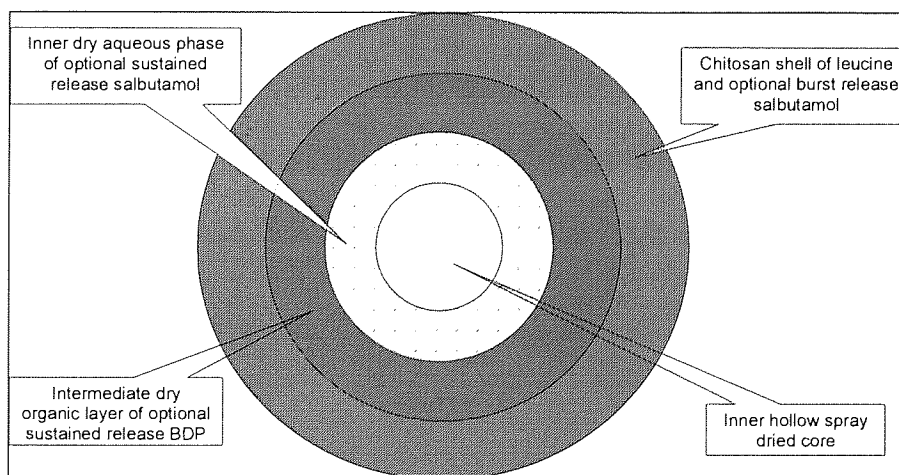
Figure 6.3: Illustration of the hypothetical structure of spray dried double emulsions utilizing the GRAS polymers chitosan and PLGA 50:50.

The structural integrity of the micro-particulates appeared to stay intact during *in vitro* dissolution testing with the appearance of the characteristic chitosan gel and the release of agents over a 19-day period. However, the cilia (6 μm in length, 0.2 μm in diameter) of the 50,000 plus target bronchioles perpetuate in an upward motion at 1000 beats per minute, a velocity far faster than the 50 rpm of the rotating basket used during *in vitro* dissolution (Kim & Folinsbee, 1997). The intense movement could theoretically damage the integrity of the micro-particulates during residence in the lung affecting dissolution with the possibility of dose dumping.

The release from the chitosan emulsion spray dried particulates depended upon the phase location of the agent. All drug molecules regardless of phase had to navigate the matrix of the chitosan gel layer, with hydrophilic entities contained in the primary and hydrophobic entities in the secondary layer also having to diffuse slowly through the slowly eroding PLGA 50:50 polymer. The influences of the erodable PLGA layer and matrix release of chitosan upon the dissolution of terbutaline and BDP from the chitosan emulsion formulations correlated with first order and Higuchi release kinetics suggesting the inclusion of the two polymers, PLGA might confer a first order release and chitosan the Higuchi matrix kinetic. Despite a concentration peak at 19 days 100% recovery of total drug load was not achieved by these formulations, possibly due to method dilution factors, indicating the very slow release of agents from pockets of PLGA 50:50.

The long dissolution period *in vitro* (19 days) may not be attainable *in vivo* due to the limited bioadhesive property of chitosan, reported as 7.5 to 8 h (Grabovac et al., 2005). Even with deposition of the formulation in the deep lung the total residence of finer particulates could not be

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envisaged as being longer than 56 hours (48 hours being the longest journey along the mucociliary escalator, respiratory bronchioles to oropharynx: Kim & Folinsbee, 1997), although once a day dosing remains a real possibility.

The pH of the chitosan emulsion formulations before spray drying (c.pH ≈ 6.3) proved more agreeable than the chitosan containing formulations (c.pH ≈ 5.5) with the average pH of lung secretions (c.pH ≈ 7.4: Kim & Folinsbee, 1997). However, problems with chitosan gel plugging of narrower airways and the possible restriction of gaseous exchange if large amounts of the highly respirable particulates amass in the alveoli remain a concern.

The final experimental chapter focused on a dual spray drying process. Some of the limitations of this method have already been discussed at the end of the respective chapter concerning the use of higher spray drying airflow rates exceeding the standard operating process variable of 600L/h to reduce primary diameter size, and the use of leucine to de-aggregate particulates in the primary aqueous spray-drying portion. The formulations also showed poor stability with large agglomerates forming over three months storage visible by SEM, DSC also revealing the partial re-crystallisation of the formulations. Despiite the restraints, this method highlighted many possibilities for the encapsulation of agents that are difficult to formulate using simple aqueous spray drying. The technique involving two steps, open-cycle spray drying followed by closed cycle-spray drying, proved time-consuming producing variable physical characteristics. The aerosolisation results, although good in comparison to marketed typical DPI formulations and other researched spray dried formulations (Larhrib et al., 1999; Zeng et al., 1999; Taki et al.,

2006; Corrigan et al., 2006a) were comparatively poor (FPF up to 56% TD) compared to the chitosan formulations of chapter two (FPF up to 82% TD).

The dissolution of the dual spray dried formulations of chapter five all delivered the hydrophilic marker salbutamol and the hydrophobic marker BDP over and possibly beyond 28 days. The length of *in vitro* dissolution may not replicate to drug plasma levels however after pulmonary absorption despite the relative respirability of the dual spray dried formulations. The lack of a recognised bioadhesive in the formulation to anchor micro-particulates reaching the high generations of the lung mean that a maximum residency time of 48 h would be expected; assuming maximal delivery of the inspired formulation to the far end of the mucociliary escalator (Kim & Folinsbee, 1997).

With the demand for more convenient regimens in the treatment of restrictive airway disease and the advances made in the formulation of DPI devices the aim of this thesis was to produce highly respirable sustained release dry powders, which could potentially improve patient compliance and the disease management of restrictive airway disease.

The thesis objectives were as follows:

- To produce highly reproducible stable dry powder formulations using spray drying

- To produce highly respirable spray dried powders using spray drying

- To produce highly respirable spray dried powders with a degree of sustained release

- To produce multiple drug loadings within the same highly respirable controlled release powder

- To gain greater insight into the factors which govern the spray drying of respirable powders

The first objective of achieving reproducibility in physical, aerosol and solvate property has been achieved in the spray drying of controlled release powders throughout this thesis. However, the stability of the dual spray dried powders in chapter 5 was questionable, with a tendancy for particulates to undergo re-crystallisation on storage. In addition, the appearance of strands on LEU-modified chitosan spray-dried particulates and the discoloration of these samples although not appearing to affect aerosolisation in the stability study are also concerns.

High respirability was achieved in chapter 2 using the amino acid LEU, the greater understanding of leucine's affect on spray drying conditions and the powder produced enabled the production of highly dispersible and highly respirable chitosan based powders in chapter 3 and chapter 4. Conversely the production of spray dried powders with not as promising aerosolisation character which utilised polyvinyl alcohol (PVA) and a dual spray drying process showed the necessity of utilising an aerosolisation enhancer such as LEU in the production of controlled release spray dried powders.

The third objective of achieving sustained release was achieved with the use of polylactide co-glycolide (PLGA) variants in the later chapters. PLGA showed the ability to give the sustained release of hydrophilic agents over 28 days depending on formulation whereas previously examined chitosan only gave a 2 hour *t*max for a similar agent. However PLGA is not known to

be bioadhesive where as chitosan is, the double emulsions formed with a PLGA layer in a chitosan envelope in chapter 4 addressed this balance.

The double emulsion method of chapter 4 recognised the fourth objective of multiple drug loadings with the burst and sustained release of two agents depending up on which layer the drug was incorporated. The dual release of agents from the same formulation in this manner is thought to be unique and with the utilisation of the aerosolisation agent LEU in a tertiary layer of chitosan the double emulsion spray dried powders were both highly respirable and potentially bioadhesive (Illum, 1998).

Overall this thesis brings greater understanding to the process of producing spray-dried powders for inhalation, the greater understanding of the implications of thermal efficiency on the spray drying process possibly enabling the production of more highly respirable powders in future research. Studies into particle surface roughness have proved inconclusive in finding a root cause to the characteristic regarded as desirable in spray-dried powders for inhalation due to the enhancement of aerosolisaton (Chew et al., 2005a, 2005b; Rabanni & Seville, 2005; Li et al., 2005a). The excessive penetration of heat into dispersed liquid feed caused by excessive inlet temperatures or by the inclusion in an appropriate surface active agent has the potential to improve the aerosolisation of formulations previously regarded as poorly respirable. This thesis also suggested a mechanism of action of LEU in reducing primary particle diameter and aggregation, through surfactant activity at the atomiser-air interface and high heat evaporation in the drying chamber respectively, providing greater understanding to previous researchers work (e.g. Najafabadi et al., 2004; Chew et al., 2005a; Rabanni & Seville, 2005; Li et al., 2005a).

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In general the use of *in vivo* animal studies in realising the full potential of the controlled release spray dried powders could provide interesting further work and insight into the feasibility of the formulations developed during these *in vitro* studies. Small model studies offering clearance and adsorption data. The use of higher resistance dry powder inhalers over the Spinhaler would also give an extra dimension to the work.

Further work which could have been addressed following on from the work in chapter 2 includes the use of other surfactants which are solid at room temperature in the production of respirable spray dried particles, using the LD:d_{ae} aggregation ratio as a tool in assessing the surfactant's suitability whilst monitoring the surfactant's effect on dryer thermal efficiency. With the extra knowledge gained with regards to the thermal activity during spray drying the introduction of higher inlet temperatures to decrease thermal efficiency and give higher dry particulate surface fracture and improved aerosolisation could be explored.

Extensions to the development of controlled release studies for respirable powders would include the development of more appropriate *in vitro* dissolution models that more closely equate to *in vivo* lung conditions, e.g. potential sink conditions in low volumes of fluid, to test the feasibility of respirable sustained release formulations. With regard to the controlled release of spray dried powders the production and utilisation and testing of more latent bioadhesives would provide an interesting extension.

The stability of some of the spray-dried powders was in question; the omission of lactose from chitosan formulations may prevent the acid hydrolysis of the disaccharide and prevent any discolouration over time as seen with the spray dried chitosan formulations. Furthermore the

introduction of a biocompatible plasticizer in the organic spray drying section of the dual spray drying process could prevent the partial re-crystallisation of the formulations developed in chapter 5.

The work in this thesis focuses on one method of producing controlled release formulations for inhalation. Other methods of producing controlled release in inhalation could equally be explored such as the use of pro-drugs (Zhou et al., 2002), liposomal technology employed via liquid nebulized formulation or via a dry powder form (Vermehren et al., 2006; Mohammed et al., 2007), solvent evaporation methods in producing dry powder formulations (Huo et al., 2005) or through the selective targeting of ligand bound agents.

The overall aim of the thesis was to achieve highly respirable sustained release formulations. Through the co-spray drying of hydrophilic and/or hydrophobic markers with the aerosolisation enhancer leucine and the polymer/s chitosan and/or polylactide co-glycolide in either one or two stage processes dry powders with good reproducibility were formulated. The successfully spray dried powders exhibited high respirability (>82% FPF) with sustained release of up to 28 days *in vitro*.

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Appendices

Appendix 1.
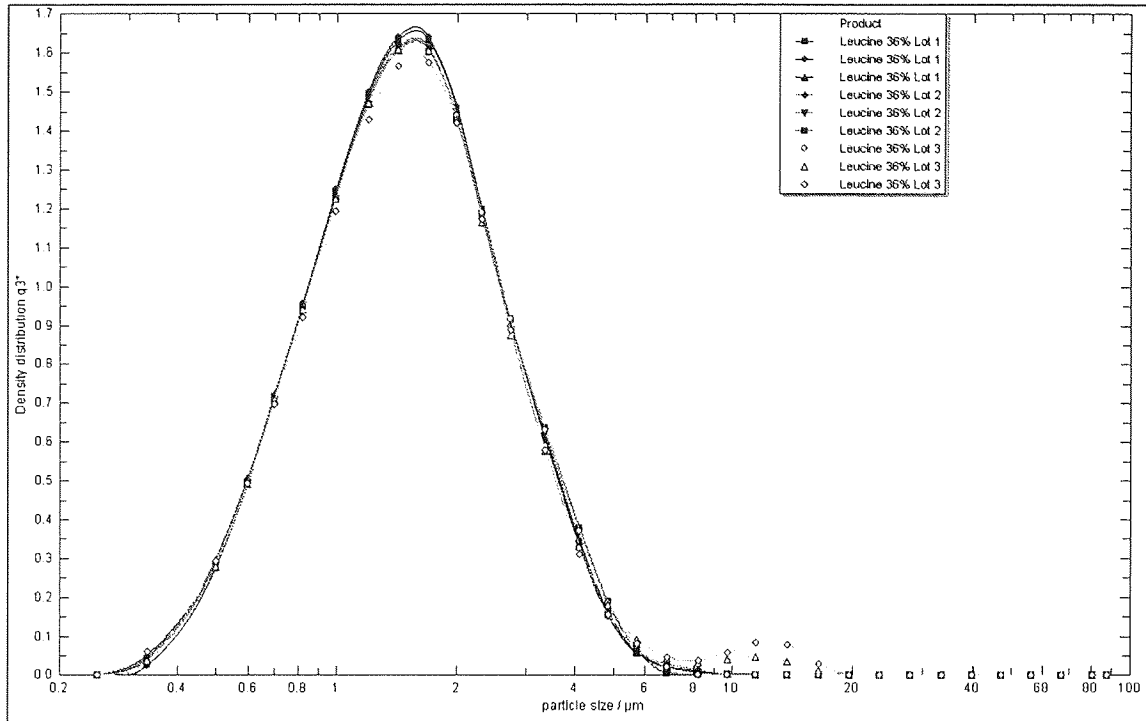
Appendix 1:   Sympatec illustration of the particle size distributions of three separate spray dry runs of 4% w/w salbutamol, 36% w/w leucine and 60% w/w lactose 2%w/v in 30% v/v aqueous ethanol used to high light the reproducibility of the Büchi B290 system. The spray drying yields of the three lots being 74%, 73% and 75% of the anticipated respectively using the high performance cyclone. The dry powder yields and the particle sizes identified showing the high reproducibility of the Büchi B290 mini lab spray drier.

Appendix 2.
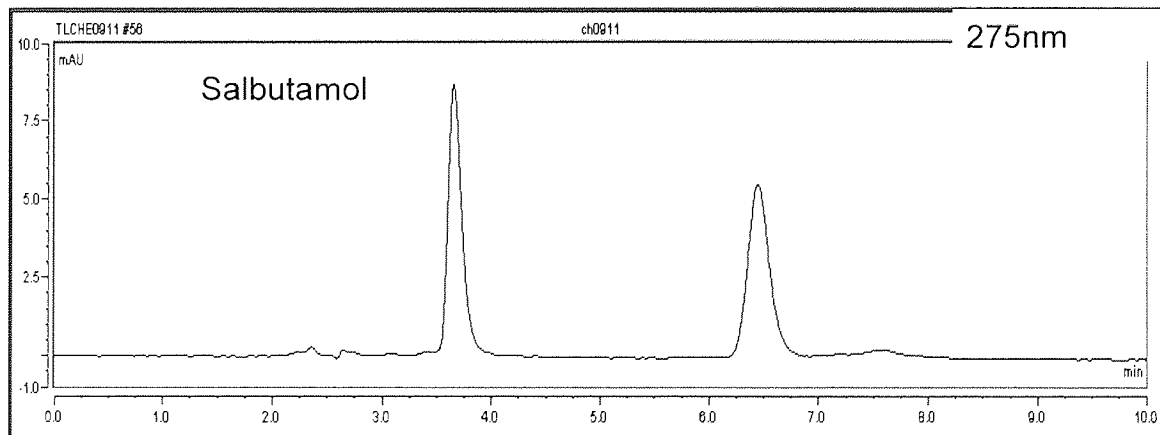
Appendix 2A: A trace of a 5mcg/ml salbutamol sulfate elution in a 15% aqueous methanol mobile phase using reverse-phase high performance liquid chromatography (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6 x 150 mm Phenomomex La Luna column (Phenomomex, USA) and 20 µl injection volume with UV detection at 275 nm.
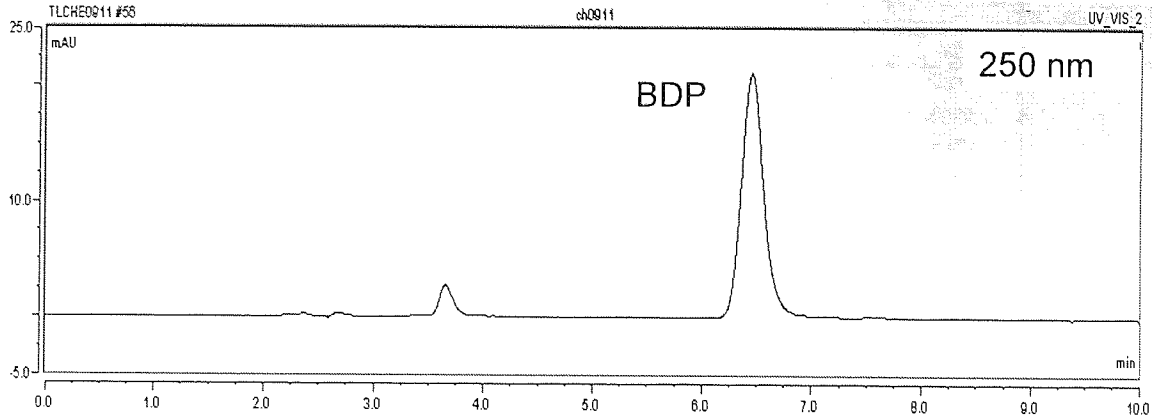
Appendix 2A: A trace of a 5mcg/ml betametasone dipropionate elution in a 15% aqueous methanol mobile phase using reverse-phase high performance liquid chromatography (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6 x 150 mm Phenomomex La Luna column (Phenomomex, USA) and 20 µl injection volume with UV detection at 275 nm.

Appendix 3.

Appendix 3: An example of linear regression of dissolution data from a spray dried sample containing 4% w/w terbutaline 36% w/w leucine, 50% w/w chitosan HMW and 10% w/w lactose. Dissolution performed as outlined in the methods section of chapter 3.

standard conc	UV abs				
(mcg/ml)	1	2	3	av	sd
5	0.11	0.11	0.11	0.11	0.00
10	0.12	0.11	0.13	0.12	0.01
15	0.16	0.13	0.16	0.15	0.02
20	0.18	0.18	0.19	0.18	0.00

Appendix 3.1: Terbutaline standards tested with UV radiation using a Jenway 6305 UVvis spectrophotometer (Jenway, Dunmow, Essex, UK).
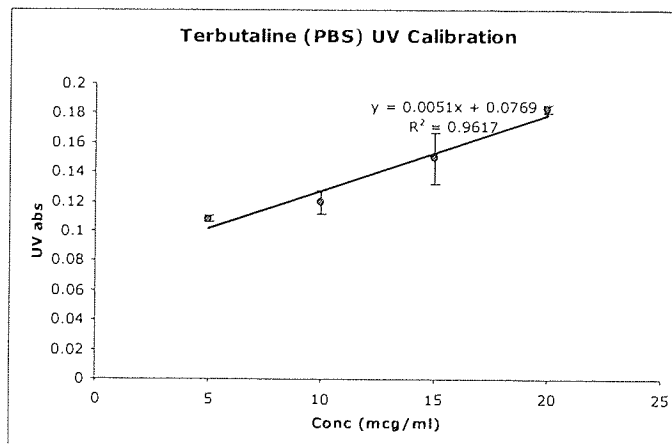

Appendix 3.2: An example of a terbutaline UV calibration set against standards displayed in Appendix 3.1.

Time (min)	UV abs				
	1	2	3	av	sd
0	0.00	0.00	0.00	0.00	0.00
2	0.09	0.11	0.07	0.07	0.02
5	0.07	0.12	0.10	0.10	0.02
10	0.09	0.14	0.13	0.12	0.02
20	0.14	0.16	0.13	0.14	0.01
30	0.15	0.18	0.19	0.17	0.03
60	0.21	0.22	0.19	0.21	0.01
120	0.29	0.28	0.27	0.28	0.01
240	0.25	0.28	0.30	0.28	0.03

Appendix 3.3: UV absorption readings of terbutaline elution released in to 1000 mL of phosphate buffer solution (PBS) by the specified chitosan formulation taken by the same Jenway 6305 UVvis spectrophotometer at the time points indicated.
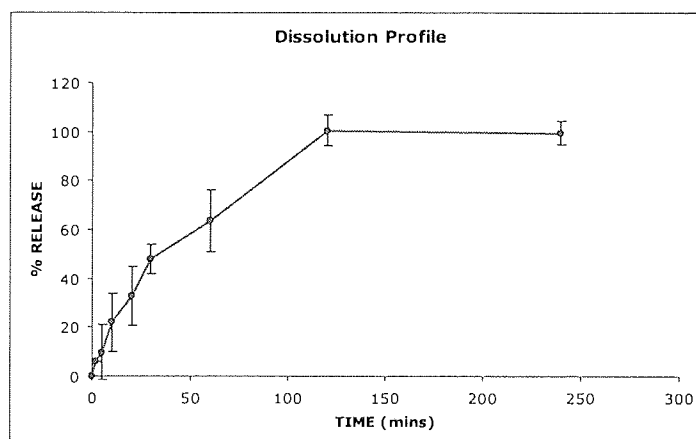
Appendix 3.4: Dissolution profile of terbutaline release obtained from the data from Appendix 3.3 interpretated in Appendix 3.5 as a percentage of the total dose displayed in Appendix 3.6.

Time (min)	% RELEASE				
	1	2	3	av	stdev
0	0.00	0.00	0.00	0.00	0.00
2	4.51	17.89	-4.41	6.00	11.22
5	-2.43	21.36	10.46	9.79	11.91
10	8.47	32.26	25.32	22.02	12.23
20	30.28	39.69	28.29	32.75	6.09
30	33.74	52.57	57.53	47.95	12.55
60	65.46	68.93	56.54	63.64	6.39
120	105.10	100.64	95.68	100.47	4.71
240	87.26	99.15	112.03	99.48	12.39

Appendix 3.5: The release of terbutaline from the specified chitosan formulation in to 1000 mL PBS expressed as a percentage of total dose outlined in Appendix 3.6.

mass AV.	UV abs AV.	Terbutaline conc	Terbutaline mcg per mg of powder	in 200mg
24.83	0.58	982.55	39.57	7914.21

Appendix 3.6: The calculation of the total loaded dose expected in 200 mg of the specified chitosan spray dried formulation calculated from the relevant calibration line (Appendix 3.2) and an approximate 25 mg aliquot of spray dried sample.

Time (min)	Q (mcg)				
	1	2	3	av	st dev
0	0.00	0.00	0.00	0.00	0.00
2	356.86	1415.69	-349.02	474.51	888.22
5	-192.16	1690.20	827.45	775.16	942.27
10	670.59	2552.94	2003.92	1742.48	968.03
20	2396.08	3141.18	2239.22	2592.16	481.89
30	2670.59	4160.78	4552.94	3794.77	993.12
60	5180.39	5454.90	4474.51	5036.60	505.77
120	8317.65	7964.71	7572.55	7951.63	372.72
240	6905.88	7847.06	8866.67	7873.20	980.65

Appendix 3.7: The data extrapolated in Appendix 3.8 calculated from the terbutaline release from the spray dried chitosan formulation related to zero order kinetics.
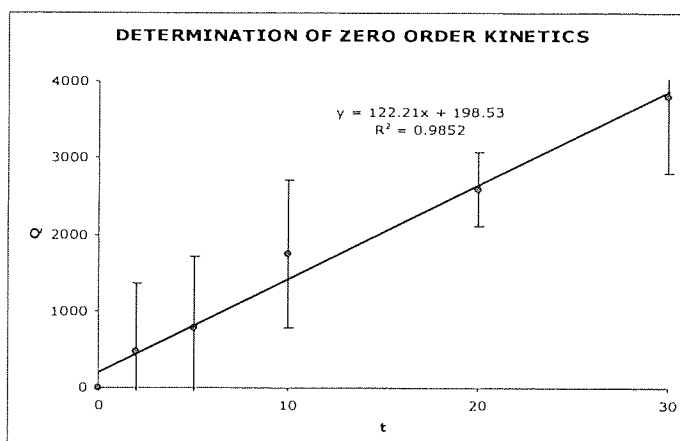
Appendix 3.8: Data from appendix 3.7 represented as zero order using linear interpolation.

Time (min)	C (conc remaining in matrix.)			log Co/C				
	1	2	3	1	2	3	Av	sd
0	7914.21	7914.21	7914.21	0.00	0.00	0.00	0.00	0.00
2	7557.35	6498.52	8263.23	0.02	0.09	0.00	0.04	0.04
5	8106.37	6224.01	7086.76	0.00	0.10	0.05	0.05	0.05
10	7243.62	5361.27	5910.29	0.04	0.17	0.13	0.11	0.07
20	5518.13	4773.03	5674.99	0.16	0.22	0.14	0.17	0.04
30	5243.62	3753.42	3361.27	0.18	0.32	0.37	0.29	0.10
60	2733.82	2459.31	3439.70	0.46	0.51	0.36	0.44	0.07
120	0.00	0.00	341.66	0.00	0.00	1.36	0.45	0.79
240	1008.33	67.15	0.00	0.89	2.07	0.00	0.99	1.04

Appendix 3.9: The data extrapolated in Appendix 3.10 calculated from the terbutaline release from the spray dried chitosan formulation related to first order kinetics.
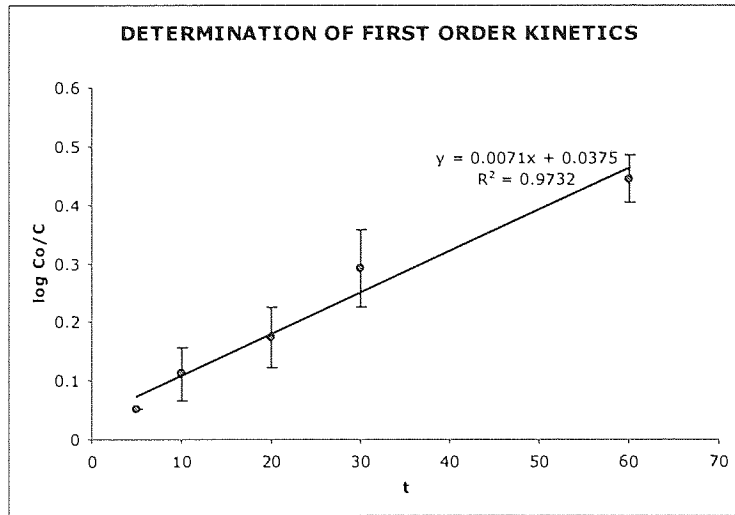
DETERMINATION OF FIRST ORDER KINETICS

$y = 0.0071x + 0.0375$
$R^2 = 0.9732$

Appendix 3.10: Data from appendix 3.9 represented as first order using linear interpolation.

	Q (mcg)				
t^1/2	1	2	3	av	st dev
0.00	0.00	0.00	0.00	0.00	0.00
1.41	356.86	1415.69	-349.02	474.51	888.22
2.24	-192.16	1690.20	827.45	775.16	942.27
3.16	670.59	2552.94	2003.92	1742.48	968.03
4.47	2396.08	3141.18	2239.22	2592.16	481.89
5.48	2670.59	4160.78	4552.94	3794.77	993.12
7.75	5180.39	5454.90	4474.51	5036.60	505.77
10.95	8317.65	7964.71	7572.55	7951.63	372.72
15.49	6905.88	7847.06	8866.67	7873.20	980.65

Appendix 3.11: The data extrapolated in Appendix 3.12 calculated from the terbutaline release from the spray dried chitosan formulation related to Higuchi matrix order kinetics.

DETERMINATION OF HIGUCHI KINETICS

$y = 590.24x + 18.376$
$R^2 = 0.9352$
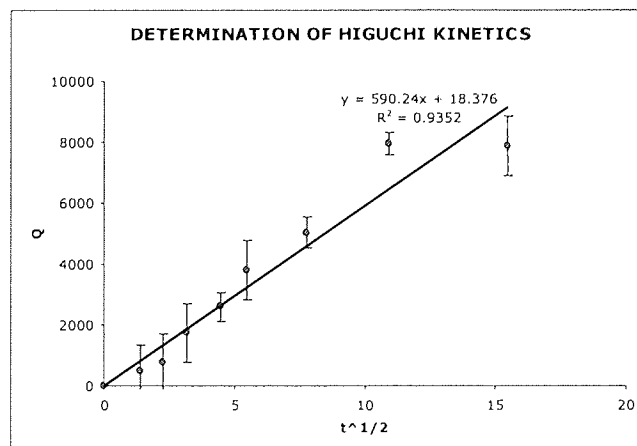
Appendix 3.12: Data from appendix 3.11 represented as first order using linear interpolation.

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The closer the r^2 correlation coefficient to 1 the more relative the release kinetic to the data (full data for the leucine modified chitosan series is contained in chapter 2, section 3.3.2.3).

Appendix 4.

The author's work on completing this thesis has lead to various accolades and publications, which are listed below:

Patents:
Learoyd, T.P., Seville, P.C., 2006. Respirable powders. International Patent Application (PCT/GB2006/003184) filed 25 August 2006, claiming priority from GB 0524194.8 filed 28 November 2005.

Prizes:
Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. APSGB commended poster, British Pharmaceutical conference.

Published research papers:
Seville, P.C., **Learoyd, T.P.,** Li, H.Y., Williamson, I.J., Birchall, J.C., 2007. Amino acid modified spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Accepted for publication. Powder Technology.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2007. Chitosan-based spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Accepted for publication. Eur. J. Pharm. Biopharm.

Seville P.C., Li H-Y., and **Learoyd T.P.,** 2007. Spray-dried powders for pulmonary drug delivery. Invited review for Critical Reviews in Therapeutic Drug Carrier Systems. Accepted for publication.

Learoyd T.P., Burrows J.L., French E. and Seville P.C., 2007. Sustained release of beclometasone dipropionate from chitosan-based spray-dried respirable powders. Submitted.

Learoyd T.P., Burrows J.L., French E. and Seville P.C., 2007. Sustained release profile exhibited by spray-dried chitosan-based respirable powders containing terbutaline sulfate and beclometasone dipropionate. In preparation.

Learoyd T.P., Burrows J.L., French E. and Seville P.C., 2007. Double emulsion sustained release spray dried powders for inhalation. In preparation.

Learoyd T.P., Burrows J.L., French E. and Seville P.C., 2007. Sustained release powders for inhalation produced by a novel two stage spray drying process. In preparation.

Published abstracts:

El-Haffar, Y., **Learoyd, T.P.**, Seville, P.C., 2005. Spray dried powders for pulmonary drug delivery: comparison with marketed products. J. Pharm. Pharmacol.18: 251.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. Chitosan based spray dried respirable powders for sustained drug delivery. J. Aerosol Med. 19: 232.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. Influence of chitosan molecular weight on drug release from respirable spray dried powders. J. pharm. Pharmacol. 58: A15-16.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. Spray dried w/o/w double emulsions can generate respirable powders for sustained drug delivery. J Pharm. Pharmacol. 58: A15.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. Sustained drug release from respirable powders prepared by spray drying w/o/w double emulsions containing PLGA, chitosan and leucine. J. Pharm. Pharmacol. 58: A67-68.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006. Spray dried respirable powders for sustained drug delivery. J. Aerosol Med. 20: 144.

Learoyd T.P., Burrows J.L., French E. and Seville P.C, 2007. Novel respirable powders for sustained drug release. Program and Proceedings of the ISAM 16th International Congress.

Learoyd T.P., Burrows J.L., French E. and Seville P.C, 2007. A novel process for preparing respirable powders for sustained drug release. Program and Proceedings of the ISAM 16th International Congress.

Learoyd T.P., Burrows J.L., French E. and Seville P.C. Controlled release formulations for pulmonary drug delivery. J. Pharm. Pharmacol. 2007; 59(Suppl. 1): A-70 – A-71.

Oral presentations:

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C. October 2005. Chitosan spray-dried respirable powders for sustained drug delivery. Pfizer, Sandwich.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., December 2005. Spray dried powders for pulmonary drug delivery. Edinburgh.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., September 2006. Sustained drug release from respirable powders prepared by spray drying w/o/w double emulsions containing PLGA, chitosan and leucine. British Pharmaceutical Conference, Manchester.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., November 2006. Spray dried respirable powders for sustained drug delivery. Drug delivery to the lungs, Edinburgh.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., March 2007. Preparation of respirable powders for sustained drug release by a novel double spray-drying technique. APS Inhalation, Bath.

Poster presentations:

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., October 2005. Chitosan spray-dried respirable powders for sustained drug delivery. Pfizer, Sandwich.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., December 2005. Spray-dried chitosan respirable powders for sustained drug delivery. Drug delivery to the lungs, Edinburgh.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., January 2006. Controlled release spray-dried powders for pulmonary drug delivery UKICRS, Loughborough.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., April 2006. Chitosan-based spray-dried respirable powders for sustained drug delivery. Respiratory Drug delivery X, Boca Raton.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., April 2006. Leucine modified PLGA-based spray dried powders for sustained drug delivery. Respiratory Drug delivery X, Boca Raton.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., September 2006. Influence of chitosan molecular weight on drug release from respirable spray dried powders. British Pharmaceutical Conference, Manchester.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., September 2006. Spray dried w/o/w double emulsions can generate respirable powders for sustained drug delivery. British Pharmaceutical Conference, Manchester.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., September 2006. Sustained drug release from respirable powders prepared by spray drying w/o/w double emulsions containing PLGA, chitosan and leucine. British Pharmaceutical Conference, Manchester.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., January 2007. Spray dried respirable powders for sustained drug delivery. UKICRS, Nottingham.

Learoyd, T.P., Li H.Y., Birchall, J., Seville P.C., March 2007. The use of amino acids in spray drying formulations for pulmonary drug delivery. APS Inhalation, Bath.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., March 2007. Preparation of respirable powders for sustained drug release by a novel double spray-drying technique. APS Inhalation, Bath.

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