Novel Phospholipid-Based Pressurised Metered Dose Inhaler Formulations

A thesis presented by

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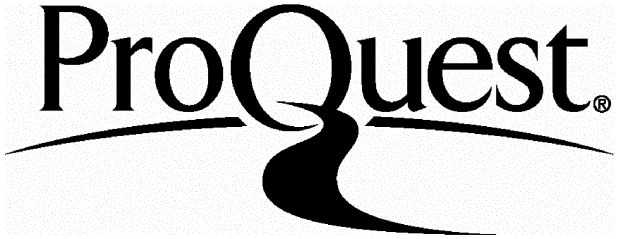
This thesis describes research conducted in the School of Pharmacy, University of London between 2003 and 2007 under the supervision of Prof. Kevin M.G. Taylor and Prof. Ian W. Kellaway. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature **17 December 2007**



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ABSTRACT

A novel formulation which involves the use of phospholipids and ethanol has been shown to produce physically stable pressurised metered dose inhaler (pMDI) solutions. Two model drugs salbutamol base and budesonide were soluble in the investigated amounts of phosphatidylcholine (PC), ethanol and 1,1,1,2-tetrafluoroethane (HFA 134a). A one phase solution system was formed in the propellant. Extending delivery of therapeutically active agents in the optimum quantity, at a desired location within the respiratory tract is the principal aim of inhalation therapy. This was achieved with the right combination of PC and cosolvent. Both drugs were entrapped within spontaneously formed liposomes *in vitro*; the rate of efflux from the liposomes determined drug availability. The results obtained from the current investigation can serve as a model for an approach that can be used to optimise phospholipid-containing pMDI formulations, thus enabling formulation of a therapeutically effective phospholipid-containing solution pMDI.

In a separate study, the stability of 1H,1H,2H,2H-perfluorooctan-1-ol (PFOH) and PEG-phospholipid suspension pMDIs containing budesonide, formoterol fumarate or terbutaline sulphate suspended in HFA 134a was investigated. The focus was on the suspension stabilising abilities of the fluorinated alcohol, PFOH. All formulations formed a stable suspension system. Settling properties of these formulations were investigated as a function of cosolvent PFOH concentration. The settling kinetics were examined by Turbiscan measurements and were also investigated with the naked eye at different temperatures. At the lowest PFOH concentration used (5% w/w), sedimentation and creaming were observed. Correspondingly, the higher PFOH concentration (15% w/w) resulted in increased 10^{th}, 50^{th} and 90^{th} percentiles (Dv10, Dv50, and Dv90) undersize values for HFA-based suspension pMDIs compared to 5% and 10% w/w PFOH, as determined using a Malvern Spraytec. PFOH cosolvent successfully stabilised PEG-phospholipid suspension pMDIs and led to the production of a finer suspension, reduction of particles adhesion to the can walls and inhibition of particle flocculation. The spray performance of PFOH and PEG-phospholipids suspension pMDIs was investigated. All formulations produced aerosol clouds in the respirable range. Suspensions containing PEG-phospholipid and PFOH produced a significantly larger ($P \leq 0.05$) stage 2 deposition in the twin impinger (TI) compared to drug alone in HFA 134a. All formulations produced aerosol clouds in the respirable range. Incomplete evaporation of PFOH may cause a reduction in fine particle fraction (FPF).

LIST OF ABBREVIATIONS

ACI	Andersen Cascade Impactor
ANOVA	Analysis of variance
APS	Aerosol Particle Sizer® or aerosol particle sizing
ARDS	Adult respiratory distress syndrome
BDP	Beclometasone dipropionate
BNF	British National Formulary
BS	Backscattering
CFC	Chlorofluorocarbon
COPD	Chronic obstructive pulmonary disease
CR	Controlled release
DCP	Dicetylphosphate
DFP	2H,3H-Decafluoropentane
DLS	Dynamic light scattering
DLVO	Derjaguin, Landau, Verwey and Overbeek
DMPC	Dimyristoylphosphatidylcholine
DPI	Dry powder inhaler
DPPC	Dipalmitoylphosphatidylcholine
DSPE-PEG-DSPE	1,2-Distearoyl-sn-glycero-3-phosphatidyletanolamine-methyl-poly(ethylene)glycol-1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine
Dv10, Dv50, Dv90	10^{th}, 50^{th}, 90^{th} percentile or 10%, 50%, 90% undersize
ECD	Effective cut-off diameter
EPC	Egg phosphatidylcholine
FPF	Fine particle fraction
FPM	Fine particle mass
GMO	Glycerol monooleate
GSD	Geometric standard deviation
HFA 134a	1,1,1,2-Tetrafluoroethane
HFA 227	1,1,1,2,3,3,3-Heptafluoropropane
HFA	Hydrofluoroalkane
HPLC	High performance liquid chromatography
IgE	Immunoglobulin E
LogP	Partition coefficient
LSD	Least Significance Difference
LUV	Large unilamellar vesicle
LW	London–van der Waals
LysoPC	Lysophosphatidylcholine
MLV	Multilamellar vesicle
MMAD	Mass median aerodynamic diameter

MMI	Marple-Miller Impactor
mPEG-DMPE	Methyl-poly(ethylene)glycol-1,2-dimyristoyl-sn-glycero-3-phosphatidylethanolamine
mPEG-DSPE	Methyl-poly(ethylene)glycol-1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine
MSLI	Multi-stage liquid impinger
MVV	Multivesicular vesicle
NRDS	Neonatal respiratory distress syndrome
P	Probability value
P-113	Trichlorotrifluoroethane
PC	Phosphatidylcholine
PCS	Photon correlation spectroscopy
PE	Phosphatidylethanolamine
PEF	Peak expiratory flow
PEG 300	Polyethylene glycol 300
PEG 400	Polyethylene glycol 400
PEG	Polyethylene glycol
PFOH	1H,1H,2H,2H-Perfluorooctan-1-ol
PI	Phosphatidylinositol
PLA	Poly(L-lactic acid)
PLGA	Poly(lactide-co-glycolide)
pMDI	Pressurised metered dose inhaler
PS	Phosphatidylserine
PTA	Phosphotungstic acid
RDS	Respiratory distress syndrome
RF	Respirable fraction
RhDNase	Dornase alpha
RI	Refractive index
SD	Standard deviation
SP-A/B/C	Surfactant proteins A, B and C
Span 85	Sorbitan trioleate
Span	(90% undersize – 10% undersize)/ 50% undersize
SPC	Soya phosphatidylcholine
SSGI	Single stage glass impinger
SUV	Small unilamellar vesicle
T	Transmission
TEM	Transmission electron microscopy
TI	Twin impinger
TOF	Time-of-flight
VMD	Volume median diameter
ΔG	Free energy

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Pulmonary drug delivery

Growing attention in pulmonary drug delivery has been fuelled by the emergence of inhaled systemically active molecules and global concern over the increased incidence of respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD). The incidence of these chronic lung diseases has increased significantly in the past decade. This has been paralleled by an increase in the prescriptions of drugs for these conditions (Strassels *et al.,* 2001). The lung is the target organ for the treatment of local respiratory diseases and is a potential administration route for systemic treatments, which makes it an attractive organ for drug delivery (Labiris and Dolovich, 2003a). There are many advantages associated with the pulmonary route over other delivery routes: treatment delivery by inhalation uses the close proximity of the alveoli to blood flow and their extensive surface area (approximately 100 m^2); it avoids gastrointestinal/first-pass metabolism which means that lower doses are required for a therapeutic effect compared with oral doses and fewer side-effects occur. Moreover, the inhalation route is an alternative to injection and it provides an option for safe, painless drug delivery to the systemic circulation, especially for macromolecules such as the newer class of protein and polypeptide drugs (Brown, 2002). However, pulmonary delivery is a complex and challenging process, as it requires a safe and efficacious drug, a carrier system, the design and engineering of a device and its correct use by the patient.

Currently, new drugs, peptides, proteins, and gene-based therapies for treating local and systemic diseases are the focus of research in pulmonary drug delivery. The delivery of drugs via the pulmonary route is a potentially effective form of therapy for patients with chronic disease, including the debilitating hereditary disease, cystic fibrosis and type I diabetes (insulin is absorbed well through the lungs) (Skyler *et al.,* 2001).

1.1.1 Anatomy and physiology

The human respiratory system is a complex organ system. It is divided into the upper and lower respiratory tracts. The upper respiratory tract consists of the nose, nasal passages, paranasal passages, mouth, Eustachian tubes, the pharynx, the oesophagus, and the larynx, sometimes the trachea and bronchi are also included. The lower respiratory tract comprises the air bronchioles and alveoli (figure 1.1) Hickey and Thompson, 1992; Washington *et al.*, 2001).

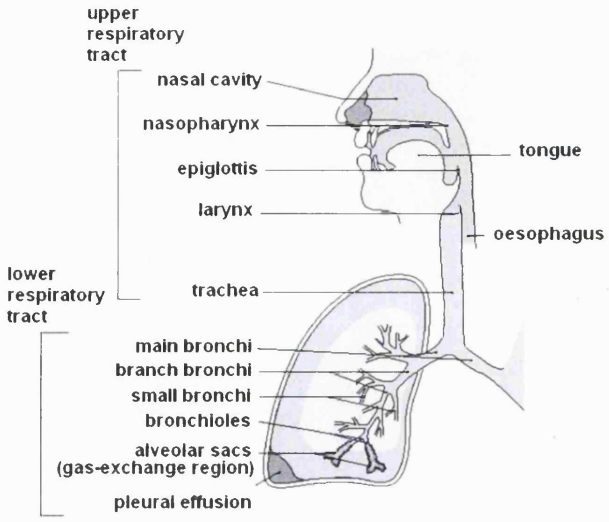


Figure 1.1 *Structure of the respiratory tract (adapted from http://www.cancer.gov).*

As the airways bifurcate repeatedly, new generations of airways are formed. The trachea divides forming the main bronchi, which then divide in turn to form smaller bronchi. The same process continues through the bronchioles, the alveolar ducts, ending in the alveolar sacs. It is estimated that the whole process consists of 23 generations. New generation airways are smaller in size, length and diameter (e.g. tracheal diameter is about 1.8 cm and alveolar diameter is about 0.04 cm), with a consequently increased cross-sectional surface area that is required for an extensive gas exchange between blood and the alveolar space (Hickey, 1992). In smaller airways, there is a large increase in resistance to flow (e.g. a 10% reduction in diameter causes an increase in resistance of more than 50%). Table 1.1 lists the main physical differences between the various airways generations.

The trachea is considered generation 0 and the alveolar sacs generation 23. The main, lobar and segmental bronchi are represented by airway generation z = 1, 2, 3, followed by intrasegmental bronchi, bronchioles, secondary bronchioles, and terminal bronchioles (z = 4-16). The terminal bronchioles mark the end of the tracheobronchial region, which is followed by the alveolar region comprising respiratory bronchioles, alveolar ducts, and alveolar sacs (z = 17-23) (Taylor and Kellaway, 2001).

The structure of the lower airways permits close contact between air and blood, a useful characteristic for the administration of drugs in the inhaled form (Hickey, 1992).

Table 1.1 Morphological changes in the airway generations (Weibel, 1963).

Generation (z)	Diameter (cm)	Length (cm)	Number	Total cross sectional area (cm^2)
0	1.80	12.0	1	2.54
1	1.22	4.8	2	2.33
2	0.83	1.9	4	2.13
3	0.56	0.8	8	2.00
4	0.45	1.3	16	2.48
5	0.35	1.07	32	3.11
16	0.06	0.17	6×10^4	180.0
17				
19	0.05	0.1	5×10^5	10^3
20				
22				
23	0.04	0.05	8×10^6	10^4

Functionally, the airways consist of two main regions, the conducting zone and the respiratory zone. The conducting zone comprises the nasal cavity and associated sinuses, nasopharynx, oropharynx, larynx, trachea, bronchi, bronchioles and terminal bronchioles and does not take part in gas exchange. The respiratory zone comprises respiratory bronchioles, alveolar ducts, and alveolar sacs, and is involved with gas exchange. The main feature distinguishing the conducting and respiratory zones is the alveolar pockets as they confer a gas exchange function (Hickey, 1992).

The conducting zone links the external environment and the respiratory zone. It also provides gas buffering and humidification (Hickey, 1992). The conducting zone is the main site of airway obstruction in obstructive lung diseases such as asthma. Consequently, if drugs are intended for the local treatment of such conditions, they are required to be delivered and deposited in the conducting zone. A third transitional zone in between is sometimes included (Washington *et al.*, 2001).

In addition to delivery of drugs intended for local action within the lung via the pulmonary route, growing attention has been given to the potential of the pulmonary route as a non-invasive administration route for systemic delivery of therapeutic agents. This is due to the fact that the lungs provide a large absorptive surface area (up to 100 m^2) but an extremely thin (0.1-0.2 μm) absorptive mucosal membrane and a

good blood supply. Pulmonary delivery is complicated, however, by the complexity of the anatomic structure of the human respiratory system and the effect on disposition exerted by the respiration process (Gonda, 1992; Clark, 2004).

1.1.2 Deposition of particles

When an aerosolised drug is inhaled, aerosol particles will deposit in different regions of the respiratory tract (from mouth through to alveoli), depending on particle size and density, lung morphology, breathing pattern and aerosol velocity. The respiratory system functions like a "filter"; a fraction of the drug present within the inspired air is removed during its movement. In the lower respiratory tract, three principal mechanisms of drug deposition take place, inertial impaction, gravitational sedimentation and Brownian motion (Taylor and Kellaway, 2001). Larger drug particles are deposited by the first two mechanisms in the airways, while smaller particles reach the peripheral region of the lungs by diffusion, as detailed later. Other factors, which directly influence the aerosol deposition by the above three mechanisms, are the physical size distribution of the aerosol particles and the density of the aerosol particles figure 1.2.

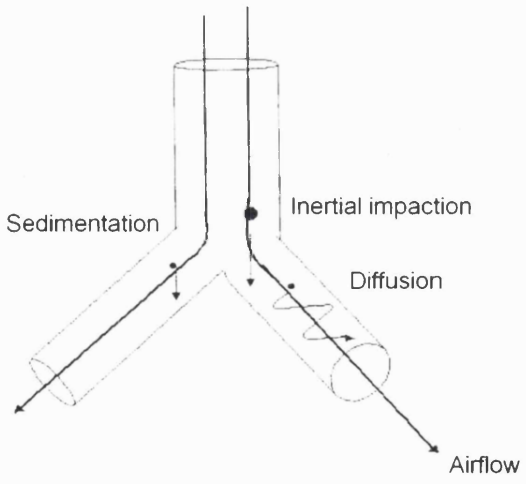


Figure 1.2 *Mechanisms of aerosol particle deposition (adapted from Hinds, 1998).*

Although there is similarity in drug absorption from the lungs and the other mucosal surfaces, the process in the lungs is further complicated. This is due to the complexity of aerosol-particle disposition and the hygroscopic properties of most therapeutic aerosols that allow the particle size to change drastically during drug transport in the highly humid atmosphere of the respiratory tract.

The upper tracheobronchial regions are where inertial impaction mainly occurs. Particles larger than 1 μm in diameter and having a large momentum (product of mass and velocity) may collide with the airway walls as they continue on their original course while the direction

of inspired air makes a sudden change at or near the bifurcation (Taylor and Kellaway, 2001). The larger the particle momentum the greater the inertial impaction, thus particle diameter, density and velocity are all factors that determine the extent of impaction. The longer a particle persists moving in the original direction thereby resisting the change in airflow direction, the more likely it is to hit airway walls (Hickey, 1992).

Sedimentation occurs in areas of the airways where airstream velocity is relatively low. It occurs when particles settle under gravity and is dependent upon how long the particles remain in airway regions with low airstream velocity (e.g. in the bronchioles and alveolar region) (Taylor and Kellaway, 2001). A particle with a density greater than that of air will experience a downward force due to gravity (Moren *et al.*, 1985). A sphere of diameter D, and density d, under the influence of gravitational force, will have a terminal settling velocity v_{ts}, governed by Stokes law (Equation 1.1). The probability of sedimentation increases with increasing particle momentum.

$$v_{ts} = \frac{d D^2 g}{18\eta} \qquad \textit{Equation 1.1}$$

where; g = gravitational acceleration, η = viscosity of air. A slip correction factor, Cc, is typically applied to the numerator for small particles which 'slip' through the air (Gonda, 1992).

Brownian diffusion is more prevalent in airway regions where airflow is very low or absent (e.g. the alveoli) and is more significant for particles smaller than 1 μm in diameter. Brownian diffusion of drug particles of this size is the result of a constant random bombardment of gas molecules with the particles, which pushes them in an irregular fashion causing them to collide with the airway walls. Unlike both previous mechanisms, the likelihood of Brownian diffusion deposition increases with decreasing particle size (Taylor and Kellaway, 2001).

Two other mechanisms may be considered. Interception occurs in small air spaces where the particle diameter is greater than the distance to a surface (Moren *et al.*, 1985). This mechanism of deposition is particularly pertinent to the deposition of elongated particles on nasal hair, in small airways, and alveoli (Hickey, 1992).

Another proposed mechanism of drug deposition is electrostatic precipitation. It occurs when particles carry an electrostatic charge when an aerosol is produced. Charged particles may induce an opposite charge in the airway wall, therefore becoming electrostatically attracted to it (Hickey, 1992). Charged particles have a greater

tendency to be deposited than neutral ones. Particles that carry the same charge will repel each other and further enhance deposition (Moren *et al.*, 1985). To date, there is little evidence that electrostatic precipitation significantly increases the deposition of drug in the human respiratory tract (Hickey, 1992).

Drug deposition in the lung is clearly determined by particle size. Particle size for inhalation is typically characterised by the aerodynamic diameter, d_a, which is the diameter of unit density sphere with the same settling velocity as the particle in question (Task Group on Lung Dynamics, 1966; McDonald and Martin, 2000). In practice, pharmaceutical inhalations are polydispersed with log-normal distributions. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) characterise such aerosol distributions. MMAD is the value for d_a, above or below which 50% of the mass of aerosol resides (Gonda, 1985) and takes into account particle size, density and shape factors. GSD is the square root of d_a at the 84.13% cumulative value divided by the 15.87% cumulative value. Theoretically, a GSD value of 1 refers to a perfectly monodisperse system; although in practice, <1.22 is accepted as monodisperse for pharmaceutical aerosols (Fuchs and Sutugin, 1966).

Particles having an aerodynamic diameter larger than 10-15 μm are entirely impacted in the upper airways (mouth down to the larynx) then quickly removed by mucociliary clearance and swallowing giving potential for systemic side effects (Hickey, 1992). Particles with smaller aerodynamic diameters of 5-10 μm impact mainly in the tracheobronchial region where a maximum tracheobronchial deposition of 20% occurs. With aerodynamic diameters of about 3 μm, a maximum alveolar deposition of about 60% occurs (Hickey, 1992). However, the size range 1-5 μm is the optimal size range for drug deposition and is thus considered the respirable size range (McDonald and Martin, 2000). Unless particles are ultrafine (<0.01 μm) and therefore deposited by diffusion to the alveolar region, submicron particles are generally exhaled under normal breathing conditions. However, breath holding for 5-10 seconds after inhalation, which allows more time for sedimentation and diffusion and hence reduction of the extent of exhaled drug, can greatly improve deposition of particles ≤ 0.5 μm (Hickey, 1992).

Other factors that affect drug deposition include lung morphology and airstream velocity (Taylor and Kellaway, 2001). Particles must constantly change direction in the airways in order for them to remain airborne. Thus lobes of the lung with the shortest average pathlength will show greatest peripheral deposition. Generally, the impaction probability increases with each branching of the airways. There are two factors that influence particle velocity: the inspiratory flow rate and the inhalation device. As the

inspiratory flow rate is increased, deposition by impaction in the first few generations of the tracheobronchial region increases. Failure to co-ordinate the inhaler actuation with inspiration will result in near total particle impaction in the oropharyngeal region. As a general rule, the particle velocity must be as low as possible in order to reduce early deposition. As the time between inspiration and exhalation is increased, the time needed for sedimentation to occur increases. Breath holding, ideally for 5-10 seconds, is therefore commonly used to optimise pulmonary drug delivery.

1.1.3 Clearance of deposited particles

Pulmonary delivered drugs are rapidly absorbed except large macromolecules, which may yield low bioavailability due to enzymatic degradation and/or low mucosal permeability. It is generally agreed that following administration of a drug substance, less than 20% of the emitted dose from the inhalation aerosol reaches the lower respiratory tract. Typically, drug particles greater than 5 μm may impact in the mouth and throat and only a small fraction of the emitted aerosol represents particles in the narrow particle size range of 1-5 μm, which reaches the trachea part and may undergo dissolution. The physicochemical and physical properties of the drug substance and the site of deposition in the respiratory tract are factors that determine the fate of the inhaled drug particles (Taylor and Kellaway, 2001).

The epithelial surfaces of the bronchi and bronchioles contain cilia. Small bronchi have a ciliated cylindrical epithelium identical to that in the large bronchi (Clary-Meinesz *et al.*, 1997). Inhaled particles stick to mucus (secreted by goblet cells) which is continuously removed from the airways by these cilia. Macrophages are present in the airways. These cells protect the airways from infection by engulfing inhaled particles and bacteria (Mortensen *et al.*, 1994). Mucociliary clearance (pertaining to the clearance of mucus and other materials from the airways by the cilia of the epithelial cells in the airways) is the principal mechanism responsible for the clearance of particles that deposit in the ciliated airways. On the other hand, drug particles that reach and deposit in the peripheral non-ciliated areas of the lungs can be cleared by many mechanisms including dissolution (Svartengren *et al.*, 1999).

Particles delivered to the respiratory tract by pressurised metered dose inhalers (pMDIs) and dry powder inhalers (DPIs) are in the solid form and need to be dissolved in order for their local and systemic effects to be facilitated. The fraction of soluble aerosol particles from the emitted dose that reach the non-ciliated regions of the lung is expected to have a very high dissolution rate because they are of very small particle size, hence having a large surface area to volume ratio of particles.

Insoluble particles, on the other hand, become engulfed by alveolar macrophages depending on size, and then transported to the mucociliary clearance system. Subsequently, drug particles will be carried to the pharyngeal region where they may be expectorated or swallowed. Dissolution of particles upon passage through the gastrointestinal tract and subsequent entering the bloodstream is possible if they are swallowed. Metabolism and excretion of the drugs (or their metabolites) via the urine or bile occurs following elicited systemic effects. Finally particles that do not undergo disintegration and dissolution would be excreted in the faeces. Sometimes, insoluble particles in the alveolar region are not engulfed by phagocytosis and become sequestered in pulmonary tissue. Some solubilisation may occur if particles become sequestered for a long time, and this sometimes results in benign pneumoconiosis with no immediate adverse response seen. Alternatively, a fibrotic response may result depending on the chemical nature of the material and adverse tissue reactions.

To date there is no single *in vitro* test system considered ideal for performing dissolution measurements in order to estimate *in vivo* solubility in the lung fluids. When soluble particles reach the pulmonary region and dissolve in the lung fluid, local toxic, irritant or pharmacological effects may result.

1.2 Pulmonary disease

1.2.1 Asthma

Asthma is the most common respiratory disease encountered in UK clinical practice (McFadden and Hejal, 1995). It is estimated that there are 100-150 million asthmatics globally, and annual asthma care costs exceed US$ 6 billion in the United States alone (WHO, 2000).

Asthma is a chronic inflammatory disorder of the lower airways that often starts in childhood. It is characterised by reversible airway obstruction and increased bronchial hyper-reactivity, i.e. increased responsiveness of the airways to specific and nonspecific bronchoconstrictor stimuli resulting in episodes of wheezing, breathlessness, chest tightness and coughing, especially at night or in the early morning (Caramori and Adcock, 2003). Severe breathlessness and wheezing are characteristics of an asthma attack, which is caused by narrowing of the airways due to three factors (Hope *et al.*, 1999): (1) bronchial muscle contraction, (2) airway inflammation, and (3) mucus formation which causes airway blockage.

Inflammatory mediators, i.e. leukotrienes and prostaglandins are released by degranulation processes of inflammatory cells such as eosinophils, neutrophils,

macrophages and mast cells that infiltrate the airway wall and cause airway inflammation. These inflammatory mediators cause bronchoconstriction due to their potent biological action, including airway smooth muscle contraction, increased airway permeability (causing mucosal edema) and increased mucus secretion (Hope *et al.*, 1999).

1.2.1.1 Treatment

Treatment of asthma consists of symptom control rather than cure (Caramori and Adcock, 2003). Asthma therapy is mainly directed towards bronchodilation and suppression of inflammation. In recent years, treatment of asthma has improved considerably with the discovery of potent compounds which prevent or alleviate some of the symptoms associated with asthma. While many of these compounds are effective orally, formulation-device combinations that facilitate their delivery direct to the airways have been developed. This 'local' aerosol administration to the site of action is one of the simpler cases of drug targeting. Typically, therapeutic strategies aim to reduce the number of asthmatic episodes and improve lung function. They include regular short β_2-adrenoceptor stimulants together with a combination of one or more drugs from the following: long acting β_2-adrenoceptor stimulants, antimuscarinic bronchodilators, inhaled corticosteroids, cromolyns, leukotriene receptor antagonists (oral), theophylline (oral), and oral corticosteroids (table 1.2).

Table 1.2 Medication used in the treatment of asthma, in addition to short acting β_2-adrenoceptor stimulants.

Therapeutic class	Examples
Long acting β_2-adrenoceptor stimulants	Salmeterol xinafoate, formoterol fumarate
Antimuscarinic bronchodilators	Ipratropium bromide, oxitropium bromide, triotropium bromide (long-acting)
Inhaled corticosteroids	Beclometasone dipropionate, budesonide, fluticasone propionate
Cromolyns	Sodium cromoglicate, nedocromil sodium
Leukotriene receptor antagonists (oral)	Montelukast sodium, zafirlukast
Theophylline (oral)	Theophylline, aminophylline
Oral corticosteroids	Prednisolone[1]

[1]used as 'rescue' therapy for periods of severe asthma.

Anti-asthmatic drugs are generally administered via inhalation directly to the site of action. This is associated with a number of advantages compared to oral administration such as:

(1) rapid onset of action relative to oral delivery (ideal for acute treatment), (2) lower doses (μg) are required as opposed to oral therapy (mg) in order to produce the same therapeutic effect, (3) reduced incidence of systemic side effects such as tremor for oral salbutamol and adrenal suppression for oral prednisolone, and (4) avoiding first-pass effects. On the other hand, the blood levels of drug that are achieved following inhalation administration are typically much lower than those during oral dosing. In terms of bronchodilation, 2 to 4 mg in a tablet formulation of salbutamol for example is equivalent to only 90-180 μg by pMDI. Oral dosing produces plasma levels in the 5-15 ng/ml range, whereas inhalation therapy produces levels which are << 1 ng/ml and are difficult to assay even though a substantial proportion of each metered dose is usually swallowed and absorbed through the gastrointestinal tract. Concentrations which are required to produce therapeutic effects at the site of action, i.e. airway smooth muscle, are similar to plasma levels following oral therapy (around 10 ng/ml), which demonstrates the ease with which small aerosol doses are able to generate effective drug concentrations in the small fluid volumes lining the airways (Buhl, 2003).

Inhaled short acting β_2-adrenoceptors stimulants, e.g. salbutamol sulphate or terbutaline sulphate are commonly used for the treatment of acute asthma exacerbations. Chronic asthma management has essentially remained unchanged in the UK over the past three decades (Lipworth, 1999) and is outlined in the guidelines of the British Thoracic Society *et al.*, (1997).

Essentially, asthma treatment generally consists of the combination of short-acting bronchodilatory β_2-agonists (e.g. salbutamol sulphate, terbutaline sulphate, fenoterol hydrobromide) or anticholinergic bronchodilartors (e.g. ipratropium bromide) with anti-inflammatory drugs such as corticosteroids (e.g. beclometasone dipropionate, budesonide, fluticasone propionate) or alternatively non-steroidal anti-inflammatory drugs such as sodium cromoglicate or nedocromil sodium (British Thoracic Society *et al.*, 1997; Caramori and Adcock, 2003). Long-acting β_2-agonists (e.g. salmeterol xinafoate and formoterol fumarate) may also be used depending on the severity of asthma.

Another treatment for asthma is the relatively new development of monoclonal antibodies, which target immunoglobulin E (IgE). IgE is responsible for moderating response to allergens such as house dust mites, pollen and spores, and is therefore associated with exacerbations of asthma. IgE antibodies, e.g. omatizumab, therefore stop the inflammatory cascade. IgE antibody administration reduces the requirement for steroids in asthma treatment (Buhl, 2003) and highlights the entry of macromolecule treatment for asthma, as opposed to traditional low molecular weight drugs.

1.2.2 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) represents one of the most common causes of morbidity and mortality worldwide (Barnes, 2003), and there are further increases in the prevalence and mortality of the disease predicted for the coming decades (Hurd and Pauwels, 2002). The disease incidence increases with age and depends on risk factors such as genetic disposition, oxidative stress, and smoking history (Puchelle and Vargaftig, 2001). COPD is a collective term for a number of conditions encompassing emphysema (destruction of the lung parenchyma, loss of lung elasticity and closure of small airways), chronic obstructive bronchiolitis (inflammation of the central and peripheral airways with obstruction of small airways) and mucus plugging (Saetta *et al.*, 2001; Barnes, 2003). These conditions are characterised by a progressive airflow limitation in the lung. The slow progressive development of airflow limitation in COPD is not fully reversible. Most COPD patients exhibit all three pathologic conditions. Tobacco smoking is considered the single most important cause of COPD (Doll *et al.,* 1994). α_1-Antitrypsin is an antiprotease enzyme that protects the epithelium from proteolytic enzymes (Calverley and Walker, 2003; MacNee, 2003; Calverley, 2003). A deficiency of α_1-antitrypsin in non-smokers is associated with emphysema. However, there is a higher risk of developing COPD in enzyme-deficient patients who smoke (Janus *et al.,* 1985). Environmental factors such as air pollution may also contribute to the development of COPD. Stopping smoking is the most important and most beneficial factor in the management of COPD (Janus *et al.,* 1985; British Thoracic Society, 1997).

Due to inflammation and hyperplasia of the mucus glands in chronic bronchitis, the thickness of the bronchiolar lining increases. These factors also result in hypersecretion of mucus which either partly or completely plugs the airway, especially since mucus clearance by the cilia is typically impaired by chronic insult to the lung such as smoking. Consequently, frequent infections develop together with a degree of bronchoconstriction because mucus can harbour bacteria. Emphysema typically occurs with chronic bronchitis and causes dilatation of the respiratory bronchioles and alveolar sacs through destruction of the walls lining the airways. Pathophysiological changes are due to reduced α_1-antitrypsin activity.

1.2.2.1 Symptoms and treatment

There are a number of symptoms associated with COPD including cough, sputum production, dyspnoea, wheeze and fever, which are precipitated by chronic inflammation and recurrent infection. For smokers in particular, only two interventions have been shown to increase survival for those that develop COPD – stopping smoking (nicotine replacement therapy has proved useful) and long term oxygen

therapy (Calverley, 2003).

COPD symptoms may be treated with β_2-adrenoceptor stimulants and antimuscarinic bronchodilators since it has a reversible element. The extent of reversibility depends on how far the disease has progressed and varies from patient to patient. Due to the lag between onset and diagnosis in the case of emphysema, the condition is unlikely to have a significant reversible element. A broad range of β-adrenergic and anticholinergic bronchodilator drugs for the treatment of COPD are available. Drug treatment is aimed at improving lung function with inhaled bronchodilators (anticholinergics, β_2-agonists and theophylline) (Barnes, 2003) of which anticholinergics are the most effective class (Gross, 1991). A relatively new long acting anticholinergic drug, tiotropium bromide, appears to be the most effective bronchodilator in the treatment of COPD (Barnes, 2000). However, β_2-adrenoceptor stimulants and antimuscarinic bronchodilator therapeutic classes are equally effective and a combination of one drug from each class is typically used, as the effects are synergistic (British Thoracic Society, 1997). It is reported that, in contrast to asthma treatment, there is little evidence that inhaled corticosteroids are beneficial in patients with COPD, and they should not be used frequently although they reduce inflammation and improve lung function in asthmatic patients (Barnes, 2003).

1.2.3 Other conditions treated by the pulmonary route

There is ongoing research in the biotechnology field for pulmonary drug delivery which is intended for systemic action despite obvious domination by drugs with local action, thus the potential for pulmonary delivery remains to be fully exploited (McDonald and Martin, 2000). Table 1.3 lists many drugs available in the UK market for use via the pulmonary route.

There are other drugs including antibiotics and mucus active agents administered as aerosols for treatment of pulmonary conditions other than asthma or COPD. Antibiotics administered via inhalers include pentamidine for the treatment of pneumocystis carinii infection (Simonds *et al.,* 1990) and ribavirin for the treatment of respiratory sincytial virus infection (BNF, 2007). Inhaled tobramycin aerosol has efficacy in cystic fibrosis (Littlewood, 1993; Franz *et al.,* 1994; Coate *et al.,* 1998) and other antibiotics, e.g. gentamicin, penicillin, amikacin, neomycin, and ceftazidime show potential for administration by inhalation (Hodson *et al.*, 1981; Littlewood, 1993; Dequin *et al.*, 1997). When administered as an aerosol, the mucus active drug dornase alpha (rhDNase) has been shown to be effective in improving sputum characteristics in cystic fibrosis (Gonda, 1996). The delivery of surfactants is another example, e.g. colfosceril

palmitate, via an endotracheal tube to the lung for the treatment of surfactant deficiency syndromes such as in respiratory distress syndrome (RDS) (BNF, 2007).

Table 1.3 Drugs administered by the inhalation route (BNF, 2007). Surfactants for respiratory distress syndrome are delivered by endotracheal tube.[1]

Condition	Asthma/COPD	Cystic fibrosis	Respiratory distress syndrome	Infections of the respiratory tract
Drug examples	BRONCHODILATORS	MUCOLYTIC	SURFACTANT	ANTIBIOTIC/ ANTIVIRAL
	β_2-agonists Salbutamol sulphate (pMDI, DPI, N) Terbutaline sulphate (pMDI, DPI, N) Fenoterol hydrobromide (pMDI) Salmeterol xinafoate (pMDI, DPI) Formoterol fumarate (DPI)	Dornase alpha (N) Acetylcysteine (N) AMINOGLYCOSIDES Tobramycin (N)	Colfosceril palmitate	Colistin (N) Pentamidine (N) Ribavirin (N) Zanamivir (DPI)
	Anticholinergic drugs Ipratropium bromide (pMDI, DPI, N) Oxitropium bromide (pMDI, DPI) Tiotropium bromide (pMDI)			
	ANTI-INFLAMMATORY DRUGS			
	Corticosteroids Beclometasone dipropionate (pMDI, DPI, N) Budesonide (pMDI, DPI, N) Fluticasone propionate (pMDI, DPI, N) Nometasone furoate (DPI)			
	Non-steroidal Sodium cromoglicate (pMDI, DPI, N) Nedocromil sodium (pMDI)			

[1]pMDI: pressurised Metered Dose Inhaler. DPI: Dry Powder Inhaler. N: Nebulised formulation.

1.2.4 Inhalation therapy

Because the alveoli have a good blood supply and a large absorptive alveolar surface with thin epithelial lining, inhalation offers not only an opportunity for pulmonary delivery of locally-acting drugs, but a desirable option for aerosol delivery to the alveoli for the systemic delivery of drugs (Patton, 1996; Groneberg *et al.*, 2003). These drugs avoid first-pass effects when directly entering the arterial circuit. Due to the lung's relatively high permeability for macromolecules and relatively low peptidase/protease activity, they are in particular a promising route for the delivery of peptidergic drugs or other macromolecules

(e.g. oligonucleotides) (Wall, 1995). Currently, the most recent investigations in this respect involve the systemic delivery of insulin via inhalation (reviewed by Patton *et al.*, 1999), but a number of other peptidergic and non-peptidergic drugs have been reported to reach the systemic circulation following aerosol administration (Table 1.4). Proteins and other large molecular weight drugs are absorbed from the lung by two general mechanisms (Patton, 1996): (1) by transcytosis (pass through the type I pneumocyte cells), or (2) paracellular through tight junctional processes between two cells. Transcytosis may be the dominant transport mechanism across the epithelia for macromolecules > 40 kDa, whereas paracellular and transcytotic processes may both be involved for macromolecules < 40 kDa. There are two processes that control pulmonary bioavailability of macromolecules (Taylor and Kellaway, 2001): (1) the diffusion through the mucus barrier in the conducting airways and (2) clearance processes, i.e. mucociliary clearance and macrophage phagocytosis. It takes a few hours for particles and dissolved drugs that become entrapped in the mucus to be removed from the conducting airways via the larynx to the gut (mucociliary escalator). Mucociliary clearance only takes place in the conducting airways whereas macrophages are found in the alveolar region and along larger and smaller airways. Macrophage phagocytosis depends on particle size, particles of 3 µm diameter are better internalised than particles with 6 µm diameter, and particles of 0.26 µm diameter are prevented from macrophage phagocytosis (Groneberg *et al.*, 2003).

Table 1.4 Existing and potential drugs for systemic delivery via the pulmonary route.

Drug class	**Example**
Migraine (α-adrenoceptor agonist)	Ergotamine
Protein / Hormone	α-antitrypsin[1]
	Insulin[2]
	Human growth hormone[3]
	Calcitonin[4]
	Parathyroid hormone[5]
	Cetrorelix[6]
	Leuprolide[7]
Steroid / Hormone	Estradiol[8]
Analgesic	Morphine[9]
Oligonucleotide[10]	

[1](Griese *et al.*, 2001); [2](Colthorpe *et al.*, 1992; Ward *et al.*, 1997a; McElduff *et al.*, 1998); [3](Patton *et al.*, 1989); [4](Kobayashi *et al.*, 1996); [5](Codrons *et al.*, 2003); [6](Lizio *et al.*, 2001); [7](Alcock *et al.*, 2002); [8](Wang *et al.*, 1999); [9](Ward *et al.*, 1997b); [10](reviewed by Wu-Pong and Byron, 1996).

1.2.5 Aerosol drug targeting

Aerosol drug targeting is crucial for optimal pulmonary drug delivery since the deposition site and pattern of inhaled drugs influence their therapeutic effect. There are many factors that make the alveolar region the most important area in the respiratory tract for drug absorption into the systemic circulation: (1) the alveolar space accounts for more than 95% of the lung's total surface area, (2) 95% of the alveolar epithelium is occupied with ultra-thin type I pneumocyte cells, (3) the alveolar space is directly connected to the systemic circulation via the pulmonary circulation, (4) significantly slower clearance processes (no mucociliary clearance), and (5) the lack of a mucus layer through which molecules have to diffuse. All these factors make it desirable for inhaled drugs for systemic delivery to target the alveolar region. The location of the disease (e.g. bronchoconstriction and inflammation) and the location of adrenergic, cholinergic, and glucocorticoid receptors determine the intended target site for locally-acting asthma drugs. Because the β_2-adrenoceptors and the M3 muscarinergic receptors are not distributed uniformly throughout the respiratory tract, the effect of the bronchodilator may be affected by the site of deposition of the inhaled drug particles (Howarth, 2001). According to autoradiographic studies, there is a more dense population of M3 muscarinergic receptors than β_2-adrenergic receptors in the trachea, whereas β_2-adrenergic receptors are predominantly found in the alveolar walls (Barnes *et al.*, 1982; Carstairs *et al.*, 1985; Mak and Barnes, 1990). However, the distribution patterns of β_2-adrenergic and M3 muscarinergic receptors does not correspond with the distribution of the smooth muscle cells in the airways which are absent in the alveoli but found in all other areas of the respiratory tract (Figure 1.3).

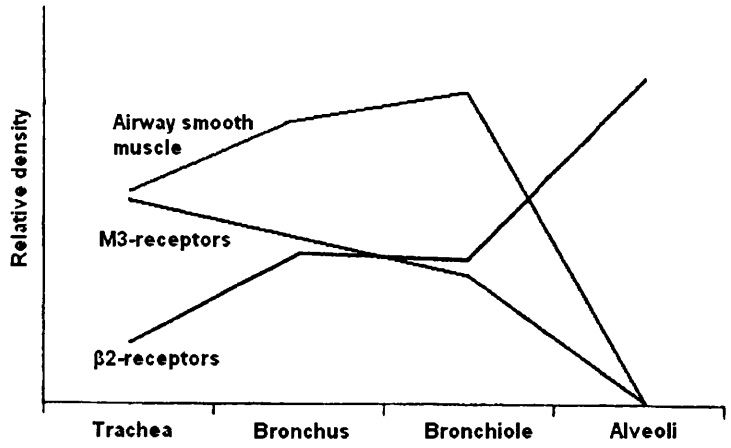


Figure 1.3 *The distribution of airway smooth muscle, M3 muscarinergic receptors, and β_2-adrenoceptors in the small and large airways (adapted from Howarth, 2001).*

Data from studies that investigated the effect of particle size of a β_2-agonist (salbutamol) and an anticholinergic (ipratropium bromide) aerosol on the bronchodilatory effect in

asthma (Zanen *et al.* 1994; 1996) showed that the most suitable particle size is 2.8 μm for optimum bronchodilation. Thus, it is proposed that bronchodilatory drugs should primarily target the compartments of the lung where smooth muscle cells are present such as the bronchioles. The inflammatory process in asthma is widespread through the large and small airways as opposed to the bronchoconstriction which mainly occurs in the tracheobronchial area. Recent studies indicate that inflammation is also present in the alveoli (Kraft *et al.*, 1996) and investigations on the location of glucocorticoid receptors show that they are ubiquitous in the airways with the highest concentration in the alveoli (Adcock *et al.*, 1996).

To date, there is no evidence relating corticosteroid particle size to site of action and efficacy. In a study comparing the deposition patterns of beclometasone dipropionate (BDP) aerosols with two different particle sizes averaging 3.5 μm and 1.1 μm, respectively, it was found that the aerosol containing particles with MMADs of 3.5 μm showed reduced drug deposition to the lung (4-7% as opposed to 55–60% with the aerosol containing particles with MMAD of 1.1 μm). The clinical effect was also decreased by a factor of two (Leach *et al.,* 1998). Whether the increased clinical effect is a result of the increased deposition to smaller airways and the alveoli because of the smaller particle size or of the improved lung deposition in general is not clear.

There are many factors that determine regional particle targeting in the lung; patient related factors include breathing pattern and pathological conditions in the lung. For instance, particles deposit mainly in the central airways in patients with constricted airways, which does not allow efficient absorption for drug intended for systemic delivery. Breathing at low flow rates (< 20 l/min), breath holding for 4-10 seconds, and increasing the inhaled volume by deep breathing, on the other hand, are believed to enhance particle deposition to the smaller airways and alveoli, especially for submicron particles. Device related factors may also influence targeting although the particle property is considered the primary factor determining particle targeting in the lung (Dolovich *et al.*, 1981; Hakkinen *et al.*, 1999).

1.3 Therapeutic aerosol generators

A drug can be administered by the pulmonary route using two techniques: aerosol inhalation (also used in intranasal applications) and intratracheal instillation. Instillation is not expensive but has a non-uniform distribution of drugs. The aerosol inhalation technique, on the other hand, achieves more uniform distribution with greater extent of penetration into the peripheral of the alveolar region of the lung; it costs more and is faced with difficulty in measuring the exact dose inside the lungs.

In order for drugs to be delivered to the lung via the inhalation technique, a therapeutic

aerosol needs to be generated for inhalation. An atomiser is the general term given to the device that produces an aerosol (Washington *et al.*, 2001). Aerosols are dispersions or suspensions of solid material and liquid droplets in a gaseous medium. An aerosol can be described as a colloidal, two-phase system, of liquids or solids (or a combination of the two) in gas (Taylor and Kellaway, 2001). Currently there are three types of aerosol generators available (Washington *et al.*, 2001): nebuliser, pMDI and DPI. The major differences between the three inhalation devices are highlighted in Table 1.5. It is generally accepted that the following specific criteria have to be met in order for a device to be accepted for clinical use (Dalby *et al.,* 1996):

- be able to generate an aerosol in the size range of 0.5-5 μm;
- be able to provide reproducible dosing;
- be able to protect the physicochemical stability of the drug;
- be able to offer portability and inconspicuousness during use; and
- be simple to operate with minimal training.

Both pMDIs and DPIs currently available are complete drug delivery systems. That is, they meter, aerosolise, and, in some cases, partly control how the patient inhales the chosen dose of drug. They can be perceived from two different viewpoints: (1) from the patient's point of view, both devices are perceived as much simpler than nebulisers, they are certainly much smaller than nebulisers and are therefore easier to carry and to conceal. Compliance and privacy can be maintained more readily during self-medication as a result. Furthermore, hygiene is usually less of a problem than it is with nebulisers and metering mechanisms offer the patient the advantage of being aware of the amount of medication they have inhaled; (2) from the pharmaceutical point of view, developing and manufacturing pMDIs and DPIs presents a more complex system and poses greater technical challenges than do nebulisers. They do a reliable job of dispensing medication in the form of a reproducible aerosol once they are correctly formulated. Nebulisers, on the other hand, offer much less reproducibility. One of the reasons for that is the variable ways in which they are used and also due to the fact that a particular drug solution may be nebulised in various different devices. Thus, following inhalation of a metered dose from a pMDI or a DPI by a given subject population, pharmacologic response vs. time profiles are more reproducible provided subjects have been correctly instructed on the use of the device.

Briefly, the rigorous pharmaceutical quality control and assurance that is presently imposed during the manufacture of a pMDI is effective in defining the clinical outcome than that which is imposed on the manufacture of a nebuliser solution. Thus, marketing a 'self-contained' pMDI which is sealed and effectively tamperproof is much more advantageous and appealing, again from a pharmaceutical perspective, than an

aqueous solution which can be contaminated and inhaled in inappropriate doses.

Table 1.5 Differences between conventional inhalation devices (adapted from Taylor and Kellaway, 2001; Washington *et al.*, 2001; Hickey, 2003).

Device	Particle Size Range (μm)	Dose Range	[air][1]	Means of delivery to the lung	Formulation	Exit velocity from device
Nebuliser	1-10	100-600 μg/min	Low	Passive	Solution/ Suspension	Fast (30 m/s)
pMDI	1-10	0-250 μg	High	Active	Solution/ Suspension	Variable
DPI	1-10	1-20 mg	High	Passive/ Active	Dry powder	Slow

[1][air] = aerosol concentration in the air during administration.

1.3.1 Nebulisers

Nebulisers are inhalation devices that convert aqueous solutions or micronised suspensions of drug into an aerosol for inhalation. Aerosols generated from nebulisers have a wide droplet size range of 1-10 μm. There are two types of nebulisers, air-jet which operate from a pressurised gas source, and ultrasonic, including mesh nebulisers. When the airflow is adjusted to the patient's inspiration rate, impaction is effectively reduced. Large amounts of drug can be inhaled with continuous nebulisation. The use of conventional nebulisers was considered inconvenient in the past due to the need for a power source and their bulky format (Taylor and Kellaway, 2001; Washington *et al.*, 2001).

1.3.2 Dry powder inhalers

DPIs are propellant free inhalers. The drug, which is generally micronised powder, is delivered from DPIs to the airways as a dry powder aerosol. Particle aggregation can be a problem with micronised particles as they have a large surface area which enhances interactions. Since DPIs are traditionally breath-actuated, the drug is withdrawn from the inhaler at a rate that depends on the patient's inspiration force. One of the inconveniences associated with DPI use is the fact that the particle flow rate is only determined by the patient's inspiratory flow rate which, albeit beneficial in terms of the impaction rate, can be a problem in cases of respiratory disease with serious airway obstructions and breathing problems (Washington *et al.*, 2001; Taylor and Kellaway, 2001). The patients are recommended to inhale as rapidly as possible from the DPI to provide the maximum force to disperse the powder (Borgström *et al.*, 2002). There are two types of DPIs: unit dose DPIs and multi-dose DPIs in which the dose can be pre-metered or metered from a reservoir upon actuation of the device (figure 1.4).

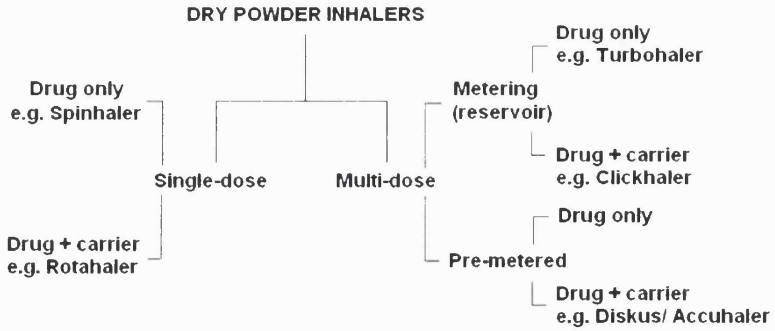


Figure 1.4 *Different types of potential DPIs.*

1.3.3 Pressurised metered dose inhalers

pMDIs for therapeutic use were first introduced in the mid 1950s. Most commonly, the formulation for a pMDI is a suspension in an aerosol propellant stabilised by added surfactants. Usually the drug, a polar solid, is dissolved or suspended in a non-polar liquefied propellant (Washington *et al.*, 2001; Taylor and Kellaway, 2001). The pMDI device consists of a metering valve crimped onto an aluminium can which is filled with drug dispersed in liquefied propellant (figure 1.5).

An individual dose is measured volumetrically upon actuation of the pMDI. It then goes through the metered valve and into a metered chamber. On actuation, due to atmospheric pressure, the propellant starts to evaporate leaving a mixture of vapour and propellant-drug blend. Evaporation results in volume expansion which causes the mixture to be forced through the nozzle thereby breaking liquid propellant into fine droplets. The resulting high speed flow generates shear forces that lead to further reductions in the droplet size. Droplet sizes between 1-5 μm with a velocity of around 30 ms^{-1} are generated (Williams *et al.*, 1998; Washington *et al.*, 2001).

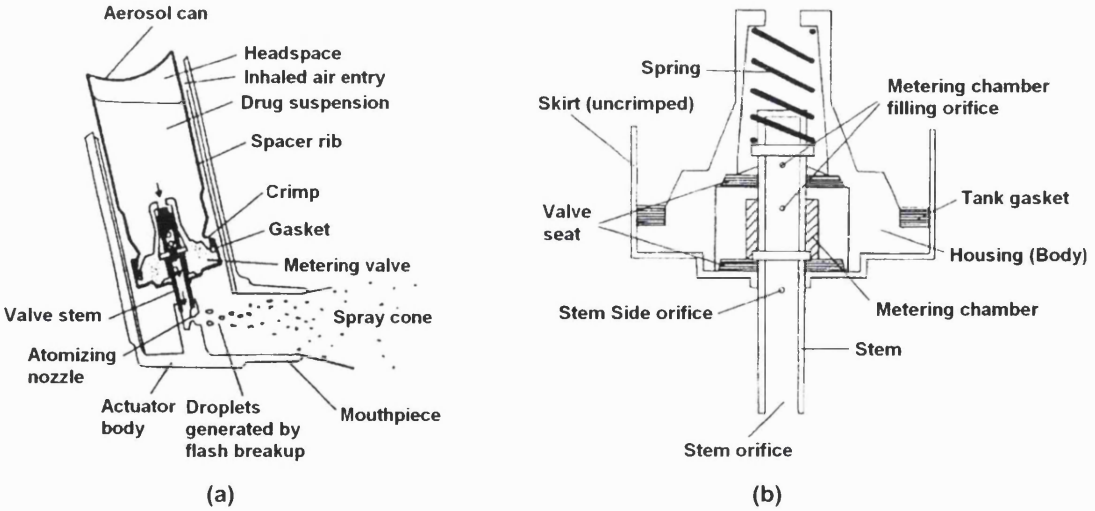


Figure 1.5 *A diagram representing (a) a metered-dose inhaler (adapted from Smith, 1995), and (b) a pMDI metering chamber (adapted from Dalby et al., 1996).*

The pMDI has generated a lot of interest since it was introduced and has been at the centre of the treatment of asthma and chronic obstructive pulmonary disease (COPD). It is considered to be a reliable dosage form, having the benefits of cost, user acceptability, and efficiency over DPIs and nebulisers (Brown, 2002). There are about 500 million pMDIs manufactured each year, which makes them the most commonly used pulmonary delivery systems (McDonald and Martin, 2000). The problem of high oropharyngeal drug deposition associated with the high droplet flow velocity due to the use of propellants, can be solved by using a spacer device. Spacers make the use of pMDIs simpler as the coordination between actuation and inhalation becomes easier and less problematic. They provide a time delay which allows more propellant to evaporate and reduces particle velocity. Thus, smaller and slower particles are generated and inhaled which in turn improves drug deposition (Washington *et al.*, 2001).

There are a few modified delivery systems available for use with pMDIs. A number of extension devices have been the subject of numerous investigations. Spacers or simple extension devices are usually small devices which are placed between the conventional actuator and the patient and require that aerosol is discharged into them at the same time as inhalation. Thus they do not provide much opportunity for an aerosol to dwell prior to inhalation. Spacers are intended to: (1) provide extra time for the aerosol propellant droplets to evaporate before they reach the patient; (2) reduce the impaction efficiency of the aerosol at the back of the throat by spacing the actuator further from the mouth which is due to a reduction in the droplet velocity; and (3) retain some of the extremely large droplets, which are unlikely to be respirable, by sedimentation and impaction on the inside walls of the spacer. They are mainly designed to overcome inhalation problems exhibited by patients who have difficulty in coordinating actuation and inhalation. Some devices have an audible mechanism incorporated, which usefully warns the patient in case they are inhaling too quickly for optimal deposition. The importance of optimising patient training (especially small children so that aerosol therapy becomes possible in the first place) is of paramount importance. The patient is required to synchronise valve actuation with the earlier part of inhalation when using a pMDI without a spacer device. When this proves difficult to achieve in some patient groups, using an inhalation triggered actuator can overcome the problem, also known as breath-actuated devices.

All pMDIs marketed before 1995 used chlorofluorocarbon (CFC) propellants as an energy source to atomise the formulation into respirable drug particles. However, the production of CFCs was phased out under the terms of the Montreal Protocol due to their depleting effect on the ozone layer and their contribution to the greenhouse effect. The

Montreal Protocol on substances that deplete the ozone layer is an international treaty which brought about a phase-out for the production and use of CFC propellants by 1996 (Whitham and Eagle, 1994; Stein and Stefely, 2003). All pMDIs were exempted from the CFC phase-out until new alternatives were available due to the fact that they are a life saving medication for asthmatics. Research shifted towards finding new propellants that comply with the requirements of toxicity, flammability, chemical stability, physical properties and environmental compatibility. Hydrofluoroalkanes (HFAs) are now replacing CFCs and 1,1,1,2-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA 227) are now considered the most suitable pMDI propellants (figure 1.6). HFAs have many desirable characteristics which make them suitable propellants for the use in pMDIs as described by the Committee for Proprietary Medicinal Products in Europe, such as being non-flammable, non-ozone depleting, chemically stable, and having suitable vapour pressure. Their contribution to the greenhouse effect is minimal (McDonald and Martin, 2000). Some of the physicochemical properties of HFAs and those of CFCs are listed below in table 1.6.

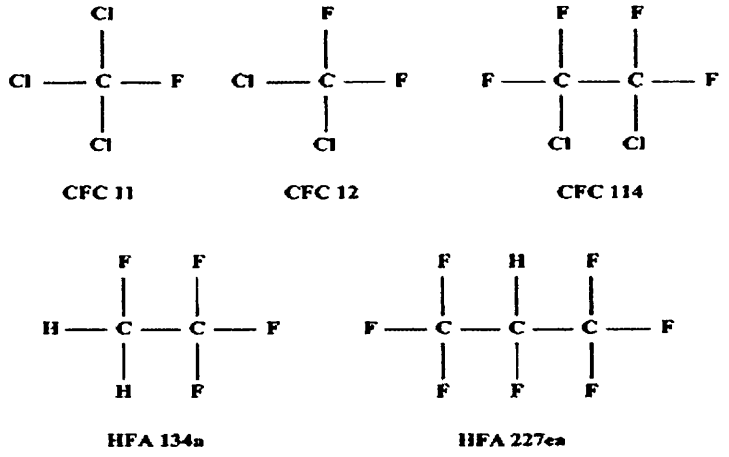


Figure 1.6 *Chemical structures of CFC and HFA propellants (Vervaet and Byron, 1999).*

As table 1.6 indicates, HFAs exhibit a higher polarity, reflected in dielectric constant and dipole moment, and a higher vapour pressure than CFCs, especially HFA 134a. Higher vapour pressure is due to the change from chlorine substituents in the hydrocarbon chain to fluorine, which possesses the highest electronegativity of all halogens. High electronegativity means that the nucleus binds its electrons more strongly, which then results in lower polarisability. Hence, the fluorine mantel in HFA propellants is difficult to polarise. Low polarisability, in turn, leads to poor dispersion forces (induced dipole-induced dipole interaction). Vapour pressure is strongly dependent on interactions between molecules. Hence, weak dispersion forces in the fluorine mantel result in higher vapour pressure in HFAs. Higher polarity can be

explained by the presence of H-atoms in HFA molecules. Fluorine, having a strong electronegativity, withdraws electrons from the H-atom leaving a partial positive charge on the hydrogen. This results in a strong moment (Byron *et al.*, 1994).

Higher polarity results in different solvency properties (Byron *et al.*, 1994), which in turn has an effect on drug and surfactant solubilities. Solubilities of the drug and surfactants are the determining factor for the pMDI formulation in the propellant as a solution or suspension.

Table 1.6 Physicochemical and atmospheric properties of chlorofluorocarbons and hydrofluoroalkanes (Byron *et al.*, 1994; Fink, 2000; Solvay Fluor und Derivate, 2006).

Propellant	HFA 134a	HFA 227	CFC11	CFC12	CFC114
Formula	$C_2H_2F_4$	C_3HF_7	CCl_3F	CCl_2F_2	$C_2Cl_2F_4$
Molecular weight ($g.mol^{-1}$)	102	170	137.4	121	170.9
Density at 20°C ($g.ml^{-1}$)	1.21	1.41	1.49	1.33	1.47
Vapour pressure at 20 °C (bar)	5.72	3.9	0.89	5.66	1.82
Boiling point (°C)	-27	-17	24	-30	4
Dipole moment (debye)	2.06	0.93	0.46	0.51	0.5
Dielectric constant (ε)	9.51	4.07	2.3	2.13	2.26
Atmospheric life years	16	33	60	125	200
Global warming potential*	0.26	0.3	1.0	3.0	3.9
Ozone-depleting potential*	0	0	1.0	0.9	0.7

*Global warming potential and ozone-depleting potential are set relative to CFC 11, which is assigned a value of 1.0.

Solution pMDIs offer many advantages, being homogeneous and having uniform spray content even after storage (Warren and Farr, 1995). Another advantage is that the actuator orifice diameter can be as small as 0.25 mm without being obstructed as would be the case with suspension formulations. Studies showed that a higher respiratory particle fraction, thus a greater extent of lung deposition, results from using the smaller orifice diameter (Warren and Farr, 1995). Generally, it has been proposed that a better pulmonary drug deposition is achieved with solution pMDIs than suspension pMDIs; a 4-fold increase has been reported (Ashworth *et al.*, 1991; Harnor *et al.*, 1993).

CFC formulations have used micelle solubilisation and cosolvents to improve solubility. Reverse micelle formation has been described in CFCs (Vervaet and Byron, 1999), whereas research into the formation and orientation of micelles has indicated an L_1 orientation (Ridder *et al.*, 2005). A disadvantage of solution pMDIs is that they are less stable chemically.

Some of the advantages of suspension pMDI formulations are chemical stability and the possibility of formulating an insoluble drug. However, due to the agglomeration of micronised drug particles, creaming or sedimentation can be a problem, which leads to poor dose reproducibility and reduced numbers of particles in the respirable size range (McDonald and Martin, 2000). In order to achieve suspension stability, surfactants in the solubility range of 0.1 to 2% have been investigated. They provide steric repulsion between the particles by adsorbing onto their solid surface, in addition to acting as lubricant for the metering valve. Oleic acid, sorbitan trioleate (Span 85) and lecithin were licensed for use in CFC pMDIs but have poor solubility in HFAs which makes the search for new appropriate surfactants in HFAs necessary (Blondino and Byron, 1998). Cosolvents such as ethanol can also be used to improve the solubility profile of surfactants. A cosolvent reduces the vapour pressure of the propellant and may cause partial drug dissolution and subsequent crystal growth.

Various liquid model propellants with similar physical properties to the CFC or HFA liquefied propellants used in pMDI formulations have been used due to the difficulty of practical analytical work with gaseous propellants. Trichlorotrifluoroethane (P-113) is a model propellant for the CFCs. 1-H-Perfluorohexane and 2H,3H-decafluoropentane (DFP) have been used as model propellants for the HFAs (Ridder *et al.*, 2005). DFP, which has an additional hydrogen atom and one carbon atom less, resembles the structure of the HFAs. Increased H-bonding due to the additional hydrogen atom influences solubility (Dickinson *et al.*, 2000).

1.4 Pulmonary sustained release

The subject of this thesis is the formation of liposomes from pMDI solution or suspension formulations. Liposomes, being microscopic lipid vesicles that encapsulate a portion of aqueous phase within their structure, may act as targeted carriers of hydrophilic or hydrophobic drugs to the respiratory tract. They may also control drug release within the lung, which leads to increased local tissue concentrations and minimised release to extrapulmonary sites (Niven, 1990).

1.4.1 Liposomes

Liposomes are formed when phospholipids or certain other surface-active amphiphiles, which contain a hydrophilic (polar) head group and two hydrophobic (non-polar) tails typically composed of long hydrocarbon chains, are swollen in excess aqueous phase (Lasic, 1988). They were first described in 1965 (Bangham *et al.*, 1965). In water, these molecules spontaneously arrange into ordered structures forming lipid bilayers, commonly known as the characteristic "onion-layer" appearance of multilamellar

liposomes (figure 1.7). The hydrophilic (polar) head groups of the phospholipids stay in contact with the aqueous environment by surrounding channels of aqueous phase while the hydrophobic (non-polar) groupings aggregate in lipid rich channels pointing inward to form the inner portion of the bilayer. The resulting spherical vesicles vary in size, as well as in the number of lamellar layers that will form. Vesicles with a single lipid bilayer (4 nm thick membrane) can range in size from 20 nm to 50 μm (Heldt *et al.*, 2000).

1.4.2 Liposomes as drug carriers

Liposomes have a closed phospholipid bilayer structure within which a portion of aqueous phase is entrapped following vesicle formation. This allows accompanying dissolved aqueous material to become encapsulated (Allen and Chonn, 1987; Woodle and Lasic, 1992).

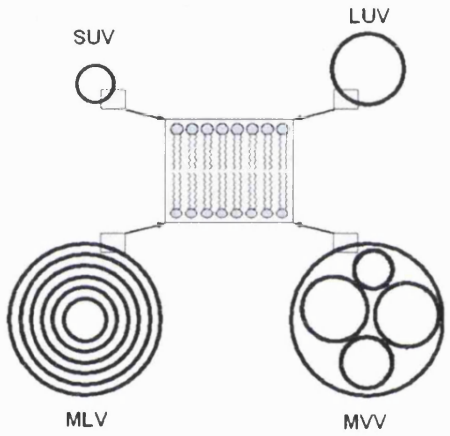


Figure 1.7 Structure of the liposome in its different forms; small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicles (MLV), and multivesicular vesicles (MVV) (adapted from AZoNanotechnology, *http://www.azonano.com)*.

The amphiphilic nature of the liposome allows drugs of varying physicochemical nature to be encapsulated in different regions. Following liposomal encapsulation, there is a dramatic reduction in the amount of agent which enters into the systemic circulation from the liposomal distribution throughout the airspace of the lung, compared with free drug (Hunt and McCasland, 2006).

Liposomes are a means of achieving sustained activity of locally active drugs within the lung and help control the availability of systemically active drugs. They offer the advantage of making a variety of drugs useful for a range of diagnostic and therapeutic applications after their encapsulation within these phospholipid structures (figure 1.8): (1) hydrophilic drugs can be entrapped within the liposome aqueous phase, their

encapsulation efficiency depends on the volume of aqueous phase that is incorporated, which is determined by the mean liposome size, number of bilayers, and the total lipid concentration (New, 2003); (2) hydrophobic compounds have the potential of partitioning within the repeating lipid bilayers upon vesicle formation when they are added to the lipid phase during liposome preparation. Here, the encapsulation efficiency is dependent on the lipid concentration, the transition state of the bilayers and the oil/water partition coefficient of the drug; and (3) amphiphilic drugs interact with both the hydrophobic bilayer regions and the hydrophilic channels. Agents may also be covalently bound to the liposome. An example is the "stealth" liposome, in which bonding of the outer liposomal phospholipids to hydrophilic polymers such as polyethylene glycols (PEGs) provides extended residence times within the circulation (Allen and Chonn, 1987; Woodle and Lasic, 1992).

1.5 The pulmonary delivery of liposomes

One advantage of liposomal use as drug carriers of drugs for the lung is that they can be formed from endogenous materials found in high quantities in the body, and for which there is considerable data regarding their fate *in vivo*. Commonly, drug-containing liposomes intended for pulmonary delivery have been composed of phosphatidylcholine (PC), dimyristoylphosphatidylcholine (DMPC), dipalmitoyl-phosphatidylcholine (DPPC), or phosphatidylglycerol and usually include cholesterol in order to promote *in vivo* and *in vitro* stability.

There are high levels of phospholipids and cholesterol in the lung (10 to 20% of the lung's dry weight) (King and Clements, 1985). These are principally contained in lung surfactant and are the most common components of liposomes. Lung surfactant functions as a complex mixture that maintains airway integrity. In fact, the smaller alveoli of the lung would remain in a deflated state following exhalation in the absence of lung surfactant, due to the elevated surface tension at the air interface (Notter and Morrow, 1975). The role of the surfactant involves reducing this surface tension by the action of the phospholipid fraction, comprising primarily saturated and unsaturated phosphatidylcholines, in particular DPPC. It also contains small amounts of phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and lysophosphatidylcholine (lysoPC). Efficient surfactant activity is also dependent on a smaller protein fraction, comprised of four apoproteins, surfactant proteins A, B, C and D (SP-A/B/C/D) (Voss *et al.*, 1991; Manz-Keinke *et al.*, 1992; Voss *et al.*, 1992; Voss *et al.*, 1998).

Briefly, the surfactant phospholipids form a monomolecular film when they adsorb at the air interface of the alveoli. During exhalation, the film is compressed, in doing so, phospholipid molecules become sequestered in the underlying aqueous phase as they leave the film. When the breathing cycle enters the inspiratory phase and the film becomes expanded, the sequestered phospholipids rejoin the interface, thus maintaining the reduced surface tension. The presence of the hydrophobic surfactant proteins accelerates this movement of surfactant to the interface, ensuring that there is no loss of film coverage during inspiration (Sorensen *et al.*, 2007). The often fatal neonatal, and adult, respiratory distress syndroms (A/NRDS) illustrate the importance of lung surfactant. NRDS occurs in neonates and is caused by under developed surfactant. ARDS, on the other hand, results from surfactant loss which may result from sepsis. The administration of exogenous phospholipids in nebulised preparations may reduce the mortality rate from ARDS and NRDS (Weg *et al.*, 1994).

As liposomes are cleared from deposition sites in the lung, the duration of drug release becomes limited (Reifenrath, 1983; Manz-Keinke *et al.*, 1992). Surfactant clearance, and by inference, that of liposomal phospholipid, may also occur via the lymphatic system, phagocytosis by alveolar macrophage ingestion, enzymatic degradation at the respiratory surface, or by reuptake into alveolar type II epithelial cells (Reifenrath, 1983; Manz-Keinke *et al.*, 1992). These mechanisms have been implicated as potential mechanisms of liposomal phospholipid removal and it has been evidenced that these can be manipulated by changes in the lipid composition of vesicles. It has been demonstrated that exogenous phospholipids become associated with the endogenous surfactant pool, following administration to rabbit lung (Hallman *et al.,* 1981).

It has been reported that liposome-containing droplets are handled by the lung in a different manner to conventional inhaled drug particles, in that processing, uptake and recycling by type II pneumocytes seems to occur as for natural surfactant. In so doing, liposomal phospholipid produces minimal disturbance of normal metabolic processes (Mihalko *et al.*, 1988).

It has been reported previously that liposomes have the potential to be formed within the respiratory tract after inhalation of microfine phospholipid aerosols derived from CFC solution pMDIs (Farr *et al.*, 1987). The use of liposomes as drug carriers for pulmonary delivery is discussed in Chapter 3.

1.6 Scope of the thesis

The aim of this work was to investigate formulating phospholipid-based pMDI formulations for use with different model drugs. Both solution and suspension pMDI systems were studied. First, the possibility of formulating salbutamol base and budesonide in HFA pMDIs containing phosphatidylcholines was investigated. The aim being to facilitate liposome formation following delivery of an aerosol dose to a moist surface in order to achieve a degree of control of drug release within the respiratory system. Drug efflux from liposomes and clearance of the liposomes would determine their availability. Using phosphatidylcholines, which are endogenous to lung secretions, for liposome formation is an advantage from a toxicological viewpoint (Heldt *et al.*, 2000). Phospholipids orientate in liposomal configurations through a spontaneous entropic process in an aqueous environment. The airways of the respiratory tract are a water-rich milieu for such a process to occur. Incorporation of a drug in such formulations should create a reservoir of liposomally encapsulated drug that can be released subsequently in a controlled-release fashion. Salbutamol, a relatively hydrophilic drug, is expected to partition within the aqueous bilayers of liposomes. Its relatively short duration of action (4 to 6 hours) following inhalation might be extended usefully by liposomal encapsulation. Initial investigations were initially carried out in the model propellant 2H,3H-decafluoropentane (DFP).

The reformulation of pMDIs with HFAs is a challenge to improve pMDI performance. The second part of the experimental work involved assessing the use of new excipients in suspension pMDIs. The aim was to establish a novel formulation for suspension pMDIs in liquefied HFAs to obtain physically stable suspensions. The method involved using PEG phospholipids, various drugs and 1H,1H,2H,2H-perfluorooctanol to stabilise the suspension. Different types and concentrations of excipients and drugs were investigated. Different concentrations of the fluorinated alcohol were investigated to determine their influence on the physical stability as well as product performance of the suspension. Budesonide, formoterol fumarate and terbutaline sulphate were used as model drugs.

Throughout this work, phospholipid-based HFA pMDI performance was investigated *in vitro*, with an emphasis on the effects of formulation variables and comparison with commercially available pMDIs.

CHAPTER 2

PHOSPHOLIPID, GLYCEROL MONOOLEATE AND DRUG SOLUBILITY IN 2H,3H-DECAFLUOROPENTANE AND HFA 134a PROPELLANT

2.1 Introduction

The pressurised metered dose inhaler (pMDI) is widely used as a device for the delivery of drugs via the inhaled route. A pMDI formulation consists of an active ingredient which is either suspended (suspension pMDI) or solubilised (solution pMDI) in a liquefied gas propellant. The system can contain further ingredients and formulation additives, such as surfactants, taste-masking agents and cosolvents. The main component in pMDI formulations is the propellant system, which serves as a dispersion medium or solvent for the drug substance and other excipients, and as the energy source for generating the aerosol cloud while the dose is emitted from the metering valve following actuation (Williams *et al.,* 1998).

The Montreal protocol has defined the environmental reasons for replacing CFC propellants. The CFC phase out and selection of the replacement HFA propellants has necessitated reformulating pMDIs to address their different solvent properties of surfactants previously used in CFCs as solution or suspension aids (Purewal and Grant, 1998; Gonda, 2000). Changing and redesigning the formulation and the pMDI device components was due to the differences in solubility of drugs and excipients in the HFA propellants (Smith, 1995; Cripps *et al.*, 2000). In order to facilitate the transition from CFC to HFA formulation, the new pMDI formulations are required to be similar in appearance and use, clinically non-inferior with the same recommended dosage and pharmaceutically comparable with a similar product performance (Haywood *et al.*, 1997).

Currently, two hydrofluoroalkanes are used: HFA 134a and HFA 227 (figure 1.6). The three digits (from left to right) refer to the number of carbon atoms in the molecule minus one, the number of hydrogen atoms plus one, and the number of fluorine atoms, respectively (Banks and Tatlow, 1994). Therefore, 'HFA xyz' has the molecular formula $C_{x+1}H_{y-1}F_z$. The unique properties of the HFAs are conferred by the boiling point, vapour pressure, and chemical structure of these propellants (table 2.1) (Rogueda, 2003).

Because a pMDI is formulated with a pressurised liquid, studying its properties proves difficult. Therefore, there is the need to use a model propellant, a non-volatile liquid under ambient temperature and pressure conditions that will be a reference for formulation properties and predicting behaviour in propellants. P113, also known as 1,1,2-trichlorotrifluoroethane was used as a model propellant for CFC systems (Clarke *et al.*, 1993; Bower *et al.*, 1996). Model propellants for CFCs have different properties from fluorinated molecules because of their chlorine atoms and therefore cannot be used as model HFAs (Rogueda, 2003).

Table 2.1 Physical characteristics of propellants HFA 134a and HFA 227 (Rogueda, 2003).

	HFA 227				HFA 134a			
Temp (°C)	Vapour pressure (bar)	Density of liquid (g/cm^3)	Dynamic viscosity (mPa s)	Surface tension (mN/m)	Vapour pressure (bar)	Density of liquid (g/cm^3)	Dynamic viscosity (mPa s)	Surface tension (mN/m)
10	2.80	1.446	0.324	8.22	4.15	1.262	0.239	10.07
15	3.32	1.428	0.302	7.64	4.89	1.245	0.225	9.38
20	3.90	1.408	0.267	6.96	5.72	1.226	0.2107	8.69
25	4.56	1.388	0.259	6.47	6.65	1.208	0.1974	8.02
30	5.29	1.367	0.237	5.88	7.70	1.189	0.1849	7.36
35	6.11	1.345	0.216	5.30	8.87	1.169	0.1731	6.70

To date, there have only been a few attempts to suggest a suitable model for the HFAs. Perfluorohexane, 1H-perfluorohexane and 2,2,2-trifluoroethanol were suggested as model propellants by Dickinson *et al.* (2000). Only 1H-perfluorohexane showed a linear relationship between solubility in the HFA propellants and in the model but at a much lower level (11% and 26% of those in HFA 134a and HFA 227, respectively). It is interesting to note that the introduction of the hydrogen appears to have an effect on solvency properties. Hydrogen bonding is believed to be crucial in solute solubility in the HFAs.

Rogueda (2003) suggested the following criteria for choosing a model propellant for the HFAs: (1) perfluoroalkanes should have a low degree of hydrogen substitution, similar to HFA 134a and HFA 227; (2) the hydrogen atom should not be easily accessible, similar to HFA 227; and (3) the carbon chain should be as short as possible in order to maintain the rigidity of the short chain HFAs, but has to be long enough to ensure a liquid state at ambient temperature. He suggested 2H,3H-decafluoropentane (DFP) as a model propellant (figure 2.1). A comparison of some physical properties with both HFAs is shown in table 2.2.

DFP was chosen as the model propellant for subsequent work to study formulation aspects of HFA pMDIs. It is a non-volatile liquid at room temperature and was shown to have solvency properties similar to the HFAs (table 2.2).

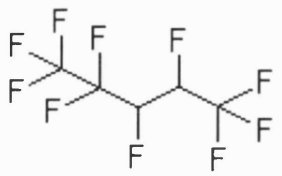


Figure 2.1 *Molecular structure of model propellant DFP (2H,3H-decafluoropentane) (adapted from http://www.chemblink.com).*

Table 2.2 Properties of DFP, HFA 134a and HFA 227 (Rogueda, 2003).

	DFP	HFA 227	HFA 134a
Molecular weight (g.mol^{-1})	252.051	170.03	102.03
Density (g.cm^{-3}) at 25°C	1.583	1.388	1.208
Boiling point (°C)	53.6	-16.5	-26.3
Melting point (°C)	-80	-131	-101
Dielectric constant (ε) at 20-25°C	15.05 at 18.66 kHz	4.071 at 3.9 bar	9.46 at 4.5 bar
Surface tension (mN m^{-1}) at 20°C	13.59	7.55	8.69
Dynamic viscosity (mPa s) at 20°C	0.537	0.266	0.211
Water solubility (ppm) at 23-25°C	390 ±40	610	2220

A variety of drugs have been formulated into pMDI delivery systems. A pharmaceutical aerosol formulation in HFA propellant can be either a solution or a suspension system. The solubility of a drug in the propellant also relates to the physical stability of the formulation, drug propensity for polymorph interconversion, crystal growth potential, and solvate formation, all of which represent important issues that need to be addressed in the early formulation development stages of pMDIs.

Suspension systems comprise a drug that is finely divided and suspended in the volatile liquefied propellant. The drug must be uniformly suspended in the propellant, in a state which is easily dispersed by the shear forces generated as the propellant vaporises, in order for a reproducible dose to be administered when the valve ejects a metered volume of the suspension. Suspension systems in HFAs have a cloud particle size that is dominated by the particle size of the suspended drug, which is defined by the milling/micronisation process.

In order to achieve such a suspension it may be necessary to incorporate a propellant-soluble surfactant in the mixture, or the particles will form tightly bound aggregates due to attractive van der Waals forces. A number of surfactants are in common use, with one of the most popular being phosphatidylcholine (PC), which is a complex mixture of phospholipids derived from egg yolk or soya beans.

The behaviour of PC in aqueous systems is well-understood; its colloid chemistry has been studied for many years, since it forms vesicular systems which can be used as model cell membranes. Its behaviour as a surfactant and emulsifier has also been widely investigated since it is one of the few materials which can be used as a surfactant in large volume parenterals, and is used for the preparation of intravenous fat emulsions (Washington, 1990). However, in non-aqueous systems its behaviour is much less well understood. Zeta potential measurements (Sidhu *et al.*, 1993)

established that it behaves as a charge-stabilising surfactant in lactose and salbutamol dispersions, despite the low dielectric constant of the propellant.

In a suspension-based pMDI the density and viscosity of the propellant system can influence the physical stability of the suspension. One approach to creating a stable suspension is to utilise different compositions of the individual propellants in order to match the density of the suspended drug. The use of large porous or hollow particles is an alternative formulation principle to avoid creaming or caking of the particles in the propellant (Bowman and Greenleaf, 1999). Other authors report the formulation of a reverse water in fluorocarbon emulsion by the use of fluorinated surfactants in HFA 134a or HFA 227 (Butz *et al.*, 2002). Techniques like spray-drying, freeze-drying or co-grinding of the drug substances in combination with surface active ingredients are also well established methods for the preparation of pMDIs in order to influence the physical properties of solid particles (Edwards *et al.,* 1997, Keller, 1999).

Solution formulations, as opposed to suspensions, do not present problems of physical stability of suspended particles and therefore could exhibit a higher dose uniformity and reproducibility. The volumetric contribution of suspended drug particles is absent and much finer liquid droplets clouds, largely defined by the drug concentration in the solution, are generated. Solution aerosol formulations also offer the advantage of being homogeneous with the active ingredient and excipients completely dissolved in the propellant vehicle or its mixture with suitable cosolvents such as ethanol. A cosolvent is used with solution aerosol formulations in HFAs in an amount that proves effective in solubilising the active ingredient in the pressurised propellant.

Despite their advantages with respect to suspensions, solution formulations present some stability problems, especially chemical stability of the active ingredient in the propellant and/or in the propellant/cosolvent system.

Ethanol is used as a cosolvent in pMDI formulations to increase the solubility of a drug in the propellants when formulating a solution-based pMDI. It is also sometimes added as a dispersing aid to facilitate the dispersion of drug particles in the propellants during the manufacturing process when a suspension-based pMDI is formulated. However, in this case, any increase in drug solubility that may result from the presence of ethanol could be deleterious to the physical stability of the suspension due to crystal growth of the drug particles. Therefore, it is critical to understand the solubility characteristics of a drug in the binary solvent system composed of propellant and ethanol in order to use ethanol as a formulation ingredient appropriately.

Alternative methods of solubilisation of drugs in pMDIs have been reported in the literature. Evans and Farr (1989) reported a propellant (CFC) aerosol formulation in which the drug is solubilised in reverse micelles. The preferred surfactant for this formulation was PC (0.025-2.5% w/v) and the resulting formulation appeared to be homogeneous.

The objective of the present study was to investigate the solubility of a series of phospholipids and glycerol monooleate (GMO) in a binary system of the model propellant, HFA 134a or 227 and ethanol in order to establish a novel formulation for solution pMDIs. A drug substance was also incorporated in the liquefied propellant system in order to create a stable system that would allow a controlled release of the drug substance from formed vesicles following delivery of an aerosol dose in an aqueous environment.

Ethanol is used to solubilise the different excipients. Both PC and GMO are well-established pharmaceutical excipients. The preparation starts with a solution of drug substance and GMO or PC in ethanol in the aerosol container. After crimping the valve onto the container, a one phase solution system is formed after addition of the propellant.

2.2 Materials

2.2.1 Chemicals

All chemicals were used as received. 1,1,1,2-Tetrafluoroethane (Solkane 134a pharma) and 1,1,1,2,3,3,3-Heptafluoropropane (Solkane 227 pharma) were purchased from Solvay Fluor und Derivate GmbH (Hannover, Germany). 2H,3H-Decafluoropentane (DFP) was purchased from Apollo Scientific Ltd. (Derbyshire, UK). Egg phosphatidylcholine (EPC) (Lipoid E-100) and soya phosphatidylcholine (SPC) (Lipoid-s-100) were gifts from Lipoid (Germany). Glycerol monooleate (GMO) was obtained from GlaxoSmithKline. Propylene glycol, polyethylene glycol 300 (PEG 300), polyethylene glycol 400 (PEG 400), and glycerol were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol absolute (99%-100%) was purchased from VWR International Ltd., UK. Salbutamol base and salbutamol sulphate were provided by GlaxoSmithKline. Budesonide was provided by AstraZeneca.

2.2.2 Equipment

Manual bottle crimper:	Model 3000-B, Aero-Tech Laboratory Company, Maryland, USA.
Pressure filling equipment:	Pressure burette, Aero-Tech Laboratory Equipment, Maryland, USA.
Zetasizer:	Malvern 3000 spectrometer, Malvern Instruments, Malvern,UK.
Sonicating bath:	XB6 Grant Instruments Ltd., UK.

2.3 Methods

2.3.1 Solubility studies with DFP and the HFAs

A commonly described method was used in order to determine solubility in liquefied gases and the model propellant (Brown and George, 1997; Blondino and Byron, 1998; Williams III and Liu, 1998a). HFAs were added by weight to a known amount of phospholipid or GMO until dissolution was visibly apparent. Dissolution was considered as achieved when one clear phase was apparent. Solubility was determined at ambient temperature (~20-23°C).

Solubility in the liquid model propellant, DFP, was carried out in screw cap glass squat vials (Scientific Laboratory Supplies Ltd., Nottingham, UK). In order to find a model cosolvent for HFA pMDI formulations, a number of solvents were used together with DFP. A known amount of GMO, SPC or EPC was weighed into the vial, a cosolvent was added and DFP was then added by weight using a Pasteur pipette. After each addition the vial was sonicated (XB6 Grant Instruments Ltd., UK) for 30 s at room temperature and judged visually for miscibility, i.e. dissolution, at ambient temperature with the naked eye (Blondino and Byron, 1998). The cosolvents used were ethanol, propylene glycol, polyethylene glycol 300, polyethylene glycol 400 and glycerol. The cosolvent was used at 0, 5, 10, 15 and 20 % w/w. Once a cosolvent was chosen, the maximum amount of each of GMO, SPC or EPC that would dissolve in DFP using that cosolvent at a particular concentration was determined.

For solubility determination in the two propellants HFA 134a and HFA 227, GMO, SPC or EPC were weighed into a plastic-coated glass bottle (Wheaton, USA), the appropriate amount of ethanol was added, and the bottle was then crimped (manual bottle crimper, model 3000-B, Aero-Tech Laboratory Equipment Company, Maryland, USA) with a continuous spray valve (Valois, France) and filled with the correct amount of propellant through the valve via a pressure burette (Aero-Tech Laboratory Equipment Company, Maryland, USA) (figure 2.2). After each propellant addition, the

bottle was sonicated for 30 s in a bath sonicator at room temperature. In order to keep the propellant in the liquid state, pressure within the burette was held between 4-6 bar with addition of nitrogen. Solubility was determined visually. The observation of phase separation or cloudiness indicated that dissolution in HFA was not complete, and a clear solution indicated dissolution in HFA.

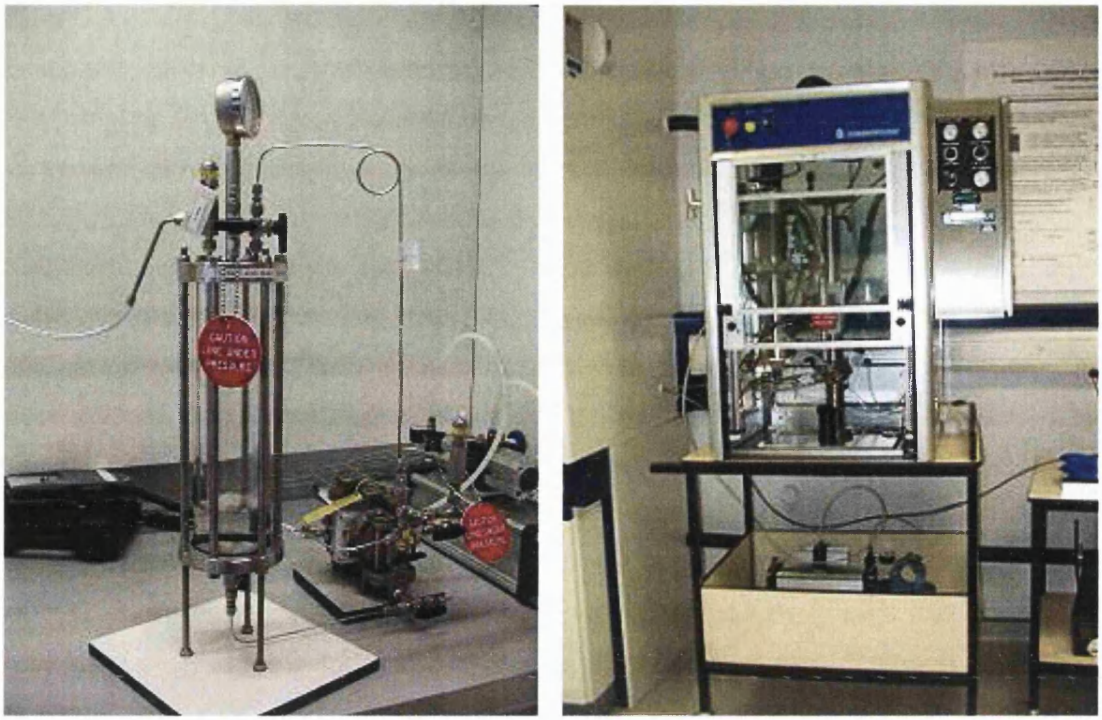
Ternary-phase diagrams were constructed for different propellant-ethanol-GMO or PC combinations. The intention was to assess the ability of the propellant/cosolvent system to solubilise GMO or PC, especially in the high propellant concentration regions, where formulations were most likely to be useful for inhalation purposes. Ethanol was used at 5, 10, 15 and 20% w/w. At each ethanol level, the propellant was added to 100% w/w after a known amount of GMO or PC was weighed into the bottle. After each addition, the bottle was sonicated for 30 s in a bath sonicator and viewed with the naked eye to detect the presence of single or multiple phases and the clarity of each phase. The percent by weight of each component was calculated and plotted on the phase diagram after each addition.

2.3.2 Incorporation of a drug in the formulation

Solubility studies to establish likely formulations of solution aerosols containing salbutamol sulphate or salbutamol base were conducted in mixtures of HFA 134a or HFA 227, ethanol and small amounts of GMO, SPC or EPC. Formulations were prepared in plastic-coated aerosol bottles sealed with continuous valves. A known amount of GMO, SPC or EPC was weighed into the bottle as well as the drug salbutamol sulphate or salbutamol base. Ethanol was then added at 5, 10, 15 or 20% w/w, and finally the propellant was added to 100% w/w. All formulations were sonicated for 30 seconds at room temperature and assessed visually and the resultant phase diagram constructed. Samples were kept for four weeks at room temperature (21 ± 2°C) to investigate formulation stability.

2.3.3 Temperature challenging experiments

Some formulations containing GMO or PC, the drug salbutamol sulphate or salbutamol base, ethanol and HFA 134a were prepared in clear glass Turbiscan tubes (as alternatives to clear aerosol vials). For the formulations prepared, all ingredients except the propellant were weighed directly into the Turbiscan tube, which was then sealed with the appropriate valve. The cold fill technique (detailed in section 4.3.2) was used to fill the tubes with HFA 134a. The entire assembly was maintained in a positive nitrogen pressure hood. All formulations were sonicated for 30 seconds and assessed visually, and then they were left to equilibrate at different temperatures in order to investigate the effect of temperature on solubility.



(a) (b)

(c)

Figure 2.2 *Pressure filling equipments used both in AstraZeneca labs (a) and (b) and at the School of Pharmacy lab (c).*

2.3.4 Water incorporation

The effect of water on solubility was studied. Formulations were challenged with small amounts of water. Water was added to salbutamol sulphate formulations to see whether it could enhance solubility of the drug. It was also added to chosen formulations containing salbutamol sulphate or salbutamol base in order to establish the tolerance of the formulations to small amounts of water that arise from components or by absorption during processing or from water ingress during storage. Formulations were prepared in plastic-coated aerosol bottles crimped with continuous valves. A known amount of GMO, SPC or EPC was weighed into the bottle as well as the drug salbutamol sulphate or salbutamol base. Ethanol and water were then added, and finally the propellant was added to 100% w/w. All formulations were sonicated for 30 seconds at ambient temperature and assessed for solubility with the naked eye.

2.3.5 PCS experiments

Photon correlation spectroscopy (PCS) of GMO- and PC-based formulations was performed using a Malvern 3000 spectrometer (Malvern Instruments, UK) equipped with a 120-mW ion laser, a 64-channel digital correlator and a computer-controlled stepper-motor-driven variable-angle detection system (the theoretical basis of the equipment is detailed in section 3.3.3.2). The refractive index and viscosity of the medium was assumed to be that of HFA 134a, i.e. 1.1846 and 0.205 $mPa.s^{-1}$ respectively (Solvay Fluor und Derivate, 2006). Subsequent PCS measurements were restricted to scattering angle 90°. Measurements were performed in triplicate. In order to perform PCS in the volatile system, a PCS pressure cell was used.

2.4 Results and Discussion

2.4.1 Solubility studies with the model propellant DFP

2.4.1.1 Choice of a cosolvent

Visual assessment of surfactant solubility in DFP was chosen due to the experimental difficulty of quantitative excipient analysis in pressurised systems. This method, however, only allows the determination of approximate solubility values.

Tables 2.3-2.7 show the solubility in DFP of GMO, SPC and EPC using one of the cosolvents ethanol, propylene glycol, polyethylene glycol 300 (PEG 300), polyethylene glycol 400 (PEG 400), or glycerol. Each cosolvent was used at 0, 5, 10 and 15% w/w. A higher concentration of cosolvent would not be desirable for inhalation purposes (Gupta and Stein, 2003). In the absence of an appropriate cosolvent, GMO, SPC and EPC are insoluble in the model propellant DFP. Solubility was considered to be achieved, if one clear phase was formed in the admixture. Solubilities are expressed as percent by weight.

DFP was chosen as a model propellant to study formulation aspects of HFA pMDIs as it has solvency properties similar to the HFAs (Rogueda, 2003). Among the tested cosolvents, only ethanol showed good solubility for GMO, SPC and EPC. Only GMO was solubilised in DFP at 0.1% w/w with cosolvent PEG 300 and PEG 400 at a cosolvent concentration as low as 5% w/w and 10% w/w for PEG 300 and PEG 400, respectively. Neither propylene glycol nor glycerol was a good cosolvent as they led to the formation of two-phase systems.

GMO (0.1% w/w) was insoluble in HFA 134a when cosolvent PEG 300 or PEG 400 was used at the same concentrations as in DFP. Ethanol on the other hand, showed reasonable reproducibility of solubility data for GMO, SPC and EPC in DFP and HFA 134a.

The suitability of DFP as a liquid model propellant in these studies has not been demonstrated due to the contradicting data regarding GMO solubility using PEG 300 and PEG 400. In DFP, the solubility of GMO, SPC and EPC was similar to that in HFA 134a when ethanol is used.

Ethanol was chosen as the model cosolvent for subsequent experiments. The levels at which it was used were 5, 10 and 15% w/w. Ethanol is widely used as an excipient in pMDI formulations for pMDIs because of its miscibility with the HFA propellant and the positive influence on the solubility of organic molecules due to its higher polarity. The addition of ethanol increases the polar/hydrophobic characteristics of a formulation (Solvay Fluor und Derivate, 2006).

Table 2.3 Solubility of GMO, SPC and EPC in DFP with cosolvent ethanol (n=3).

Formulation	GMO % w/w	SPC % w/w	EPC % w/w	Ethanol % w/w	DFP % w/w	Observation after 24 h[1]
1	0.1	-	-	0	99.9	I
2	0.1	-	-	5	94.9	S
3	0.1	-	-	10	89.9	S
4	0.1	-	-	15	84.9	S
5	0.1	-	-	20	79.9	S
6	-	0.1	-	0	99.9	I
7	-	0.1	-	5	94.9	S
8	-	0.1	-	10	89.9	S
9	-	0.1	-	15	84.9	S
10	-	-	0.1	20	79.9	S
11	-	-	0.1	0	99.9	I
12	-	-	0.1	5	94.9	S
13	-	-	0.1	10	89.9	S
14	-	-	0.1	15	84.9	S
15	-	-	0.1	20	79.9	S

[1]S denotes soluble and I insoluble.

Table 2.4 Solubility of GMO, SPC and EPC in DFP with cosolvent propylene glycol (n=3).

Formulation	GMO % w/w	SPC % w/w	EPC % w/w	Propylene glycol % w/w	DFP % w/w	Observation after 24 h[1]
16	0.1	-	-	0	99.9	I
17	0.1	-	-	5	94.9	T
18	0.1	-	-	10	89.9	T
19	0.1	-	-	15	84.9	T
20	0.1	-	-	20	79.9	T
21	-	0.1	-	0	99.9	I
22	-	0.1	-	5	94.9	T
23	-	0.1	-	10	89.9	T
24	-	0.1	-	15	84.9	T
25	-	0.1	-	20	79.9	T
26	-	-	0.1	0	99.9	I
27	-	-	0.1	5	94.9	T
28	-	-	0.1	10	89.9	T
29	-	-	0.1	15	84.9	T
30	-	-	0.1	20	79.9	T

[1]I denotes insoluble; T refers to a two-phase system.

Table 2.5 Solubility of GMO, SPC and EPC in DFP with cosolvent PEG 300 (n=3).

Formulation	GMO % w/w	SPC % w/w	EPC % w/w	PEG 300 % w/w	DFP % w/w	Observation after 24 h[1]
31	0.1	-	-	0	99.9	I
32	0.1	-	-	5	94.9	S
33	0.1	-	-	10	89.9	S
34	0.1	-	-	15	84.9	S
35	0.1	-	-	20	79.9	S
36	-	0.1	-	0	99.9	I
37	-	0.1	-	5	94.9	I
38	-	0.1	-	10	89.9	I
39	-	0.1	-	15	84.9	I
40	-	0.1	-	20	79.9	I
41	-	-	0.1	0	99.9	I
42	-	-	0.1	5	94.9	I
43	-	-	0.1	10	89.9	I
44	-	-	0.1	15	84.9	I
45	-	-	0.1	20	79.9	I

[1]S denotes soluble and I insoluble.

Table 2.6 Solubility of GMO, SPC and EPC in DFP with cosolvent PEG 400 (n=3).

Formulation	GMO % w/w	SPC % w/w	EPC % w/w	PEG 400 % w/w	DFP % w/w	Observation after 24 h[1]
46	0.1	-	-	0	99.9	I
47	0.1	-	-	5	94.9	I
48	0.1	-	-	10	89.9	S
49	0.1	-	-	15	84.9	S
50	0.1	-	-	20	79.9	S
51	-	0.1	-	0	99.9	I
52	-	0.1	-	5	94.9	I
53	-	0.1	-	10	89.9	I
54	-	0.1	-	15	84.9	I
55	-	0.1	-	20	79.9	I
56	-	-	0.1	0	99.9	I
57	-	-	0.1	5	94.9	I
58	-	-	0.1	10	89.9	I
59	-	-	0.1	15	84.9	I
60	-	-	0.1	20	79.9	I

[1]S denotes soluble and I insoluble.

Table 2.7 Solubility of GMO, SPC and EPC in DFP with cosolvent glycerol (n=3).

Formulation	GMO % w/w	SPC % w/w	EPC % w/w	Glycerol % w/w	DFP % w/w	Observation after 24 h[1]
61	0.1	-	-	0	99.9	I
62	0.1	-	-	5	94.9	I
63	0.1	-	-	10	89.9	I
64	0.1	-	-	15	84.9	I
65	0.1	-	-	20	79.9	I
66	-	0.1	-	0	99.9	I
67	-	0.1	-	5	94.9	I
68	-	0.1	-	10	89.9	I
69	-	0.1	-	15	84.9	I
70	-	0.1	-	20	79.9	I
71	-	-	0.1	0	99.9	I
72	-	-	0.1	5	94.9	I
73	-	-	0.1	10	89.9	I
74	-	-	0.1	15	84.9	I
75	-	-	0.1	20	79.9	I

[1]I denotes insoluble.

While CFCs are completely halogenated, HFA 134a has two and HFA 227 has one small, asymmetrically positioned, hydrogen atoms in their mantles. The enhanced electronegativity (fluorine is more electronegative than chlorine) in the halogen mantle of the HFAs creates a distinct dipole on the hydrogen-carbon bonds in both propellants. This is reflected in increased polarity in the HFAs compared to the CFCs (Vervaet and Byron, 1999).

HFAs have higher water solubility than the CFCs. The solubility of water in HFA 134a is significantly greater than that in HFA 227 (2220 ppm compared to 610 ppm), reflecting the larger number of available hydrogen carbon dipoles in HFA 134a (Figure 1.6). The enhanced water solubility (HFA 134a > HFA 227 > CFC12) explains why amphiphilic GMO (HLB = 3.8, Griffin, 1979) and PC (HLB ~ 7.6, Weiner and Carpenter-Green, 1997) are effectively insoluble in HFAs, although PC has been used for years in CFC formulations. Such molecules can only be used effectively in propellant blends containing cosolvents. Ethanol enables their dissolution.

From a thermodynamic standpoint, dissolution of a solute in a solvent implies that the free energy of the solution be minimised. Generally, entropy favours dissolution because of increased disorder, while enthalpic contributions result from the interplay of intermolecular forces between the solvent and the solute (Byron *et al.*, 1994).

Intermolecular attraction, and thus cohesion, is believed to be the sum of a number of forces, all of which are dependent on the distance between molecules and their atomic components. Attraction may originate from, in decreasing order of strength, ionic, dipole-dipole, dipole-induced-dipole, and induced-dipole-induced-dipole interactions between the atomic constituents. Hydrogen bonding is one of a number of dipole-dipole interactions (Byron *et al.*, 1994; McDonald and Martin, 2000).

One reason for the poor solubility of GMO and PC in HFA 134a results from the hostile polar propellant environment causing collapse of associated hydrophobic (non-polar) chains. GMO, SPC and EPC are soluble in ethanol, which in turn is soluble in HFA 134a; therefore ethanol favours the dissolution of these molecules in HFA 134a.

2.4.1.2 Maximum amount of GMO, SPC and EPC dissolved in DFP/ethanol blends

Using ethanol 15% w/w as a cosolvent in DFP, the maximum amount of GMO that could be incorporated in the formulation was found to be 0.49% w/w, higher than that of SPC and EPC, 0.16% and 0.11% w/w respectively. When PEG 300 was used as a cosolvent at 5% w/w, only GMO was dissolved upto 0.14% w/w as an upper limit. No more than 0.14% w/w GMO was dissolved in DFP and cosolvent PEG 400 at 10% w/w (figures 2.3 and 2.4).

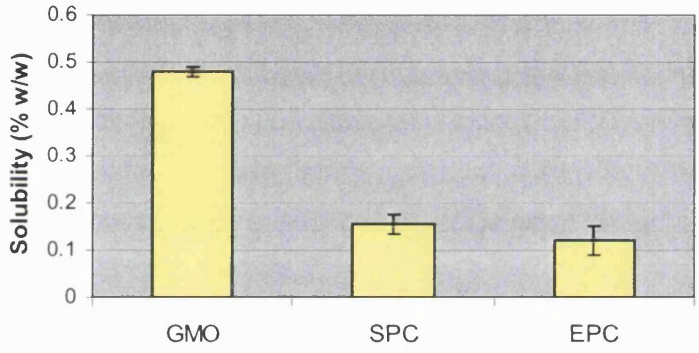


Figure 2.3 *Solubility of GMO, SPC and EPC in DFP with 15% w/w ethanol (n=3 ±SD).*

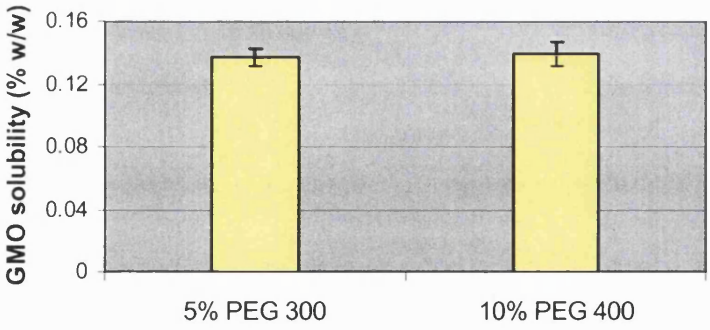


Figure 2.4 *Solubility of GMO in DFP with 5 and 10% w/w PEG 300 and PEG 400, respectively (n=3 ±SD).*

2.4.2 Solubility studies in propellant HFA 134a

Ternary-phase diagrams for different propellant-ethanol-GMO or PC combinations were constructed to assess the ability of the propellant/cosolvent system to solubilise GMO or the PCs, especially in the high propellant concentration regions, where formulations were most likely to be useful for inhalation purposes. As discussed above, ethanol was chosen

as the cosolvent in solubility studies in propellant HFA 134a.

Addition of ethanol resulted in solubilisation of GMO and the PCs. The solubility of GMO, SPC and EPC increased with increasing ethanol concentration. There was a 480-, a 150-, and a 120-fold increase in solubility of GMO, SPC and EPC, respectively, when the ethanol concentration was increased from 0 % to 20% w/w ethanol (figure 2.5). Ethanol was rarely used above 15% w/w as it has been previously demonstrated that any increase in ethanol concentration, albeit beneficial for drug solubility, decreases the respirable deposition, which suggests no net gain in the effective respirable mass (Gupta and Stein, 2003).

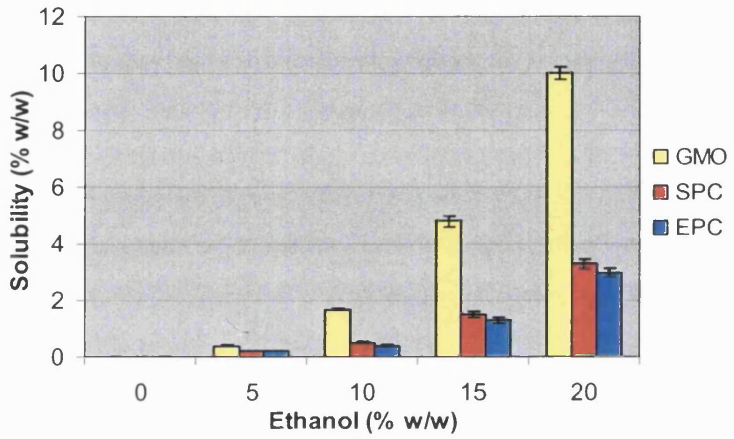


Figure 2.5 *GMO, SPC and EPC solubilities as a function of ethanol concentration in HFA 134a (n=3 ±SD).*

All ternary-phase diagrams (figures 2.6-2.8) show systems containing ≥77% propellant by weight and thus ≤ 23% ethanol and PC or GMO (please note that ethanol was rarely used above 15% w/w due to its effect on vapour pressure). Diagrams were constructed conventionally and their interpretation is straightforward. In pressurised systems, analytical determination of the constitution of the phases which are in equilibrium with each other is impractical because of the volatile nature of the blends. There is also a difference between conventional ternary-phase diagrams (at fixed temperature and pressure) and those describing blends of liquefied propellants that are maintained as liquids in closed vessels in equilibrium with their own vapour. The vapour pressure at each point in these phase diagrams is fixed, yet it varies throughout the diagram (temperature is constant at 21 ± 2°C throughout). Even though pressure is a variable throughout the diagrams (generally increasing in the direction of 100% propellant), at any given point in a diagram, temperature and pressure are fixed.

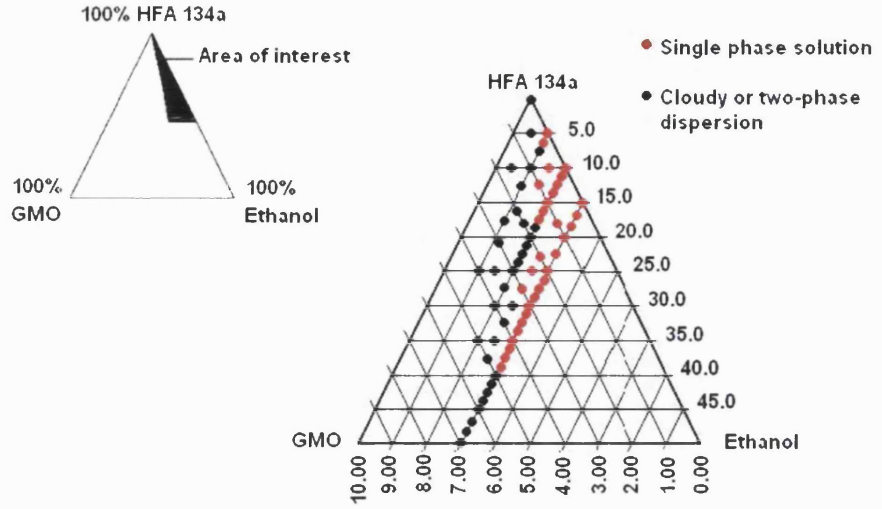


Figure 2.6 *Ternary-phase diagram for GMO-ethanol-HFA 134a (n=3).*

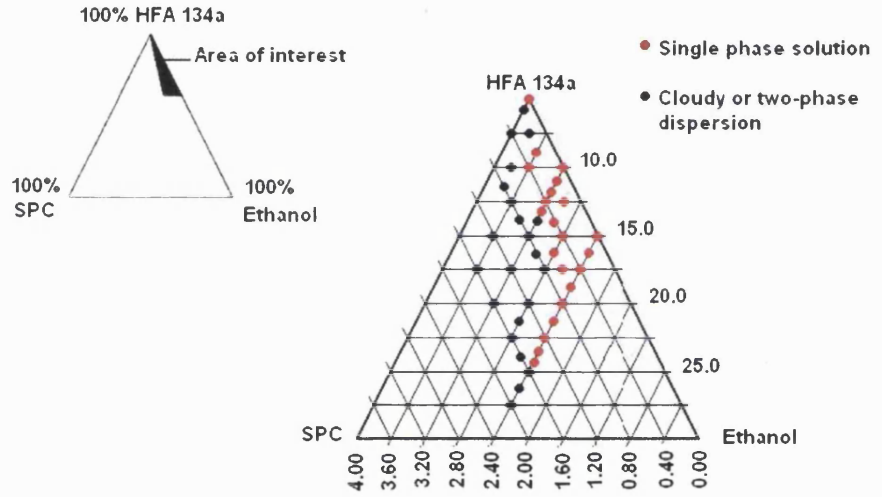


Figure 2.7 *Ternary-phase diagram for SPC-ethanol-HFA 134a (n=3).*

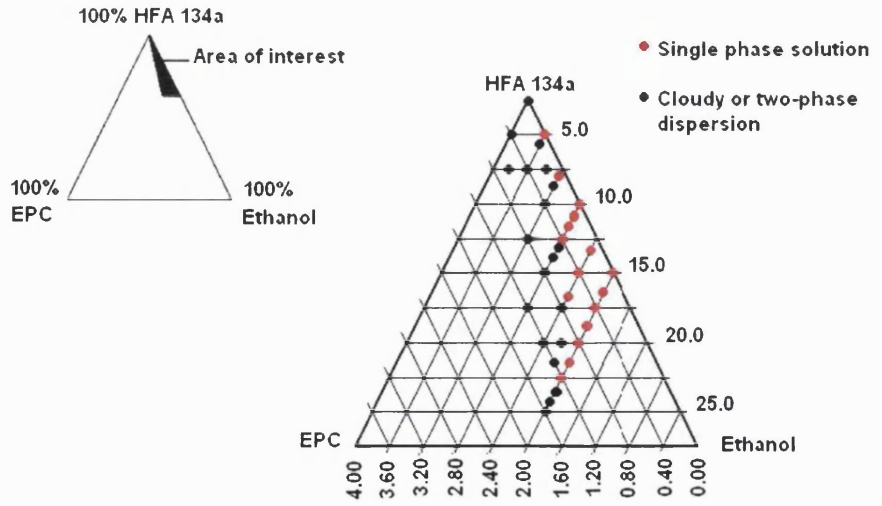


Figure 2.8 *Ternary-phase diagram for EPC-ethanol-HFA 134a (n=3).*

2.4.3 Comparing propellants HFA 134a and HFA 227

It has been reported that formulations with propellant HFA 227 require smaller amounts of excipients such as cosolvent ethanol, compared to HFA 134a (Solvay Fluor und Derivate, 2006). This is due to the more favourable lower vapour pressure and surface tensions as well as the higher density, viscosity and solubility of propellant HFA 227.

Results from our studies show that more ethanol was needed to solubilise the same amount of GMO or PCs in HFA 227 than for HFA 134a. For example, 4.8% w/w GMO was the maximum amount dissolved in HFA 134a with 15% ethanol. Using the same ethanol concentration in HFA 227, it was not possible to dissolve 4.8% w/w GMO and the resulting formulation was cloudy. This may be due to the higher polarity and water solubility of HFA 134a compared with HFA 227 which can be extended to ethanol solubility. Ethanol is more soluble in HFA 134a and ethanol-dependent dissolution of other formulation components would be more favourable in HFA 134a than HFA 227.

Results from this study favour the use of HFA 134a, thus this propellant was used for subsequent drug incorporation solubility studies. Moreover, HFA 134a is preferable in terms of spray characteristics when a dose is actuated. This is because HFA 134a and HFA 227 differ significantly in vapour pressure; 570 and 390 kPa at 20°C, respectively. Higher pressure leads to more efficient atomisation and finer sprays. Addition of cosolvent and non-volatile additives lower the propellant vapour and pack pressures, although those for HFA 134a systems remain higher than for equivalent HFA 227 systems (Brambilla *et al.*, 1999).

2.4.4 Incorporation of a drug in the pMDI formulation

2.4.4.1 Salbutamol sulphate

To investigate the usefulness of the formulation for inhalation purposes, a drug was included. Salbutamol sulphate has the chemical formula: (base)(2:1)(salt), a molecular weight of 576.7, and its empirical formula is $C_{13}H_{21}NO_3 \cdot H_2SO_4$ (www.eGeneralMedical.com). It is soluble in 4 parts of water and slightly soluble in ethanol (96%) (http://www.gsk.com). Solubility of salbutamol sulphate in the three-component system (HFA 134a-ethanol-GMO, SPC or EPC) was investigated. Formulations were considered useful when the four-component system, including the drug, formed a clear one-phase system.

It was found that salbutamol sulphate was insoluble in the combination of HFA 134a-ethanol-GMO or PC. Salbutamol sulphate particles sedimented. Upon shaking, it was possible to redisperse the drug particles but the formulation obtained was an unstable suspension with GMO and an aggregated one with SPC and EPC. One explanation is that salbutamol sulphate is a salt and does not dissolve in ethanol. Ethanol is present in our formulation to dissolve GMO and the PCs and caused the formation of an unstable system. Sedimentation occurred due to density differences between the drug particles and the propellant system.

2.4.4.2 Salbutamol base and budesonide

The incorporation of salbutamol base or budesonide in the formulations led to the formation of a clear one-phase system depending on the concentrations of the other components. Salbutamol base has a molecular weight of 239.3 and its empirical formula is $C_{13}H_{21}NO_3$. It is soluble in ethanol and sparingly soluble in water. Salbutamol sulphate on the other hand is soluble in water and slightly soluble in ethanol (www.eGeneralMedical.com). Budesonide is soluble in ethanol. The solubility of micronised budesonide in ethanol is 31.756 mg/g (Lewis *et al.*, 2004).

Increasing the concentration of ethanol in the formulation increased the amount of salbutamol base or budesonide that could be dissolved. For example, in the formulation containing 0.1% w/w GMO, 5% w/w ethanol and HFA 134a, salbutamol base was soluble at 0.01% w/w. When ethanol concentration was increased to 10% w/w and GMO concentration kept constant, salbutamol base was soluble at 0.04% w/w, i.e. a 4-fold increase in solubility. Further increasing ethanol concentration to 15% w/w enabled salbutamol base to dissolve up to 0.1% w/w, when GMO concentration was kept at 0.1% w/w. Increasing GMO concentration, however, decreased the amount of drug that went into solution. For example, only 0.05% w/w salbutamol base dissolved at 15%w/w ethanol when 0.5% w/w GMO was used (tables 2.8-2.10 and figures 2.9-2.13).

Formulations containing SPC followed the same pattern. At 5% w/w ethanol and 0.1% w/w SPC, salbutamol base did not dissolve at 0.1% w/w. The same amount of salbutamol base dissolved at the same SPC level when ethanol concentration was increased to 10% w/w. At 15% w/w ethanol, the same amount of salbutamol base (i.e. 0.1% w/w) dissolved even after increasing SPC concentration to 0.5% w/w. Further increase in SPC caused less drug to dissolve, for example, at 0.8% w/w SPC and 0.1% salbutamol base, the formulation was cloudy. The results obtained with SPC and EPC were similar. Consequently, only one PC was chosen for future experiments, EPC in this case because it was slightly more soluble.

The results obtained suggested that salbutamol, GMO and PCs compete for H-binding sites on ethanol molecules to remain in solution. When PC concentration is increased, more PC molecules associate with cosolvent ethanol, which enables their disssolution; this would inhibit the drug from binding to ethanol and causes its lack of solubility. This also explains why less GMO or PC could go into solution when salbutamol base is incorporated in the formulation compared to data obtained with the three-component system, i.e. without the drug (tables 2.8-2.10; figures 2.6-2.8 vs. 2.9-2.11). More salbutamol base can be dissolved by increasing ethanol concentration even further (to 20% w/w) but the formulation would become unlikely to be useful for inhalation purposes (Gupta and Stein, 2003). Budesonide ternary phase diagrams are shown in figures 2.12 and 2.13.

Table 2.8 Solubility data of GMO and salbutamol base in HFA 134a with cosolvent ethanol at 5, 10 and 15%w/w (n=3).

Formulation	GMO % w/w	Salbutamol base % w/w	Ethanol % w/w	HFA 134a % w/w	Observation[1]			
					30 s	19 h	At 4°C for 5 h	4 weeks
1	0.09	0.09	4.91	94.91	I	I	I	I
2	0.10	0.01	5.00	94.89	S	S	S	S
3	0.10	0.01	5.01	94.89	S	S	S	S
4	0.10	0.04	5.00	94.86	I	I	I	I
5	0.10	0.10	4.99	94.80	I	I	I	I
6	0.10	0.09	6.52	93.30	I	I	I	I
7	0.23	0.12	7.62	92.02	I	I	I	I
8	0.10	0.01	9.99	89.90	S	S	S	S
9	0.10	0.03	10.07	89.80	S	S	S	S
10	0.10	0.07	10.00	89.83	I	I	I	I
11	0.10	0.10	9.98	89.82	I	I	I	I
12	0.14	0.14	11.26	88.46	I	I	I	I
13	0.08	0.08	13.32	86.52	I	I	I	I
14	0.05	0.15	15.01	84.79	I	I	I	I
15	0.10	0.11	15.03	84.76	I	I	I	I
16	0.10	0.13	15.02	84.75	I	I	I	I
17	0.11	0.05	15.87	83.97	S	S	S	S
18	0.11	0.10	15.89	83.90	S	S	S	S
19	0.18	0.10	15.04	84.69	I	I	I	I
20	0.20	0.05	15.04	84.71	S	I	I	I
21	0.20	0.12	15.00	84.68	I	I	I	I
22	0.20	0.15	14.99	84.66	I	I	I	I
23	0.30	0.02	15.10	84.58	S	S	S	S
24	0.30	0.04	15.03	84.63	S	S	S	S
25	0.30	0.05	15.02	84.63	S	I	I	I
26	0.30	0.10	15.02	84.58	I	I	I	I
27	0.50	0.02	15.05	84.43	S	S	S	S
28	0.50	0.04	15.03	84.43	S	S	S	S
29	0.50	0.05	15.00	84.45	S	I	I	I
30	0.50	0.05	15.04	84.41	S	I	I	I
31	0.50	0.10	15.00	84.40	I	I	I	I

[1]S denotes soluble and I insoluble.

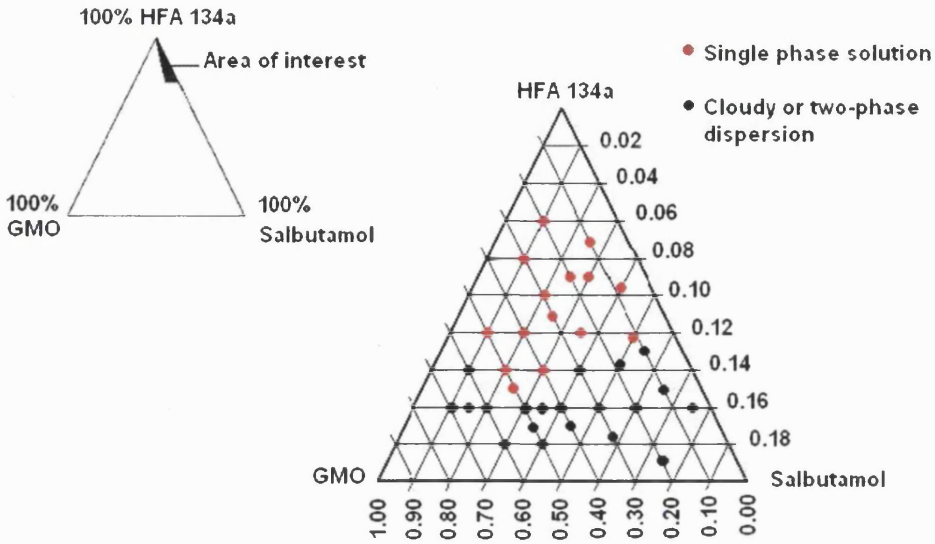


Figure 2.9 *Solubility phase diagram for salbutamol base with 15% w/w ethanol in a formulation containing GMO, ethanol and HFA 134a at ambient temperature (n=3).*

Table 2.9 Solubility data of SPC and salbutamol base in HFA 134a with cosolvent ethanol at 5, 10 and 15%w/w (n=3).

Formulation	SPC % w/w	Salbutamol base % w/w	Ethanol % w/w	HFA 134a % w/w	Observation[1]			
					30 s	19 h	At 4°C for 5 h	4 weeks
32	0.03	0.10	5.02	94.85	I	I	I	I
33	0.10	0.10	5.00	94.80	I	I	I	I
34	0.10	0.02	10.04	89.84	S	S	S	S
35	0.10	0.05	10.01	89.84	S	S	S	S
36	0.10	0.07	10.04	89.78	S	I	I	I
37	0.10	0.10	9.98	89.82	S	I	I	I
38	0.10	0.12	9.97	89.81	I	I	I	I
39	0.11	0.11	9.50	90.29	I	I	I	I
40	0.10	0.10	15.03	84.77	S	S	S	S
41	0.10	0.15	14.98	84.77	I	I	I	I
42	0.10	0.25	14.99	84.67	I	I	I	I
43	0.20	0.10	14.99	84.71	S	S	S	S
44	0.30	0.10	14.98	84.62	S	S	S	S
45	0.50	0.10	14.97	84.43	S	S	S	S
46	0.50	0.20	15.00	84.30	S	I	I	I
47	0.60	0.10	15.04	84.25	I	I	I	I
48	0.70	0.10	15.03	84.17	I	I	I	I
49	0.80	0.10	15.04	84.05	I	I	I	I

[1]S denotes soluble and I insoluble.

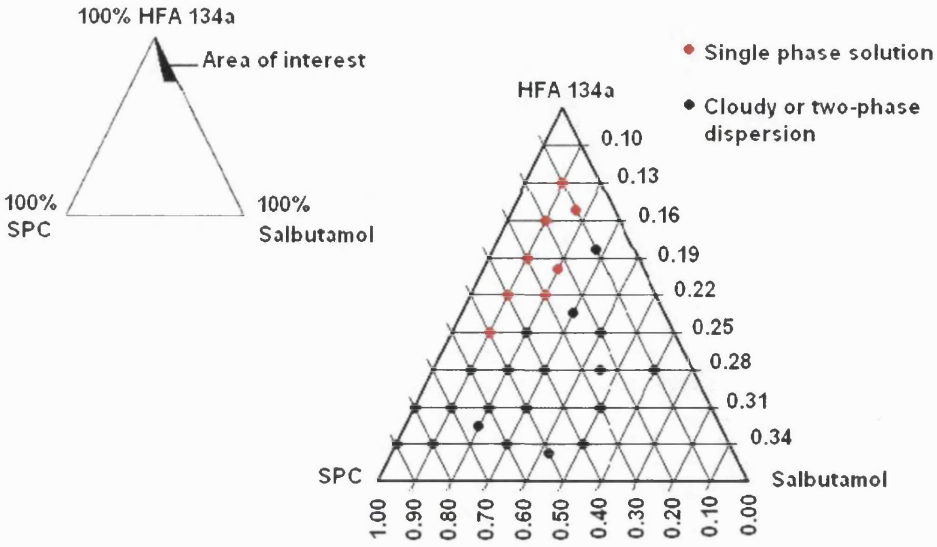


Figure 2.10 *Solubility phase diagram for salbutamol base with 15% w/w ethanol in a formulation containing SPC, ethanol and HFA 134a at ambient temperature (n=3).*

Table 2.10 EPC and salbutamol base solubility in HFA 134a with 5, 10 and 15% w/w ethanol (n=3).

Formulation	EPC % w/w	Salbutamol base % w/w	Ethanol % w/w	HFA 134a % w/w	Observation[1]		
					30 s	17 h	4 weeks
50	0.01	0.05	5.01	94.93	I	I	I
51	0.05	0.10	5.00	94.85	I	I	I
52	0.10	0.10	5.00	94.80	I	I	I
53	0.01	0.05	10.00	89.94	I	I	I
54	0.05	0.10	9.97	89.88	I	I	I
55	0.10	0.10	10.00	89.80	I	I	I
56	0.20	0.10	9.99	89.71	I	I	I
57	0.30	0.05	9.99	89.66	I	I	I
58	0.30	0.10	10.01	89.59	I	I	I
59	0.40	0.11	10.01	89.49	I	I	I
60	0.10	0.07	12.50	87.33	S	S	S
61	0.10	0.08	12.54	87.27	I	I	I
62	0.10	0.10	12.48	87.32	I	I	I
63	0.30	0.07	12.52	87.11	S	S	S
64	0.10	0.07	15.04	84.79	S	S	S
65	0.10	0.08	15.04	84.78	S	S	S
66	0.10	0.10	15.00	84.80	I	I	I
67	0.40	0.10	15.03	84.47	I	I	I
68	0.70	0.10	15.00	84.20	I	I	I
69	1.00	0.07	15.04	83.89	S	S	S
70	1.00	0.10	15.00	83.90	S	S	S
71	1.30	0.10	15.01	83.59	S	S	S
72	1.50	0.05	14.99	83.46	S	S	S
73	1.50	0.07	15.01	83.42	S	S	S
74	1.50	0.08	15.01	83.41	S	S	S
75	1.50	0.10	15.00	83.40	S	S	S
76	1.50	0.15	15.00	83.35	I	I	I
77	1.70	0.10	15.01	83.19	S	S	S
78	1.81	0.10	14.99	83.11	S	S	S
79	2.01	0.10	15.06	82.83	I	I	I

[1]S denotes soluble and I insoluble.

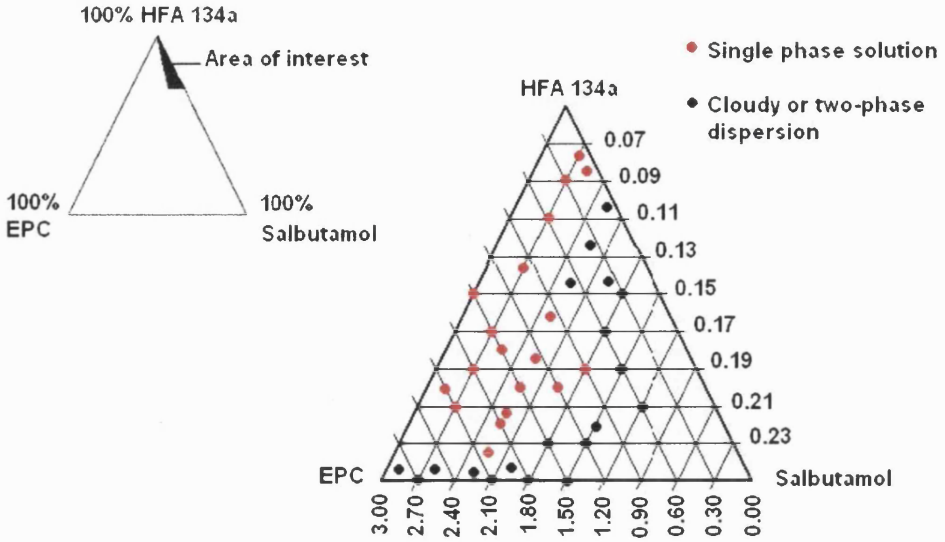


Figure 2.11 *Solubility phase diagram for salbutamol base with 15% w/w ethanol in a formulation containing EPC, ethanol and HFA 134a at ambient temperature (n=3).*

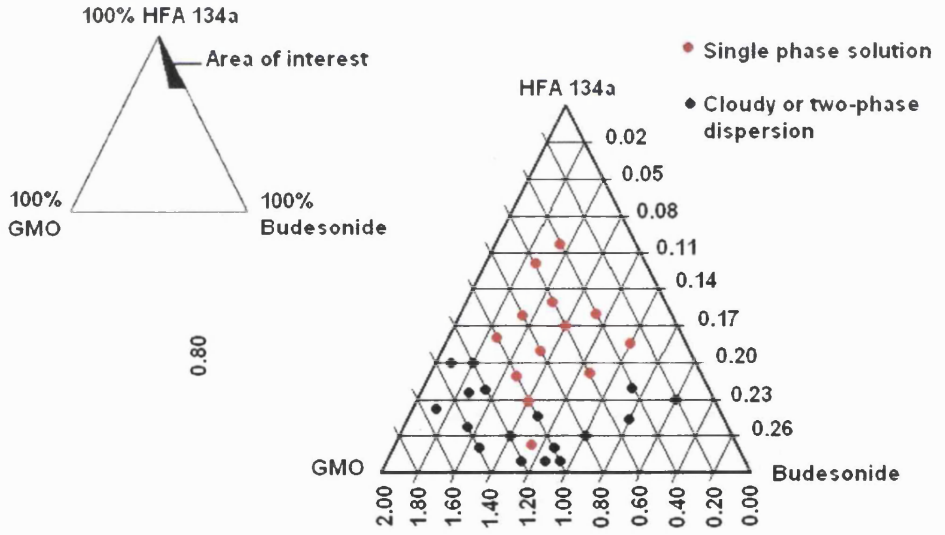


Figure 2.12 *Solubility phase diagram for budesonide with 15% w/w ethanol in a formulation containing GMO, ethanol and HFA 134a at ambient temperature (n=3).*

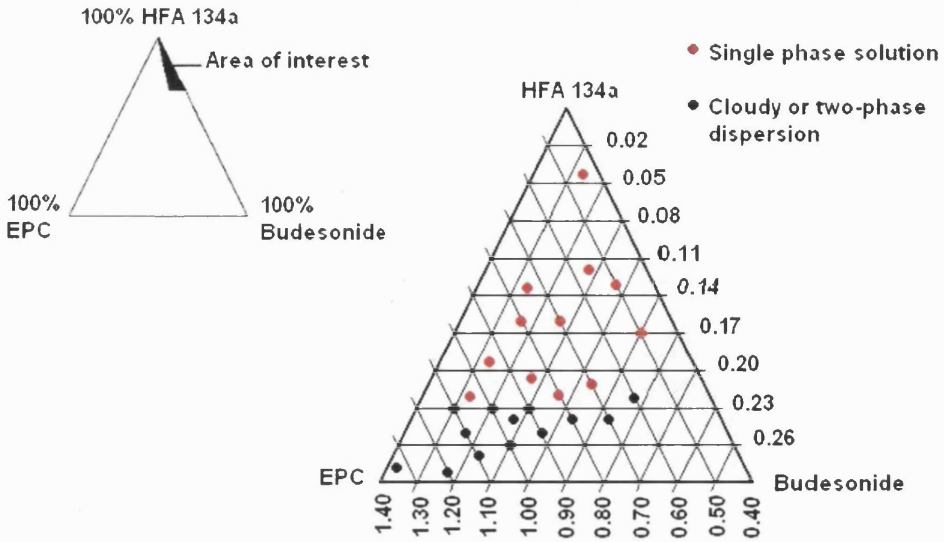


Figure 2.13 *Solubility phase diagram for budesonide with 15% w/w ethanol in a formulation containing EPC, ethanol and HFA 134a at ambient temperature (n=3).*

All pMDI solution systems formulated showed good physical stability over 4 weeks when stored at room temperature (21 ± 2°C).

2.4.5 PCS results

Nine formulations containing GMO, SPC and EPC were prepared for PCS analysis. The Zetasizer can measure particles from 5 to 5000 nm using the method of photon correlation spectroscopy (PCS). Particles in this size range display constant random thermal motion, known as Brownian motion. The motion causes the amount of light scattered by the particles to vary with time. The larger the particles the slower the motion and hence the smaller the variation in intensity of light scattered. PCS uses the rate of change in the light intensities to determine the size distribution of particles. The results shown in table 2.11 (kilo counts.sec^{-1}) represent the speed of measurements. For particles between 5 to 5000 nm, kilo counts should be in the range 50 to 300. Results from this study indicate that the formulations are of particle size smaller than 5 nm, which is that of solution formulations.

Table 2.11 PCS analysis results (n=3).

Formulation	Salbutamol base % w/w	Ethanol % w/w	HFA 134a % w/w	Kilo counts/sec
GMO % w/w				
0.10	0.06	15.01	84.83	1 to 2
0.10	0.10	14.96	84.84	1 to 2
0.30	0.05	14.99	84.66	1 to 2
SPC % w/w				
0.10	0.05	10.00	89.85	1 to 2
0.10	0.10	15.00	84.80	1 to 2
0.50	0.10	14.98	84.42	1 to 2
EPC % w/w				
0.10	0.07	12.49	87.34	1 to 2
0.10	0.07	14.99	84.84	1 to 2
0.50	0.07	15.00	83.43	1 to 2

2. 4.6 Temperature challenging experiments

It was found that where formulations were soluble, temperature variations over the range 3.5 to 50 °C did not affect solubility. On the other hand, temperature did not improve solubility of formulations where dissolution was not achieved (table 2.12).

Table 2.12 Effect of temperature on solubility of formulations containing salbutamol base, GMO, soya or EPC and ethanol in the propellant 134a (n=3).

Formulation[1]	Temperature (°C)									
	3.5	8.5	13.5	20	25	30	35	40	45	50
1, 6, 7, 12, 13, 39, 41, 47, 49, 53, 62, 68, 76	I[2]	I	I	I	I	I	I	I	I	I

[1]Formulations are the same as those in tables 2.8-2.10.
[2]I denotes insoluble.

The lack of temperature dependence can be interpreted as an indication of low enthalpy of solution in propellant HFA 134a, i.e. the enthalpy of fusion of the solids is nearly compensated by that due to mixing the solute and propellant. Therefore, the dissolution process must be driven to a significant extent by gains in entropy.

When formulations containing salbutamol sulphate were investigated, there was no effect of temperature on solubility over the temperature range investigated. The lower solubility no doubt is a result of the more tightly bound solids in the crystal matrix, as evidenced by the much higher melting point for the salts (melting with decomposition around 155°C) (http://www.gsk.com). This may represent an aspect of formulation stability. An advantage is that the problem of crystal growth during temperature-cycled storage may be diminished with the new propellants due to the reduced dependence of solubility on temperature over the range usually considered (Byron *et al.*, 1994).

2.4.7 Water incorporation

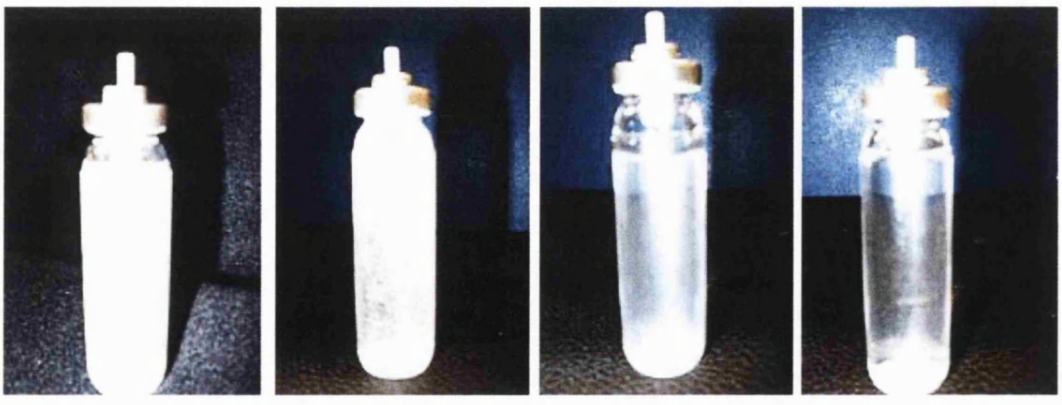
2. 4.7.1 Salbutamol sulphate

Small amounts of water were added to formulations containing salbutamol sulphate, which is a water soluble drug, therefore the intention was to investigate whether the drug's solubility can be improved with water (table 2.13). Water was added at different levels (0.26 to 1.98% w/w). The resulting formulations containing salbutamol sulphate, ethanol, GMO or PC and small amounts of water were not clear one-phase systems. However, it was found that water had an effect on the sedimentation rate of salbutamol sulphate as shown in figure 2.14. Water delayed sedimentation probably by adsorbing on drug molecules. Water is soluble in HFA 134a at 2220 ppm and water-salbutamol sulphate complexes would remain stable for a little longer than salbutamol sulphate

particles alone. It is reported that salbutamol sulphate partitions into water more strongly than does its basic form (Fitzpatrick and Corish, 2005). Drug particles would eventually sediment.

Table 2.13 Effect of water on salbutamol sulphate stability in HFA 134a pMDIs (n=3).

Formulation	Salbutamol base % w/w	Ethanol % w/w	Water % w/w	HFA 134a % w/w	Observation
GMO % w/w					
0.05	0.03	5.28	0.26	94.38	stable
0.10	0.06	9.97	0.50	89.37	stable
0.08	0.08	12.49	0.83	86.51	stable
0.10	0.10	14.76	0.10	84.95	stable
0.10	0.10	15.12	0.30	84.37	stable
0.10	0.10	15.02	0.50	84.28	stable
0.10	0.10	15.01	0.80	83.99	stable
0.15	0.14	22.00	1.46	76.24	stable
SPC % w/w					
0.05	0.03	5.29	0.26	94.36	stable
0.10	0.06	10.01	0.50	89.33	stable
0.08	0.08	12.45	0.83	86.55	stable
0.10	0.10	15.14	0.10	84.56	stable
0.10	0.10	15.08	0.30	84.42	stable
0.10	0.10	15.38	0.51	83.91	stable
0.10	0.10	15.18	0.81	83.81	stable
0.10	0.10	14.87	1.98	82.94	stable
0.14	0.15	21.94	1.46	76.30	stable
EPC % w/w					
0.05	0.02	5.28	0.26	94.37	stable
0.10	0.05	10.00	0.50	89.35	stable
0.08	0.08	12.45	0.83	86.55	stable
0.10	0.10	14.81	0.10	84.89	stable
0.10	0.10	15.15	0.30	84.35	stable
0.10	0.10	15.28	0.51	84.00	stable
0.10	0.10	14.80	0.79	84.22	stable
0.15	0.14	21.94	1.46	76.30	stable



(a) (b) (c) (d)

Figure 2.14 *Effect of water on sedimentation rate of salbutamol sulphate (a) with water after shaking; (b) without water after shaking; (c) with water after 5 min; and (d) without water after 5 min.*

2.4.7.2 Salbutamol base

Small amounts of water were added to salbutamol base formulations in order to challenge their stability (table 2.14). Formulations were all clear solutions in the absence of water. It has been reported previously that, although polar, propellant HFA 134a has difficulty in dissolving added water (Blondino and Byron, 1998).

Because ethanol solubility (and for that matter drug, PC, and GMO solubility) in pure HFA is heavily reliant upon the ability to form dipole-dipole interactions between the solvent and the liquid propellant, it is not surprising that the addition of small amounts of competing dipolar molecules (like water) can cause rapid, irreversible precipitation (phase separation) (Vervaet and Byron, 1999). Results from this study indicated, however, that water at levels between 0.1% w/w and 1.09% w/w did not apparently affect the system's stability.

Water solubility data from the literature shows that there is a difference between the HFAs and the CFCs. Water solubility is reported to be 2220 ppm, 610 ppm, 91 ppm and 100 ppm for HFA 134a, HFA 227, CFC 12/114 and CFC 11/12, respectively (Solvay Fluor und Derivate, 2006).

Theoretically, HFAs have a greater attraction to the high polarity solute, water. This property is carried through into HFA-ethanol blends, implying that dipolar interactions between HFAs and ethanol itself (at 10 and 15% by weight) were not capable of completely displacing HFA-water attractions in this liquid milieu.

Table 2.14 Effect of water on salbutamol base stability in solution HFA 134a pMDIs.

Formulation	Salbutamol base % w/w	Ethanol % w/w	Water % w/w	HFA 134a % w/w	Observation
GMO % w/w					
0.10	0.05	10.01	0.10	89.74	stable
0.10	0.05	15.21	0.10	84.54	stable
0.10	0.10	15.01	0.10	84.69	stable
0.11	0.05	16.07	1.07	82.70	stable
0.11	0.10	16.04	1.07	82.68	stable
SPC % w/w					
0.09	0.05	10.00	0.11	89.74	stable
0.30	0.10	15.14	0.10	84.35	stable
0.33	0.11	16.34	1.09	82.13	stable
EPC % w/w					
0.11	0.05	9.99	0.10	89.75	stable
0.10	0.07	15.16	0.10	84.57	stable
0.11	0.07	15.89	1.06	82.87	stable

2.5 Conclusion

This thesis proposes a novel formulation by including phospholipid or GMO as a component of the pMDI system with the aim of achieving a controlled release of a slightly hydrophilic drug, salbutamol base, and a hydrophobic one, budesonide (Chapter 3). In this research, investigation of the preparation, characterisation, encapsulation efficiency and *in vitro* release of the drug are discussed in Chapter 3. In this chapter, the different formulations with various ratios of phospholipid, drug, cosolvent and propellant were evaluated and optimised. Results from this initial study are encouraging. The study has established that it is possible to formulate amphiphilic PCs and GMO in pMDI systems, and that a cosolvent enhances their dissolution. Results from this study demonstrated that ethanol could be an efficient cosolvent for solubilisation of different formulation components and hence achieve a solution pMDI system.

For compounds used in the treatment of respiratory diseases, solution pMDI systems appear to be attractive formulations compared to suspension pMDIs. One advantage is better physical stability. This is the first time in the literature that phospholipids and drugs solubilised with a cosolvent have been used in pMDI HFA systems. They were prepared by adding propellant HFA 134a to a known amount of drug and phospholipid or GMO dissolved in cosolvent ethanol. This combined approach of a cosolvent system enhances the solubility of the pMDI ingredients.

Different solvents were investigated in terms of their solubilisation potential for drug and phospholipid in the model propellant DFP. Ethanol, propylene glycol, polyethylene glycol 300, polyethylene glycol 400 and glycerol were studied. Ethanol appeared to contribute more favourably to drug, GMO and PC solubility than the other cosolvents used, especially at high levels (15-20% w/w). Ethanol was therefore chosen as the model cosolvent in the HFA system. The following were observed:

- First, the favourable contribution of ethanol to solubility of drug, GMO, SPC or EPC is concentration-dependent.
- Second, the favourable contribution of ethanol to solubility of drug is diminished as the amount of phospholipid or GMO in the formulation increases and even becomes unfavourable at very high levels of phospholipid.
- Third, PCs contribute much less favourably than GMO in forming a solution system, but still exhibit the same ethanol-dependent solubilisation pattern.
- Fourth, salbutamol sulphate didn't form a solution system in HFA 134a, probably due to its poor solubility in ethanol as well as the propellant.
- Fifth, more EPC dissolved in the HFA system compared to SPC when salbutamol

base was incorporated in the formulation and will therefore be the PC used in subsequent experiments.

- Lastly, the contribution of ethanol to drug or phospholipid solubility appears to be mainly determined by their H-bonding ability.

The results obtained with cosolvent ethanol may permit a sufficient amount of phospholipid and drug to dissolve in HFA 134a in order to enable vesicle formation in an aqueous environment.

PC and GMO are insoluble in 100% propellant. However, their solubility in propellant formulations increased significantly upon inclusion of ethanol. The investigations into drug/phospholipid solubility showed that HFA-solubility was enhanced by ethanol with solubility significantly higher in HFA 134a than in HFA 227 and DFP. The solubility of salbutamol base in propellant HFA 134a with the copresence of phospholipids (SPC and EPC) or GMO and cosolvent ethanol was investigated. The solubility of drug or phospholipid in the liquefied propellant and model propellant increased in a non-linear fashion with the increase of the cosolvent concentration. More GMO and PC dissolved in HFA 134a compared to the model propellant DFP. For example, the solubilisation capacities of EPC with ethanol 15% w/w in DFP and HFA 134a were 0.49 and 4.8% w/w, respectively. Addition of the cosolvent ethanol to the drug/phospholipid in propellant–cosolvent mixtures generally offered a great advantage from the viewpoint of improving solubility. Salbutamol solubility was greatly enhanced (0.01 to 0.10% w/w) by including 15% w/w ethanol as compared to the case of 5% ethanol. Solubility in HFA increases with increasing ethanol concentration from 0 to 15% w/w, but a further increase to 20% w/w would be disadvantageous from an inhalation point of view. Thus higher amounts of ethanol (>15% w/w) were rarely used. The observed solubility enhancement achieved using the cosolvent results from intermolecular interactions such as H-bonding interactions formed between the polar alcohol molecule and the polar salbutamol solute molecule and can be used to create a one phase system containing drug and phospholipids. Benefits of cosolvent addition must be balanced against its disadvantages of poorer respiratory deposition which are investigated in Chapter 3.

The strong electron withdrawing effect of the fluorine atoms in HFAs, leaving a partial positive charge on the hydrogen substituents, allows a considerable charge separation and hence the possibility of H-bonding (Byron *et al.*, 1994). Interaction between phospholipid and ethanol has been studied (Yamamoto *et al.*, 2006). At low concentration, the ethanol hydrates adsorb into the phospholipid/propellant interface and saturate the interface. A further increase in concentration causes multilayer

formation of hydrates and/or penetration of hydrates into the monolayer core. Ethanol is easily soluble in HFA 134a. In HFA 134a, propellant molecules form a hydration cluster surrounding the alkyl chain of ethanol (the hydrophobic hydration). As the ethanol concentration increases, more alcohol molecules become confined in one hydration cluster and that cluster becomes larger (Miki *et al.*, 1999; Nakagawa, 2002). Simultaneously, the hydrophobic interaction between ethanol molecules in the cluster becomes intense (Nishikawa and Iijima, 1993; Miki *et al.*, 1999; Nakagawa, 2002). Once the ethanol hydrate multilayer is formed, ethanol hydrates adsorb into the initial semi-ethanol hydrate layer formed on the monolayer/propellant interface; the new ethanol hydrate layer is formed additionally. An increase in ethanol concentration at the interface causes an interaction between adsorbed ethanol hydrates. Consequently, ethanol hydration clusters form at the interface. When the monolayer/propellant interface is occupied by ethanol hydrates, the interface loses the function as a barrier against the ethanol hydrates and ethanol thereby penetrates more easily into the monolayer core. The amount of penetrating ethanol increases with an increase in ethanol concentration. The amphiphilic monolayer that is formed on the interface maintains the structure through interaction between hydrophobic moieties and by the hydrogen-bond network between hydrophilic moieties and propellant molecules.

A very central property is the shelf-life, or stability. In the present study, some initial stability investigations were performed with different formulations containing varying concentrations of drug and phospholipid or GMO. The pMDI solution systems showed good physical stability for over 4 weeks and will be studied further in subsequent work.

CHAPTER 3

CONTROLLED-RELEASE COLLOIDS GENERATED FROM SALBUTAMOL BASE AND BUDESONIDE PRESSURISED METERED DOSE INHALER SOLUTION FORMULATIONS

3.1 Introduction

The highly vascular nature of the lung and its large surface area have been shown to facilitate rapid absorption of a variety of compounds of different physicochemical characteristics (Farr *et al.*, 1987). Pharmaceutical aerosols provide an excellent mode of delivering drugs directly to the lung and can be achieved by metered dose inhalers (pMDIs), dry powder inhalers (DPIs), or nebulisers (Newman and Pavia, 1985; Gonda, 1990; Crowder *et al.*, 2001; Finlay, 2001). Controlled release (CR) drug delivery within the respiratory tract can be achieved by modifying the formulation of inhaled drugs. There are various drugs with high therapeutic efficacy in the worldwide market for the treatment of respiratory conditions and controlled release products for such compounds would bring about huge benefits in pulmonary drug delivery (Cook *et al.*, 2005). The concept of using liposomes as carriers for the delivery of drugs is well established.

There are many advantages associated with controlled release drug delivery to the lungs, which makes it an attractive option for delivery of inhaled therapy. These include extending the duration of action of actives, reducing the dosing frequency, improving management of therapy, reducing side effects and improving patient compliance (Zeng *et al.*, 1995; Hardy and Chadwick, 2000), as well as enhancing cost effectiveness (Saks and Gardner, 1997).

Four times and twice daily dosing frequencies for asthma have been compared (Malo *et al.*, 1989; Mann *et al.*, 1992). Results showed that four times a day dosing frequency for asthma with a corticosteroid achieved less nocturnal cough, attacks and relapses compared to twice a day therapy, which is characterised by less constant drug levels. There was no difference in the side effect profile of both treatment regimens (Malo *et al.*, 1989). However, the higher dosing frequency might be limited by patient compliance problems (Derom and Thorsson, 2002). This is supported by the work by Mann *et al.*, (1992) who reported in their findings that patient compliance was worse with four times daily schedule than twice daily treatment (57.1% versus 20.2%). In another study, it was reported that patient compliance was low even with twice daily dosing and despite an extensive educational program in the self-management of asthma at the study onset, only 40% of patients given the twice daily dosing protocol complied with the treatment (Chmelik and Doughty, 1994).

The development of liposomal formulations for inhaled administration has expanded the potential for more effective use of a range of potent and effective drugs (Weinstein and Leserman, 1984; Schreier *et al.*, 1992; Taylor and Farr, 1993). Colloidal carriers, such as liposomes, have many advantages including carrier suitability for hydrophilic and lipophilic drugs, increased drug retention time, prevention of local irritation,

increased potency, and reduced toxicity of drugs post administration (Weinstein and Leserman, 1984; Gregoriadis, 1988), as well as offering the advantage of uniform deposition for locally active drugs (Parthasarathy *et al.*, 1999).

Short acting beta-2-adrenoceptor stimulants, having a short plasma half-life, typically in the range 4-6 h, are ideal candidates for controlled release formulations as they require frequent dosing. Controlled release formulations for such drug molecules offer the advantage of providing available drug over an extended period of time, hence extending their duration of action, which in turn may enhance control of disease states such as asthma symptoms.

One of the desirable characteristics of bronchodilator therapy is the ability to correspond to a nocturnal decline cover because asthma is usually characterised by a worsening at night. An overall 24 h improvement in bronchial patency is ideal. This coincides with the greatest airway hyper-reactivity to allergens and other stimuli (Smolensky *et al.*, 1987) and corresponds to a trough in the circadian rhythm for bronchial patency (0400 hours) (D'Alonzo *et al.*, 1999). A controlled release inhaled formulation for asthma, administered ideally once daily, may therefore offer the advantage of a reduced dosing frequency and more convenience. It would provide a possible solution to non-compliance in patients and would be beneficial by providing a cover for nocturnal decline and improving therapy.

Controlled release formulations for inhaled asthma therapy are required to have the following: (1) a small MMAD and high FPF so as to minimise central/tracheobronchial deposition, as well as bypass the effects of mucociliary clearance, and (2) surface characteristics which will reduce alveolar macrophage recognition, uptake and clearance. This is due to the efficient clearance mechanisms of the lung for foreign particles, which may jeopardize the potential of a controlled release formulation to release drug over extended periods.

Juliano and McCullough (1980) demonstrated the concept of liposomal delivery to the respiratory tract. They demonstrated a more specific pharmacological activity and minimised systemic exposure, including a reduction in gastrointestinal and myelotoxic side effects, for the liposome-entrapped chemotherapy agent cytosine arabinoside as a result of prolonged retention within the lung. Their research showed that the liposome-encapsulated chemotherapy agent exhibited a longer half-life in the lung than free drug (8 h versus 1 h, respectively).

Taylor *et al.*, (1989) demonstrated the applicability of liposome-mediated pulmonary controlled release in humans. Their findings suggested there were detectable amounts of drug present in plasma over an extended period of time when the drug was entrapped within liposomes. Liposomal sodium cromoglicate (DPPC:cholesterol, 1:1) was administered to healthy human volunteers. The results showed that at 24 h, cromoglicate levels were measured whereas an equivalent dose of drug inhaled as a solution was not detected at that time.

Controlled release formulations for poly(lactide-co-glycolide) (PLGA) and poly(L-lactic acid) (PLA) microparticles have also been developed for pulmonary delivery. Following administration of PLGA/isoprenaline microspheres, a prolonged protection against bronchoconstriction challenge in rats for at least 12 h was reported (Lai *et al.*, 1993). In another study, El-Baseir and Kellaway (1998) used PLA microparticles to entrap beclometasone diproprionate and nedocromil sodium. *In vitro* data showed a controlled release effect for 8 and 6 days, respectively. However, *in vivo* studies by Armstrong *et al.*, (1996) did not show positive results; pulmonary administration of PLA microspheres to rabbits resulted in inflammation at sites adjacent to microparticle deposition, increased neutrophil count and incidence of haemorrhage. PLA and PLGA microparticles also show significant reduction in cell viability compared with lipid particles in cell-based toxicity screens (Muller *et al.*, 1996) and long residence due to slow degradation might lead to pulmonary accumulation of polymers, especially with daily administration (Dunne *et al.*, 2000).

Aqueous nebulisation (Finlay and Wong, 1998; Lange *et al.*, 2001) and dry powder formulations (Schreier *et al.*, 1994) are commonly used to deliver liposome aerosols. Nebulisers generated a lot of interest for the delivery of liposomes because unlike pMDIs or DPIs, liposomes may be delivered from nebulisers without further processing (Saari *et al.*, 1999; Lange et al., 2001). However, the aqueous liposomal dispersions required for delivery via nebulisation are usually associated with long-term stability problems (Niven *et al.*, 1992). On the other hand, DPIs offer the advantages of ease of use, portability and patient acceptability. Liposomal dry powder formulations have been prepared by freeze-drying (lyophilisation) of the aqueous liposome dispersions, followed by micronisation to achieve particles in the range of 1–5 μm by jet-milling (Schreier *et al.*, 1994). It has been reported, however, that the process of lyophilisation followed by micronisation by jet-milling can lead to deleterious effects on liposome integrity, resulting in loss of the entrapped drug (Mobley, 1998; Desai et al., 2002).

Desai et al., (2002) demonstrated an approach that resulted in spontaneous formation of

liposomes upon hydration of micronised phospholipid-based powders in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs. Three model drugs were used: two antimicrobial agents (ciprofloxacin and a cationic peptide) and a bronchodilator (salbutamol sulphate). This approach of delivering liposomes in dry powder form offered the advantages of avoiding the detrimental effects of lyophilisation and jet-milling on encapsulation efficiency as well as eliminating the process cost of lyophilisation.

The present work involves a novel approach for delivering liposomes from pMDIs. The approach relies on formulating GMO or phosphatidylcholine (PC)-based solution systems that result in spontaneous formation of vesicles in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs. Two potent drugs (a bronchodilator, salbutamol, and a steroid, budesonide) were selected as model drugs to investigate this approach. Novel formulations were examined in terms of the aerodynamic dispersion properties. In order to identify a good formulation, GMO, phospholipids, drugs and cosolvent were used at different concentrations and investigated in terms of their aerodynamic particle size properties, and their ability to encapsulate drug in the fine particle fraction (FPF) upon aerosolisation. A twin impinger (TI) was used to collect the GMO or phospholipid aerosol cloud, in order to investigate vesicle formation in an aqueous environment. Subsequent *in vitro* studies involved the use of inertial sampling devices, since particle size is one of the major contributors to the efficacy of inhalation products where aerosols in the 1-5 μm size range exhibit optimal deposition profiles. *In vitro* testing was used to determine formulation factors contributing to the production of efficient, respirable aerosol clouds. In this work, a multi-stage liquid impinger (MSLI) was used to conduct aerosol characterisation in order to investigate the influence of lipid concentration, cosolvent concentration, drug concentration and vapour pressure on the respirable fraction of emitted sprays. Since knowledge of the *in vitro* release rate of an encapsulated drug is considered a necessary prerequisite to determination of the *in vivo* behaviour of a liposomal drug delivery system, the rate of release of a material from liposomes was studied. The rate of release is governed by the physicochemical properties of encapsulated compounds: liposomes are freely permeable to water, but anions are released at a faster rate than cations, whilst aqueous hydrogen bonding may determine the efflux rate of non-electrolytes (Taylor *et al.*, 1990).

It is usually necessary to separate free drug from liposomally-associated material in order to determine the amount of drug incorporated in a liposome formulation. There are many techniques employed for this purpose, including gel filtration, dialysis and centrifugation. They involve the study of drugs having medium or high aqueous solubility, whereby the non-associated drug is present as an aqueous solution in the surrounding medium. However, when examining drugs which exhibit poor aqueous solubility, the separation of

unentrapped drug becomes considerably more problematic, since excess drug may be present as suspended particles in the aqueous phase. Ultracentrifugation alone or combined with filtration can be used to separate the drug crystals from the liposomes if there is a marked difference in density between the two components.

The difficulties associated with quantifying the incorporation of materials in liposomes are compounded when the material in question exhibits low levels of incorporation and when the sizes and densities of liposomes and drug crystals are similar. Then, serious difficulties occur for separation and subsequent quantitation of entrapment. In such instances, density-gradient ultracentrifugation may be necessary to achieve separation of liposome-associated and non-associated material. In this study, a periodic centrifugation method has been used for the removal of unentrapped drug crystals which then allows assay of drug loading using HPLC (Batavia *et al.*, 2000).

However, because of the limited solubilities of steroids and steroid esters in phospholipid systems, optimal entrapment levels are known to be low, with excess drug being present in a crystalline form (Fildes and Oliver, 1978; Smith *et al.*, 1980). Hydrocortisone has been shown to interact with phospholipid head-groups whereas the palmitate ester exhibits limited affinity for the phospholipid, with the excess forming a discrete phase or acting as insoluble particles stabilised by adsorbed phospholipid (Shaw *et al.*, 1976). Crystalline drug may be either external or internal to the liposome; therefore release rates may be partially attributable to the dissolution of this excess material rather than efflux from liposomes. The initial rapid release of synthetic steroids from liposomes has also been attributed to an incompatible fit of the steroid in the liposome bilayer (Shaw *et al.*, 1976, Radhakrishman, US5049389, 1991).

In general, the knowledge base regarding the factors influencing the liposomal incorporation of drugs which exhibit low-level entrapment is limited, even to the extent that the means of quantifying entrapment levels have not been widely studied.

The hypothesis of this work is based on the possibility of spontaneously forming liposomes capable of sustained release of drug in the respiratory tract from the solubilised aerosol components. Different types and concentrations of excipients were investigated for their influence on the physical stability of the solution, moreover different phospholipid concentrations were investigated for their ability to form vesicles *in vitro* and encapsulate different drugs. In addition, the performance of pMDI units containing the novel formulations were assessed for fine particle fraction (FPF) as well as time-profile assessment of drug release.

3.2 Materials

3.2.1 Chemicals

All chemicals were used as received. 1,1,1,2-Tetrafluoroethane (Solkane 134a pharma) and 1,1,1,2,3,3,3-Heptafluoropropane (Solkane 227 pharma) were purchased from Solvay Fluor und Derivate GmbH (Hannover, Germany). Egg phosphatidylcholine (EPC) (Lipoid E-100) and soya phosphatidylcholine (SPC) (Lipoid-s-100) were a gift from Lipoid (Germany). Glycerol monooleate was obtained from GlaxoSmithKline. Salbutamol base, budesonide, Becotide® beclometasone dipropionate (Allen & Hanbury's) and Qvar® beclometasone dipropionate (IVAX) were kindly supplied by AstraZeneca (Charnwood, Loughborough, UK). Dicetylphosphate (DCP), Triton-X and sodium hexane sulphonate, orthophosphoric acid and sodium dihydrogen orthophosphate dehydrate were purchased from Sigma-Aldrich, UK. Phosphotungstic acid (PTA) 1% was analytical grade reagent and was purchased from TAAB laboratories Equipment Limited, UK. Ethanol absolute (99%-100%) and glacial acetic acid (99-100%) were purchased from VWR International Limited, UK. Water, methanol and acetonitrile used in HPLC assay were all HPLC-grade and purchased from Fisher Scientific Limited, UK.

3.2.2 Equipment

Manual bottle crimper:	Model 3000-B, Aero-Tech Laboratory Company, Maryland, USA.
Pressure filling equipment:	Pressure burette, Aero-Tech Laboratory Equipment, Maryland, USA.
Sonicating bath:	XB6 Ultrasonic Bath, Grant Instruments Limited, Royston, Hertfordshire, UK.
Twin Impinger:	Copley Instruments, Copley Scientific Limited, Nottingham, UK.
Multi-Stage Liquid Impinger:	Apparatus C, *Ph. Eur.* Supp. 2002 (Copley, UK).
Photon Correlation Spectrometer:	Malvern 2000 Mastersizer, Malvern Instruments Limited, Malvern, Worcestershire, UK.
TSI Impactor Inlet:	Model 3306 Impactor Inlet, TSI Instruments Limited, Buckinghamshire, UK.
TSI Aerodynamic Particle Sizer®:	Spectrometer Model 3321, TSI Instruments Limited, Buckinghamshire, UK.
Malvern Spraytec:	Malvern Instruments Limited, Worcestershire, UK.
HPLC:	Instrument HP 1050 Series HPLC with a UV-Visible detector, Hewlett-Packard Co., USA.
Centrifuge:	3K30 bench centrifuge, Sigma Laboratory Centrifuge, Germany.

3.3 Methods

3.3.1 Preparation of phospholipid-based pMDI units

Aerosol formulations comprising different phospholipids were prepared by mixing phospholipids, cosolvent and the drug at appropriate concentrations. The method has been described in more detail in section 2.3.1. Dicetylphosphate (DCP) was added at 10, 20, 30 and 40% molar concentration of PC when appropriate (table 3.1).

Table 3.1 Lipid composition of EPC-based formulations containing DCP at 0, 10, 20, 30 and 40% molar concentration of EPC.

	Lipid composition	
	EPC (Mol Wt. 825 g/mol)	**DCP (Mol Wt. 547 g/mol)**
EPC at 0.1% w/w	100 mol % 10 mg	0 mol % 0 mg
	100 mol % 10 mg	10 mol % 0.663 mg
	100 mol % 10 mg	20 mol % 1.326 mg
	100 mol % 10 mg	30 mol % 1.989 mg
	100 mol % 10 mg	40 mol % 2.651 mg
EPC at 1.0% w/w	100 mol % 100 mg	0 mol % 0 mg
	100 mol % 100 mg	10 mol % 6.628 mg
	100 mol % 100 mg	20 mol % 13.256 mg
	100 mol % 100 mg	30 mol % 19.885 mg
	100 mol % 100 mg	40 mol % 26.514 mg

3.3.2 *In vitro* performance

3.3.2.1 Two-stage impinger

The two stage impinger known as the twin impinger (TI), or the Single Stage Glass Impinger (SSGI), comprises two stages, the upper stage representing the upper respiratory tract and the lower stage representing the lower respiratory airways with a cut-off aerodynamic diameter of 6.4 µm at 60 l/min flow rate (Hallworth and Westmoreland, 1987). The TI operates on the principle of impingement to divide the dose emitted from the inhaler into the non-respirable dose, impacting on the mouth and oropharynx, and the respirable fraction. The back of the glass throat and the upper impingement chamber, collectively described as Stage 1 represent the non-respirable portion. The remaining respirable dose is collected in the lower impingement chamber (sometimes referred to as Stage 2).

In this work, the TI consisted of a filtering flask containing a beaker partly filled with a known volume of aqueous receptor fluid. Deionised water was used as a collection medium, 30 and 7 ml were placed in the lower and upper stages, respectively. An intake tube with a smooth 90° bend protruded through the neck, with one end located just above the fluid surface and the other end fitted with a medicinal aerosol oral adaptor and an inverted pressurised inhaler. To ensure delivery of a sufficient fraction of aerosolised dose to the receptor fluid, an air flow through the device of 60 l/min was achieved via a tube connected to a vacuum pump. The assembled apparatus was positioned in a pre-equilibrated laminar air flow cabinet to avoid contamination of receptor fluid with airborne particulates.

For each formulation analysis, the pMDI was shaken vigorously for 5 s and actuated to waste. This was repeated four times. Each aerosol unit was then examined by depressing the valve firmly seated in the oral adaptor at 10 s intervals for up to 50 actuations. Aliquots of the receptor fluid were then withdrawn with a syringe, transferred to a glass cell and examined at 37°C with a photon correlation spectrometer (PCS) (Malvern Instruments, UK) to measure liposome size. Samples were also viewed by transmission electron microscopy (TEM) (TAAB Laboratories Equipment Limited, UK).

3.3.2.2 Multi-stage liquid impinger

A multi-stage liquid impinger (MSLI) (Apparatus C, Ph. Eur. Supp. 2002) was used to assess the aerodynamic particle size distribution. It was calibrated at a flow rate of 60 l/min in terms of the effective cut-off diameter (ECD) for each stage using a flow meter type DFM. The MSLI consists of 4 impaction stages. At 60 l/min the cut-points of the stages are as follows: stage 1 - 13.0 µm, stage 2 - 6.8 µm, stage 3 - 3.1 µm, stage 4 - 1.7 µm and final filter - <1.7 µm. Following assembly, the glass impinger with associated throat was secured in a vertical position and each stage filled with 10 ml deionised water. The filter stage was covered with a 53 mm diameter filter (Whatman Int. Limited, Maidstone, UK). A rubber sealing (Copley Limited, UK) was used to connect the induction port (USP metal throat) with the mouthpiece of the metered dose inhaler then the pressurised pack and associated oral adaptor were firmly supported in a position to direct emitted aerosol into the throat device. When air is drawn through the instrument, it flows over the different stages where aerosol particles impact on the wet surface according to their aerodynamic size. To minimise particle loss inside the throat, it should always be kept clean and dry (May, 1966).

For each formulation analysis, the pMDI was shaken vigorously for 5 s and actuated to waste. This was repeated four times. Afterwards, the units were actuated 50 times over approximately 500 s (vigorous shaking in between), thus 50 consecutive doses were released into the impinger at an air flow of 60 l/min generated by a vacuum pump

downstream of a glass-fibre filter (type GF/A, Whatman, UK). Material deposited in the oral adaptor, throat, filter and various stages of the impinger was washed off and transferred to volumetric flasks. The induction port, mouthpiece and filter were carefully rinsed with an aliquot of deionised water. The impinger itself was tightly sealed with Parafilm to avoid losses due to evaporation. After dissolution of drug substance all fractions were analysed by HPLC. The mass median aerodynamic diameter (MMAD) and the fine particle fraction (FPF) were calculated. The FPF is defined as the percentage mass of the delivered drug dose smaller than 6.8 µm. This procedure was repeated three times (n = 3) for each of the produced formulations.

3.3.3 Liposome size characterisation

3.3.3.1 Transmission electron microscopy (TEM)

The method involved placing a drop of liposomal dispersion on carbon-coated copper grids (400 mesh) (TAAB Laboratories Equipment Limited, UK), negative staining with 1% phosphotungstic acid (PTA), and then viewing and taking photographs using a Philips CM 120 Bio-Twin TEM (Philips Electron Optics BV, the Netherlands).

3.3.3.2 Particle size analysis

PCS is a dynamic light scattering (DLS) technique that determines particle size by examining the diffusion rates (i.e. Brownian motion) of suspended particles. Fluctuations in the intensity of scattered light are a direct result of particle movement (i.e. Brownian motion). The technique utilises a light source to illuminate the samples and scattered light is collected at a detector typically positioned at a fixed scattering angle. For particles moving under the influence of Brownian motion, the measured scattering intensity will fluctuate with time. Across long time intervals, the intensity trace will appear to be representative of random fluctuations about a mean value. When viewed on a much smaller time scale however, it becomes evident that the intensity trace is in fact not random, but rather composed of a series of continuous data points. This absence of discontinuity is a consequence of the physical confinement of the particles to a position very near to that occupied a very short time earlier. In other words, on short time scales, the particles have had insufficient time to move very far from their initial positions, and as such, the intensity signals are very similar. The rate that the signal changes depends on the rate of change of the position of the particles, with large slow moving particles leading to slow fluctuations and small fast moving particles leading to fast fluctuations.

The smaller the particle, the more likely it is to be subject to random bombardment by solvent molecules, thereby exhibiting faster Brownian motion than larger particles.

Particle size is therefore inversely proportional to the rate of Brownian motion. The values of temperature and viscosity must be known and controlled since any change in these factors would affect Brownian motion. The method is useful for characterising particles ranging in size from a few nm to approximately 3 µm (Muller *et al.*, 2000).

The hydrodynamic diameter, d(H), is the diameter of a sphere that has the same translational diffusion coefficient as the test particle and is given by the Stokes-Einstein equation (equation 3.1).

$$d(H) = kT / 3\pi\eta D \qquad \textit{Equation 3.1}$$

where; k = Boltzmann's constant, T = absolute temperature, η = viscosity, D = translational diffusion constant (calculated by the instrument software).

As the PCS instrument analyses the movement of scattered light it records a very complex and constantly changing intensity pattern of 'speckles' at constant viscosity and temperature. The particle size is the determining factor for the rate of change of the intensity pattern. The intensity pattern signal changes slowly for large particles, therefore correlation between the signals is observed for a long time. The correlation disappears more rapidly, however, for small particles moving at a higher rate compared to larger ones. A correlator measures the correlation of the signals versus time, and uses algorithms to generate a particle size distribution for the sample from these data. The distribution is typically characterised by a mean diameter (z-average diameter, z_{ave}) and width (polydispersity) for the size distribution.

3.3.4 Aerosol cloud characteristics

3.3.4.1 TSI Impactor Inlet model 3306 and Aerosol Particle Sizer® (APS) model 3321

TSI Model 3306 Impactor Inlet combining a single stage impactor and an APS sampling probe was used with an Aerodynamic Particle Sizer® (APS) spectrometer Model 3321. The Impactor Inlet directs a small aerosol sample to the APS for size distribution measurement. Aerosol presentation to the Model 3306 is through a *USP/Ph Eur* inlet, at a flow rate of 28.3 l/min, and the majority of the sample passes through a single stage impactor, which collects the non-respirable fraction of the sample and then through a filter which collects the respirable fraction of the sample for analysis. For this study, the target cut-point of the impactor stage was 4.7 µm, thereby allowing for the collection of a fine particle mass < 4.7 µm ($FPM_{4.7\ \mu m}$) from the filter. The larger aerosol particles that do not flow through to the filter are collected on the impaction plate.

A small portion of the aerosol that penetrates through the *USP/Ph Eur* inlet (0.2% of the sample) is sampled isokinetically by the APS at 0.062 l/min for size measurement via the Model 3321 (figure 3.1). The remaining 99.8% of the aerosol passes through a single stage impactor with a cut-point, which captures the non-respirable fraction of the sample. A 47 mm glass-fibre after-filter collects the respirable fraction of the sample for analysis. The Model 3321 analyses particles as they pass individually through the measurement zone, where their aerodynamic size is determined by time-of-flight (TOF).

For testing, each vial was shaken and then primed by actuating 3 times to waste. Five actuations were delivered for each test, and 3 tests were conducted for each vial. The MMAD was calculated using the TSI software, Aerosol Instrument Manager (2004). The plate and filter were assayed via HPLC-UV.

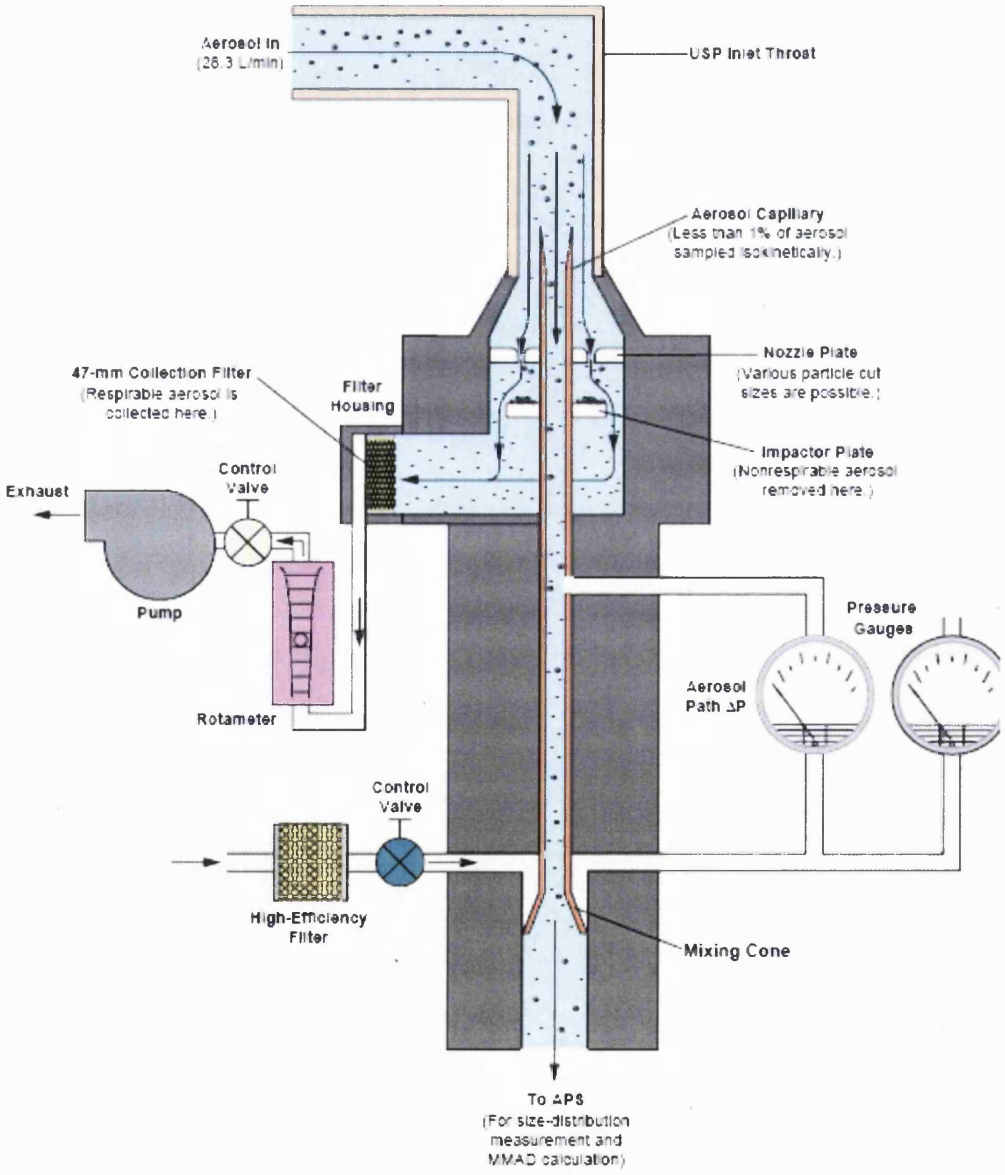


Figure 3.1 *Cross-section representation of the Impactor Inlet Model 3306. Courtesy of TSI Inc.*

Aerodynamic diameter is the most important size parameter because it determines a particle's airborne behaviour. Time-of-flight particle sizing involves measuring the acceleration of aerosol particles in response to the accelerated flow of the sample aerosol through a nozzle. The aerodynamic size of a particle determines its rate of acceleration, with larger particles accelerating more slowly due to increased inertia.

The APS 3321 measures both aerodynamic diameter and light-scattering intensity, providing accurate count size distributions for particles with aerodynamic diameters from 0.5 to 20 μm and detects light-scattering intensity for particles from 0.3 to 20 μm. In the instrument, particles are confined to the centreline of an accelerating airflow to pass through two broadly focused laser beams, scattering light in so doing. Side-scattered light is collected by an elliptical mirror that focuses the collected light onto a solid-state photodetector, which converts the light pulses to electrical pulses. By electronically timing between the peaks of the pulses, the velocity can be calculated for each individual particle. As particles exit the nozzle, TOF between the instrument's two laser beams is recorded and converted to aerodynamic diameter using a calibration curve.

3.3.4.2 Malvern Spraytec

The Malvern Spraytec measures droplet size distributions using the technique of laser diffraction. This requires the angular intensity of light scattered from a spray to be measured as it passes through a laser beam. The recorded scattering pattern is then analysed using an appropriate optical model to yield a size distribution.

The technique relies on the fact that the passage of light is affected by changes in the refractive index (RI) of the material through which it is transmitted. In a spray, the particulate phase normally has a significantly different RI compared to the gas phase. This causes light to be diffracted from the interface of the spray particles, producing a characteristic light scattering pattern. A laser diffraction system measures the intensity changes observed within this scattering pattern in order to calculate the spray particle size.

A low power He–Ne laser illuminates the flow field, and particles passing through the beam scatter light. At small forward angles, the scattering is predominantly due to diffraction and the angle of the scattered light is inversely proportional to the size of the drops. The scattered light is detected by a set of concentric annular detectors placed at the focal point of a Fourier lens, which converts the incoming rays of scattered light into a far-field diffraction pattern. Thus, the detector picks up light scattered at a specific angle independent of the position of the drops. The instrument was equipped with a 450-mm lens which gives a particle size measurement range from 2.25–850 μm.

3.3.5 Determination of the concentration of salbutamol base or budesonide by High Performance Liquid Chromatography (HPLC)[1]

3.3.5.1 Salbutamol base

Chromatography conditions (table 3.2) were constant throughout all experiments. The HPLC equipment was assembled from components (adapted from Elhissi, 2006).

3.3.5.1.1 Mobile phase preparation

The chromatographic system comprised the HPLC instrument (HP 1050 Series HPLC with a UV-Visible detector, Hewlett-Packard Co., USA) and a Symmetry® C18 column (150 mm x 4.6 mm; 5 µm; Waters Limited, UK) (table 3.2). Samples were analysed at 276 nm. A buffer containing 5 mM sodium hexane sulphonate in water was mixed with methanol in the ratio of 75:25 (v/v) to make up the mobile phase. Glacial acetic acid was added at 1% w/w of the total volume of the mobile phase. The mobile phase was mixed well, filtered and degassed for 15 min by placing the bottle in a sonicating bath (XB6 Ultrasonic Bath, Grant Instruments Limited, Royston, Hertfordshire, UK). When not required, the stock buffer was refrigerated (< 4 °C) to prolong shelf-life.

The flow rate of the mobile phase was set at 1 ml/min at 40 °C and injection volume was 10 µl. A calibration curve of salbutamol base was constructed using drug concentrations 1 to 200 µg/ml and drug in sample was quantified accordingly. Drug entrapment was expressed as entrapment efficiency (% of available drug entrapped in liposomes).

3.3.5.1.2 Chromatography

The column oven and UV detector were switched on at least 30 min prior to the initial run in order to allow stabilisation. Mobile phase solution was pumped through the system at a constant rate of 1 ml/min for 15 min prior to initiating each run. Each run consisted of triplicate injections for each drug working standard solution, followed by the samples from the TI or MSLI experiments (maximum 20 samples between standard repeats). Each sample was tested in triplicate.

3.3.5.2 Budesonide

A validated method developed at AstraZeneca R&D Charnwood was used for the reverse-phase, isocratic assay of drug concentration. Chromatography conditions (table 3.2) were constant throughout all experiments.

[1]Brief details disclosed. For specific HPLC method, please contact AstraZeneca R&D Charnwood, who will release test method on a case-by-case consideration.

3.3.5.2.1 Mobile phase preparation

The chromatographic system comprised the HPLC instrument (HP 1050 Series HPLC with a UV-Visible detector, Hewlett-Packard Co., USA) and a Supelcosil LC-Si C18 column (5 cm x 4.6 mm; 3 µm) (table 3.2). Samples were analysed at 248 nm. One litre of 300 mM phosphate buffer solution was prepared and used to make up the 2.3 mM phosphate buffer solution in 29:71 v/v acetonitrile:water mobile phase. Mobile phase was mixed well, filtered and degassed for 15 min by placing the bottle in a sonicating bath (XB6 Ultrasonic Bath, Grant Instruments Limited, Royston, Hertfordshire, UK). When not required, the stock buffer was refrigerated (< 4°C) to prolong shelf-life. Because budesonide solutions are light sensitive, exposure to light was avoided, solutions were stored in the dark immediately upon preparation.

The flow rate of the mobile phase was set at 1.5 ml/min at ambient temperature and injection volume was 250 µl. A calibration curve of budesonide was constructed using drug concentrations 1 to 200 µg/ml and drug in sample was quantified accordingly. Drug entrapment was expressed as entrapment efficiency (% of drug entrapped in liposomes).

Table 3.2 A summary of HPLC run conditions for salbutamol base and budesonide.

Salbutamol base	
Column	Symmetry® C18
Mobile phase	Methanol and 5 mM sodium hexane sulphonate in water mixture (25:75) with 1% glacial acetic acid
Mobile phase filter	0.2 µm cellulose nitrate
Flow Rate	1 ml/min
Injection Volume	10 µL
Column Temperature	40 °C
Detection Wavelength	276 nm
Run Time	7 min
Retention time	5.7 min
Budesonide	
Column	Supelcosil LC-Si C18
Mobile phase	2.3 mM phosphate buffer solution in 29:71 v/v acetonitrile:water
Mobile phase filter	0.2 µm cellulose nitrate
Flow Rate	1.5 ml/min
Injection Volume	250 µL
Column Temperature	Ambient
Detection Wavelength	248 nm
Run Time	10 min
Retention time	5 min

3.3.5.2.2 Chromatography

Mobile phase solution was pumped through the system at a constant rate of 1.5 ml/min for 15 min prior to initiating each run. Each sample was tested in triplicate and each run consisted of triplicate injections for each drug working standard solution, followed by the samples from the TI or MSLI experiments (maximum 20 samples between standard repeats).

3.3.6 Determination of drug entrapment by, and release from, vesicles

To demonstrate the concept of spontaneous formation of liposomes, various formulations containing PC or GMO, model drugs salbutamol base or budesonide, and cosolvent ethanol in HFA 134a were actuated into the TI. Subsequently, encapsulation efficiencies of drug for these various formulations were determined. To study the effect of ethanol concentration on the encapsulation efficiency, PC or GMO, drug, and ethanol were prepared at different cosolvent concentrations and subsequently studied. To study the effect of phospholipid or GMO concentration on encapsulation of the two model drugs, different SPC, EPC or GMO concentrations were studied. Salbutamol base was added at a concentration of 0.1% w/w and budesonide at 0.14% w/w (the maximum amount that could be solubilised in the formulation). The encapsulation efficiencies of both drugs in phospholipid or GMO were determined by actuating the solution pMDI formulations into the TI. The collection medium in both lower and upper compartments was then collected and samples analysed. Appropriately weighed formulations (to achieve the desired concentration) were studied and TI analysis was carried out at room temperature (21 ± 2°C). In order to allow HPLC analysis of the entrapment of drug in liposomal formulations, it was necessary to remove the excess solid drug; hence a separation method was required. The method was adapted from previous work (Taylor *et al.*, 1989; Batavia *et al.*, 2000; Elhissi and Taylor, 2005). Assessment of the incorporation of salbutamol base and budesonide in liposomes formed in the upper and lower compartments of the TI was carried out by HPLC assay of centrifuged samples collected at each stage (Elhissi and Taylor, 2005). This method is based on the density difference between the liposomes and the drug solutions/suspensions. Drug release from vesicles following dilution on stages was assessed by periodic centrifugation every 15 min for 8 h, followed by HPLC assay using 3 replicates. At the end of aerosolisation, the upper and lower stages of the TI were washed separately with NaCl (0.9%) and made up to 50 ml. Diluted preparations were shaken in a water bath at 37°C. Samples were then centrifuged for 60 min at 40,000 g and 4°C using a 3K30 bench centrifuge (Sigma Laboratory Centrifuge, Germany) to separate unencapsulated drug from the hydrated liposomes by separating the resultant supernatant and residue. Vesicles containing budesonide were suspended in D_2O and centrifuged for 60 min at

40,000 g and 4°C, the floating layer was removed and resuspended in D_2O, then centrifuged for a further 1 h at 40,000 g and 4°C (adapted from Batavia *et al.*, 2001). After dilution with NaCl (0.9%), supernatant aliquots in salbutamol base samples and pellets in budesonide samples were directly analysed and used for quantification of unentrapped drug. The entrapped drug within the multilamellar vesicles was determined after solubilisation of the samples in 0.5% w/v Triton X-100 solution according to the method of Rossi *et al.*, (2004) and the drug released was analysed by HPLC as the liposome-entrapped fraction. Error was expressed as standard deviation (±SD).

A knowledge of the total drug amount in the formulation before centrifugation and in supernatant after centrifugation, as determined by HPLC, allowed the amount of drug associated with the liposomes to be calculated by difference (equation 3.2). The percentage of drug encapsulated was expressed as the ratio of the drug in the pellet (salbutamol)/supernatant (budesonide) to the sum of the drug in the pellet and the drug in the supernatant and reported as % drug content. The reported values of encapsulation efficiencies indicate the mean of three sample replicates.

$$\text{Drug entrapment (\%)} = \frac{\text{drug}_{\text{total}} - \text{drug}_{\text{unentrapped}}}{\text{drug}_{\text{total}}} \times 100 \qquad \textit{Equation 3.2}$$

This has been the method of choice for many researchers (Meisner *et al.,* 1989; Taylor *et al.,* 1990; Ma *et al.,* 1991). The phospholipids were expected to hydrate in the aqueous environment of the TI at room temperature before centrifugation. The basis behind separation of the material by centrifugation is that vesicles containing entrapped material will be more dense than the suspending medium for salbutamol samples. Salbutamol has density of 0.8 g/ml (Vatanara *et al.*, 2007). Thus, when such vesicles are subjected to a high gravitational field, they would be expected to sediment, whilst any free material would remain in the supernatant. Budesonide on the other hand has higher density of 1.24 g/ml (De Boer, 2005) and budesonide-containing vesicles will be less dense than the drug crystals. This is based on previous work by Batavia *et al.*, (2001) which demonstrated that deuterated water (D_2O) is a better suspending medium than H_2O for efficiently separating BDP crystals from liposomes by centrifugation. D_2O is a higher density water in which the hydrogen atoms are replaced with deuterium atoms, deuterium is an isotope of hydrogen that is twice as heavy due to an added neutron. H_2O and D_2O have respective densities of 0.9982 g/ml and 1.053 g/ml (Batavia *et al.*, 2001).

3.3.7 Statistical analysis

3.3.7.1 Comparison of two pMDI formulations

For the comparison of two pMDIs, a two-sided unpaired t-test at the 95% significance level was carried out using SPSS® statistical software program (SPSS UK Limited, UK).

3.3.7.2 Comparison of more than two pMDI formulations

In order to investigate differences between more than two pMDIs, a one-way analysis of variance (ANOVA) for unpaired samples at the 95% significance level was performed using SPSS® statistical software program (SPSS UK Limited, UK). In the case of a significant difference of variances within a group ($P < 0.05$) Fisher's Least Significance Difference (LSD) test was carried out between each mean and every other mean in order to determine which of the aerosols within the group differ significantly. Fisher's LSD is the variance of the difference between two means multiplied by t calculated from the t-test (equation 3.3).

$$LSD = t\sqrt{(2s^2/n)} \qquad \textit{Equation 3.3}$$

where; the t value is two-tailed and s^2 is the *error* from the ANOVA summary table.

3.4 Results and Discussion

3.4.1 Vesicle characterisation

3.4.1.1 Microscopic examination

The aforementioned solubility data (section 2.4.4.2) described the solubility of salbutamol base as a function of ethanol concentration. For initial vesicle characterisation, the presence of spontaneously formed vesicular carriers/liposomes as well as their morphology was examined by transmission electron microscopy (TEM). Following actuation of solution pMDI formulations into the TI, the spontaneously formed liposome samples in the collecting medium of both lower and upper compartments were immediately studied by TEM after hydration in deionised water and equilibrating for 10 min at room temperature (21 ± 2°C).

The electron micrographs of samples collected from the lower TI compartment derived from SPC and EPC-based formulations encapsulating salbutamol base and budesonide are shown in figures 3.2-3.5. It is evident from these micrographs that multilamellar vesicles (MLVs) are formed spontaneously on hydration of phospholipids within the impinger, which potentially act as a reservoir for encapsulating drugs. PC-based formulations were MLVs and LUVs and sometimes SUVs were also present. Samples collected from both upper and lower TI compartments derived from GMO-based formulations with both drugs did not show the formation of vesicles (figure 3.5). The presence of multilamellar vesicles was predominant in SPC- and EPC-based formulations.

Negative stain TEM indicated that the vesicles produced in this study comprised two liposome populations: large unilamellar liposomes and multilamellar vesicles. Each type of liposome has a large internal aqueous core relative to its diameter and we would expect a more efficient entrapment of aqueous volume in large unilamellar liposomes than MLVs.

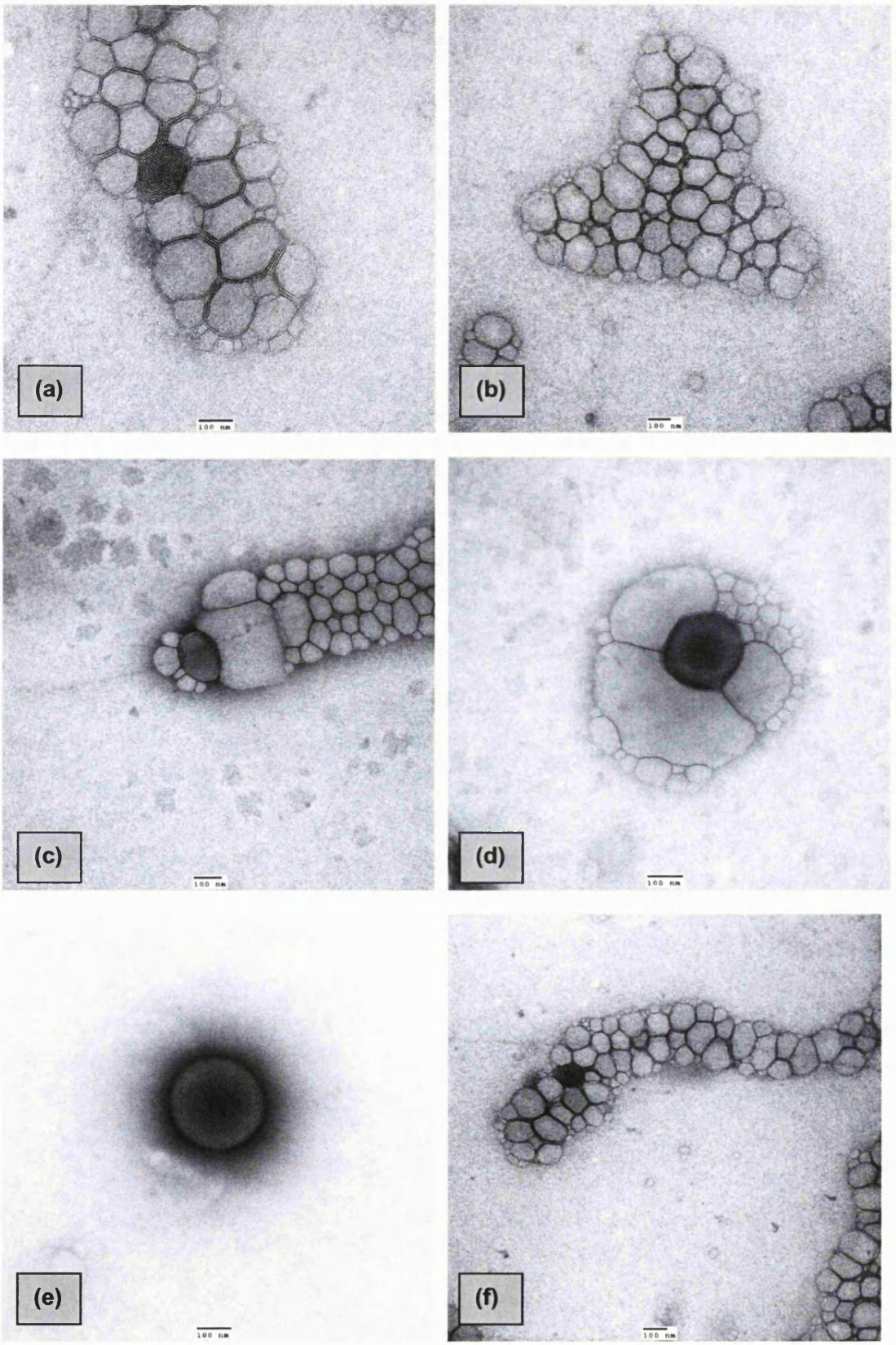


Figure 3.2 *TEM of vesicles formed from 0.1% w/w salbutamol base and 15% w/w ethanol pMDIs deposited in the lower compartment of the twin impinger; SPC at (a) 0.3% w/w, (b) 0.4% w/w, (c) 0.5% w/w, (d) 0.5% w/w, (e) 0.7% w/w, and EPC at (f) 0.5% w/w.*

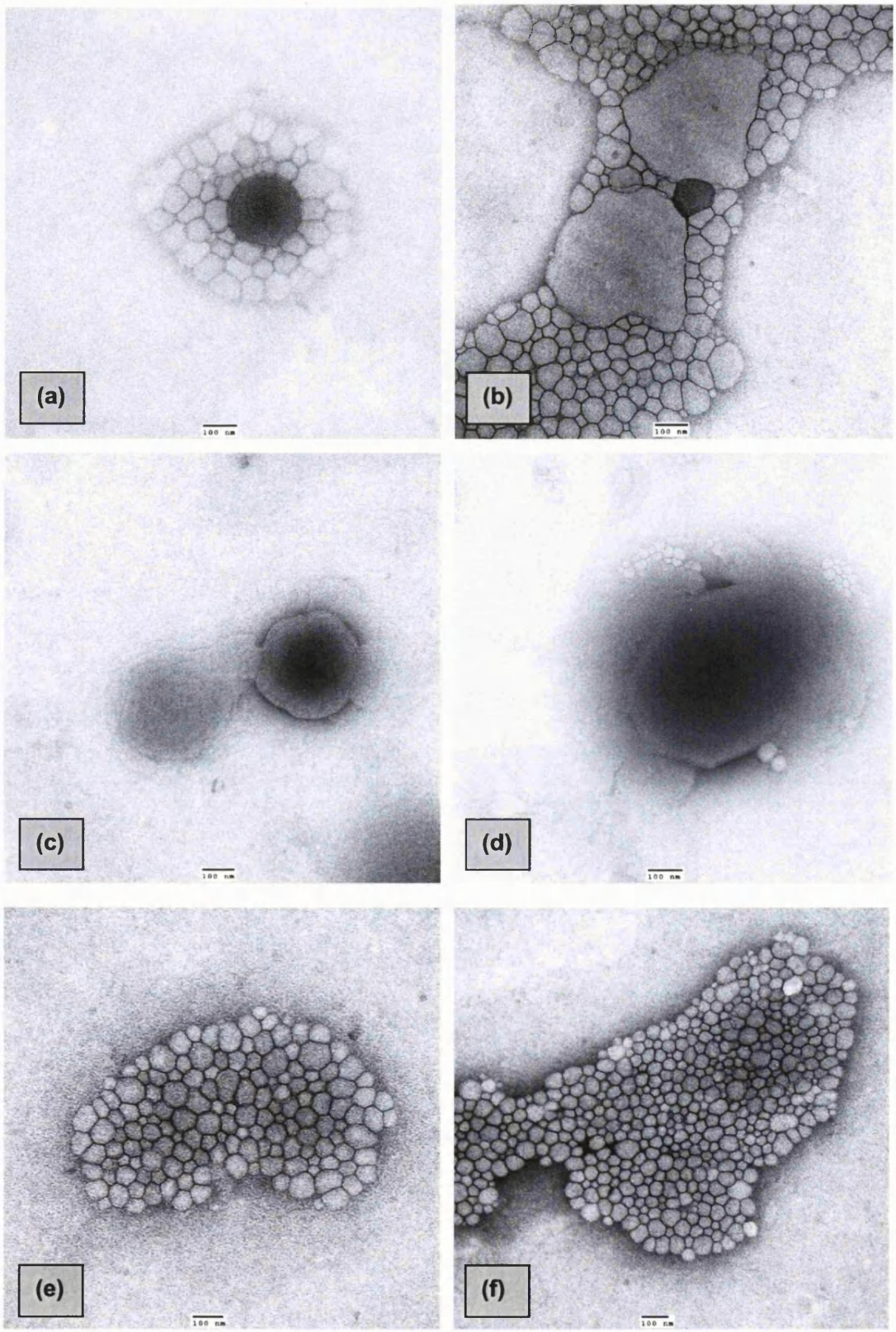


Figure 3.3 *TEM of vesicles formed from 0.1% w/w salbutamol base and EPC at (a) and (b) 0.6% w/w, (c) 0.7% w/w, (d) 1.0% w/w, and 0.1% budesonide at 0.3% w/w SPC (e) and (f).*

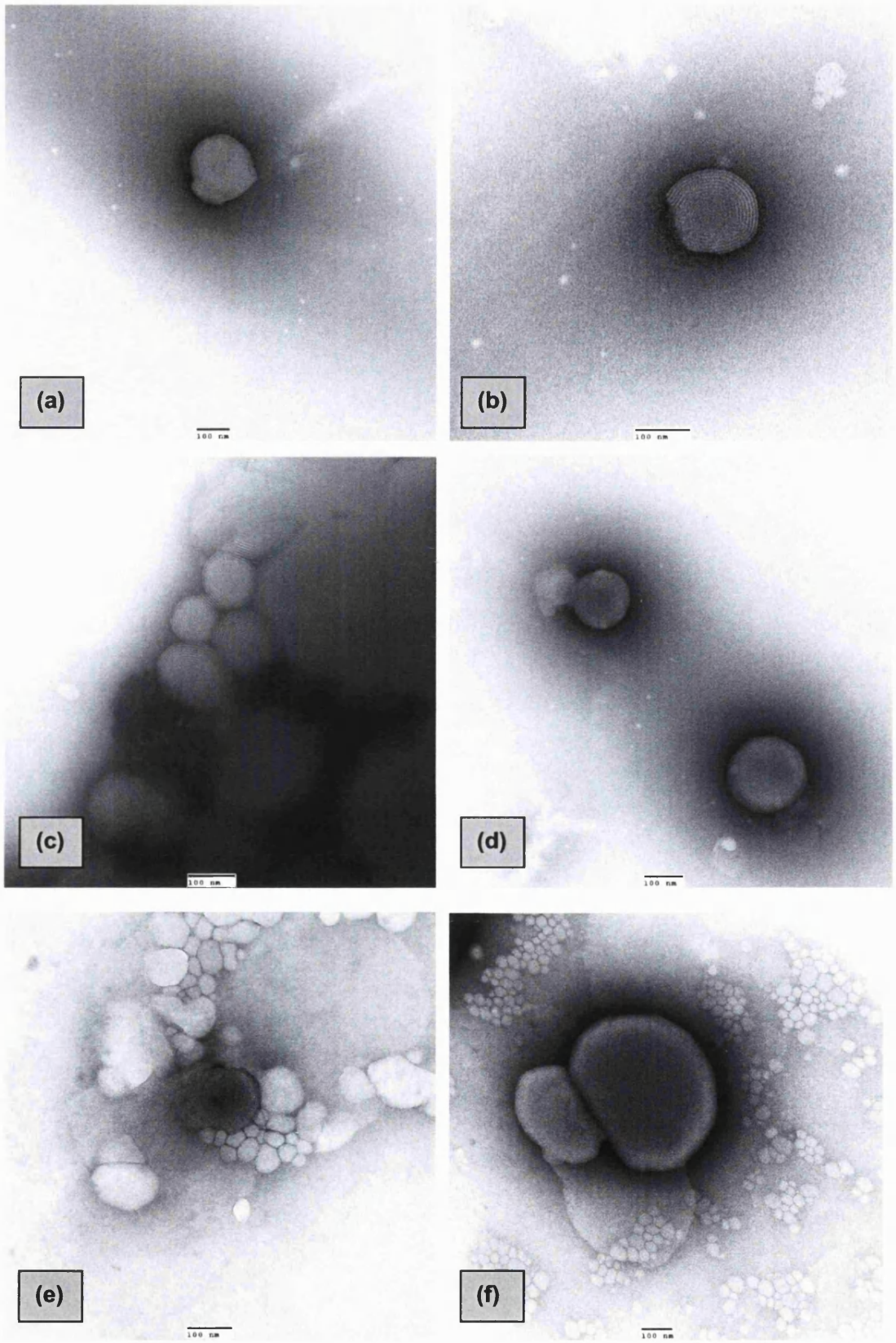


Figure 3.4 *TEM of vesicles deposited in the lower compartment of the twin impinger formed from 0.1% w/w budesonide and 15% w/w ethanol and SPC at (a) 0.4% w/w, (b) 0.5% w/w, (c) 0.8% w/w, and EPC at (d) 0.3% w/w, (e) 0.3% w/w, and (f) 0.8% w/w.*

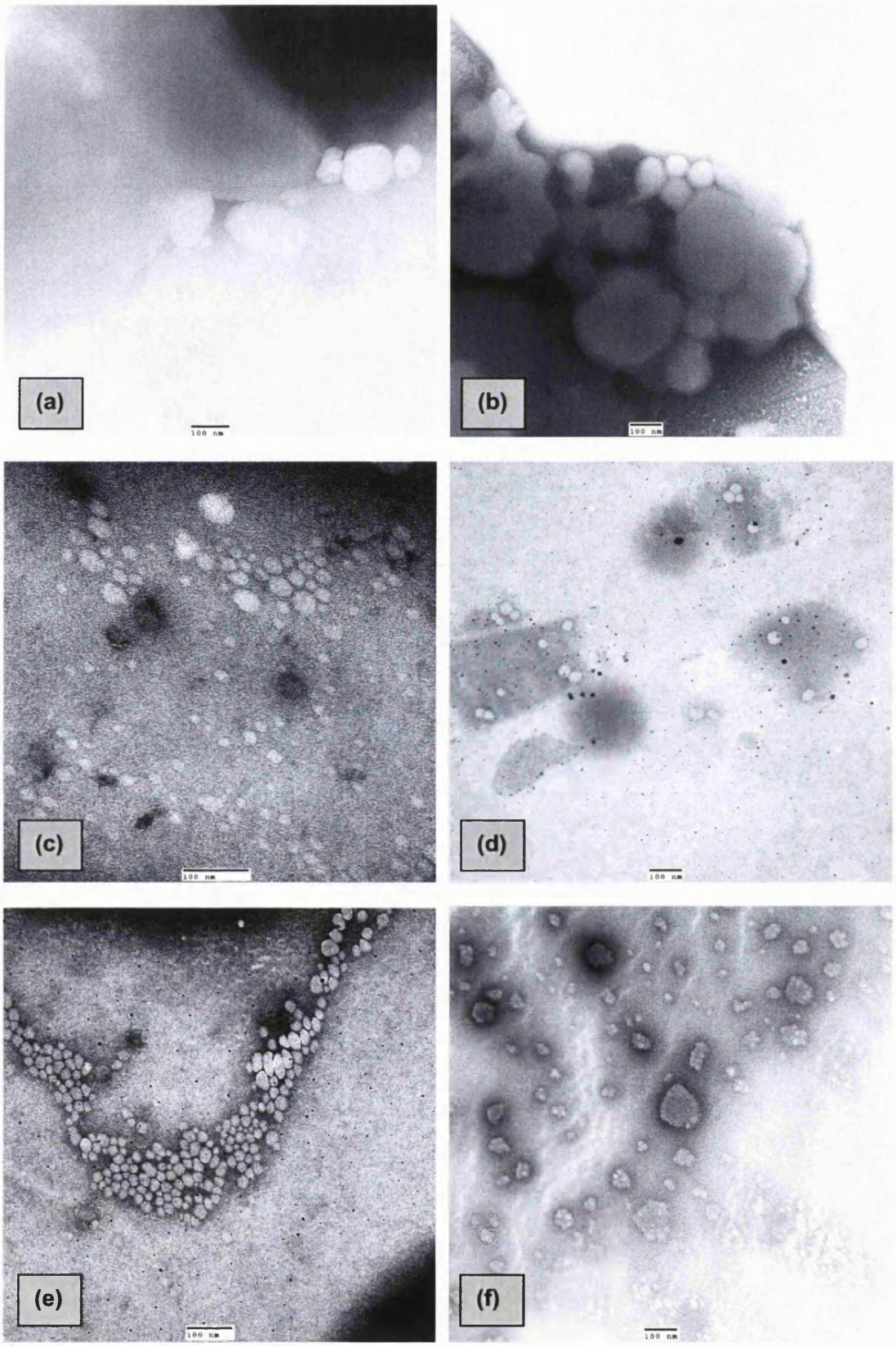


Figure 3.5 *TEM of vesicles deposited in the lower compartment of the twin impinger formed from 0.1% w/w budesonide and EPC at (a) 1.0% w/w, (b) 1.2% w/w, and GMO at 0.5% w/w (e) and (f), and 0.1% w/w salbutamol base and GMO at 0.5% w/w (c) and (d).*

3.4.1.2 Vesicle size distribution

The vesicle size distribution was determined using PCS at room temperature (21 ± 2°C). The measurements were performed five times (n=5). The resolution of PCS is determined by laser wavelength allowing particles of 10 nm and greater to be measured accurately.

The results of the sizing measurements of the various vesicles are summarised in tables 3.3 and 3.4. The tables show the z-average diameters (the mean diameters based upon the intensity of scattered light) and the polydispersity index (an estimate of the width of the distribution) obtained for the various hydrated liposome samples. PCS results showed no difference in size between liposomes formed from SPC and EPC formulations ($P > 0.05$). SPC and EPC formulations resulted in vesicles that ranged in diameters from 231 to 376 nm and 210 to 453 nm, respectively, for salbutamol base formulations. For budesonide formulations, SPC and EPC vesicles ranged from 229 to 345 nm and 257 to 400 nm, respectively.

Table 3.3 Mean diameters for SPC- and EPC-based budesonide formulations. Mean ± SD ($n \geq 3$).

Formulation (PC% w/w, budesonide 0.1% w/w, ethanol 15% w/w)	Z_{ave} Mean size (nm)	Polydispersity index
SPC		
0.1	229.7 ± 15	0.234 ± 0.043
0.2	265.3 ± 28	0.151 ± 0.034
0.3	312.7 ± 16	0.330 ± 0.056
0.4	298.2 ± 14	0.306 ± 0.025
0.5	280.1 ± 25	0.491 ± 0.037
0.6	345.2 ± 19	0.212 ± 0.011
0.7	321.0 ± 20	0.331 ± 0.039
0.8	337.6 ± 21	0.493 ± 0.027
EPC		
0.1	257.8 ± 16	0.348 ± 0.049
0.2	260.0 ± 22	0.287 ± 0.021
0.3	294.3 ± 13	0.246 ± 0.061
0.4	257.7 ± 19	0.289 ± 0.029
0.5	320.5 ± 25	0.441 ± 0.014
0.6	385.6 ± 23	0.225 ± 0.032
0.7	323.1 ± 19	0.190 ± 0.053
0.8	369.9 ± 32	0.395 ± 0.034
0.9	354.7 ± 21	0.234 ± 0.054
1.0	321.8 ± 26	0.421 ± 0.077
1.1	372.0 ± 22	0.275 ± 0.032
1.2	400.3 ± 17	0.310 ± 0.063
1.3	356.9 ± 16	0.278 ± 0.037
1.4	337.2 ± 23	0.297 ± 0.028

Table 3.4 Mean diameters for SPC- and EPC-based salbutamol base formulations. Mean ± SD (n ≥ 3).

Formulation (PC% w/w, salbutamol base 0.1% w/w, ethanol 15% w/w)	Z_{ave} Mean size (nm)	Polydispersity index
SPC		
0.1	231.6 ± 22	0.286 ± 0.017
0.2	324.6 ± 31	0.345 ± 0.023
0.3	289.9 ± 27	0.314 ± 0.027
0.4	321.6 ± 14	0.247 ± 0.034
0.5	310.5 ± 19	0.404 ± 0.021
0.6	367.4 ± 21	0.319 ± 0.040
0.7	338.9 ± 12	0.244 ± 0.061
0.8	376.0 ± 11	0.155 ± 0.058
EPC		
0.1	248.7 ± 12	0.333 ± 0.079
0.2	226.4 ± 16	0.315 ± 0.052
0.3	210.4 ± 16	0.254 ± 0.045
0.4	260.0 ± 24	0.426 ± 0.037
0.5	337.6 ± 32	0.164 ± 0.024
0.6	324.5 ± 35	0.453 ± 0.012
0.7	424.3 ± 15	0.313 ± 0.033
0.8	367.8 ± 27	0.262 ± 0.049
0.9	321.4 ± 24	0.243 ± 0.039
1.0	345.4 ± 23	0.321 ± 0.023
1.1	443.5 ± 33	0.356 ± 0.044
1.2	349.2 ± 31	0.401 ± 0.031
1.3	453.1 ± 11	0.219 ± 0.056
1.4	432.9 ± 23	0.345 ± 0.044
1.5	428.4 ± 21	0.310 ± 0.095
1.6	399.2 ± 17	0.136 ± 0.063
1.7	399.4 ± 13	0.248 ± 0.036
1.8	419.0 ± 21	0.410 ± 0.068

The mean vesicle size generated from each formulation in the TI is illustrated in figures 3.6 and 3.7. The mean vesicle size was affected by a change in phospholipid concentration, producing a mean droplet size increase of 144 and 108 nm for salbutamol base and budesonide formulations, respectively, with an increase in SPC concentration from 0.1 to 0.8% w/w. Increasing EPC concentration from 0.1 to 1.8% w/w for salbutamol base formulations and from 0.1 to 1.4% w/w for budesonide formulations resulted in mean droplet size increase of 170 and 79 nm, respectively. The response of the polydispersity values to the increased phospholipid concentration was less well defined than the vesicle size changes (figures 3.8 and 3.9). There was shown to be no significant change in the polydispersity of the size of vesicles in response to an increase in PC concentration over the studied concentration range. This suggests that different PC concentrations give similar distribution widths, i.e. polydispersity is independent of PC concentration.

A polydispersity index of between 0.0 and 0.1 is considered to indicate a unimodal size distribution. Index values ranging from 0.1 to 1.0 are believed to show a wide size distribution or a multi-modal size distribution (Ledis *et al.*, 2006; Stanley-Wood and Lines, 1992). Polydispersity index values greater than 0.1 in this work point to the presence of aggregates in the liposome samples as evidenced by TEM. Hydrated liposomes showed a bimodal distribution, indicating aggregation.

The differences in size among all the low and high concentrations of SPC and EPC formulations studied were statistically significant ($P < 0.05$); so, it could be concluded that phospholipid concentration has a significant effect on the vesicle mean size of the studied formulations, probably through increased multilamellarity and increased LUV size. On the other hand, vesicles produced by this method were in the nanometer range and TEM showed that they tend to aggregate; this could explain the variability in results obtained. Increasing the amount of PC in the formulations increased the amount of material available for vesicle formation; this led to an increase in liposome population, hence it is expected that there is a larger liposome pool for drug encapsulation. This was the next investigation carried out.

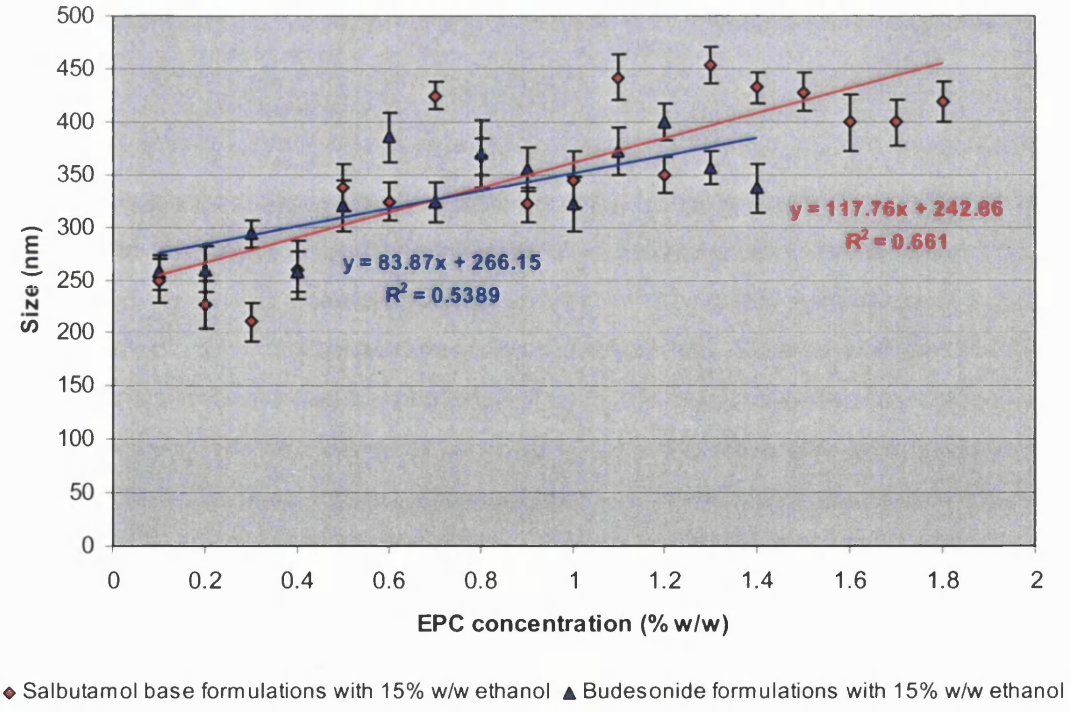


Figure 3.6 *Effect of EPC concentration on the mean size of vesicles from salbutamol base and budesonide formulations (n=5 ±SD).*

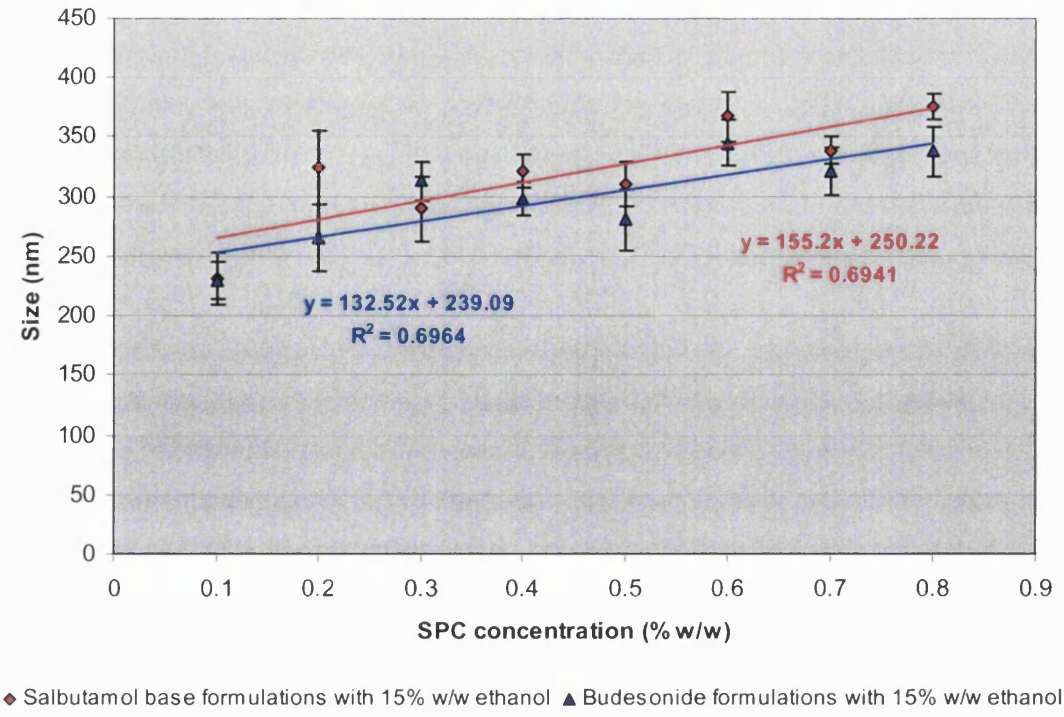


Figure 3.7 *Effect of SPC concentration on the mean size of vesicles from salbutamol base and budesonide formulations (n=5 ±SD).*

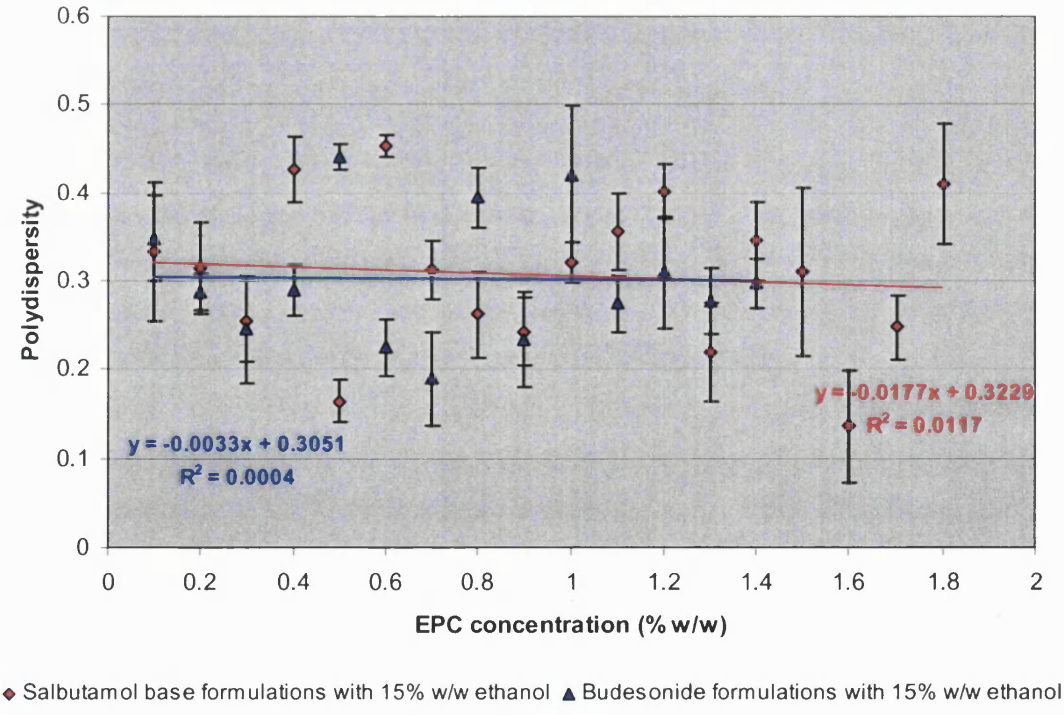


Figure 3.8 *Effect of EPC concentration on the polydispersity of vesicle size in salbutamol base and budesonide formulations (n=5 ±SD).*

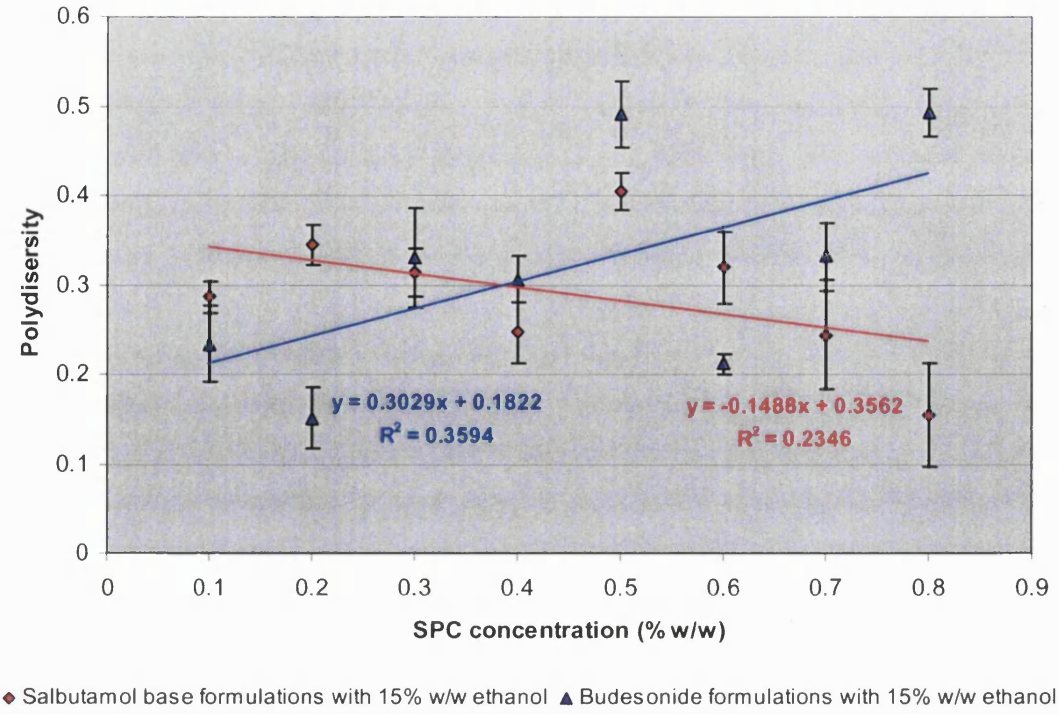


Figure 3.9 *Effect of SPC concentration on the polydispersity of vesicle size in salbutamol base and budesonide formulations (n=5 ±SD).*

3.4.2 Encapsulation efficiency

SPC- and EPC-based salbutamol base formulations showed encapsulation of 12.5 ± 1.3% and 20.1 ± 1.5%, respectively whereas SPC- and EPC-based budesonide formulation showed encapsulation of 13.3 ± 1.4% and 19.9 ± 2.1%, respectively. PC was included at the maximum concentration that can be incorporated, i.e. 0.8% w/w SPC and 1.8% w/w EPC in salbutamol base aerosols and 0.8% w/w SPC and 1.4% EPC in budesonide aerosols. This result indicates the spontaneous formation of liposomes on hydration with entrapment of drug.

The effect of lipid concentration and the nature of lipid on the encapsulation efficiency of drug are shown in figures 3.10 and 3.11. As the concentration of SPC was increased from 0.1 to 0.8% w/w and that of EPC from 0.1 to 1.8% w/w, the maximum encapsulation of SPC- and EPC-based salbutamol base and budesonide formulations (12.5 ± 1.3% and 19.9 ± 2.1%, respectively) were achieved. The results also indicate that GMO-based formulations at a concentration up to 0.5% w/w and 1.1% w/w for salbutamol base and budesonide formulations, respectively, had zero encapsulation efficiency.

3.4.2.1 Effect of charge on entrapment

Salbutamol and budesonide entrapment within liposomes increased with increasing SPC and EPC content of the formulations and was further increased by the inclusion of the negatively charged compound dicetylphosphate (DCP) at 0, 10, 20, 30, and 40% molar concentration of PC (figures 3.10 and 3.11). There was a 33.3% and a 40.0% increase in entrapment of salbutamol base within SPC and EPC vesicles, respectively, when DCP was increased from 0 to 40% molar concentration of PC. Refer to table 3.1 for calculations of different amounts of DCP used, please note that only calculations based on EPC concentrations 0.1% w/w and 1.0% w/w are shown, however the work was carried out at 0.1-1.8% w/w EPC and 0.1-0.8% w/w SPC for both drugs.

The encapsulation efficiency of budesonide in various solution formulations prepared with various concentrations of EPC is shown in figure 3.11. Budesonide exhibits lower encapsulation efficiency in SPC formulations (13.3 ± 1.4% at 0.8% w/w SPC) than EPC formulations (19.9 ± 2.1% at 1.4% w/w EPC) as more EPC could be included in the formulation than SPC at a constant ethanol concentration.

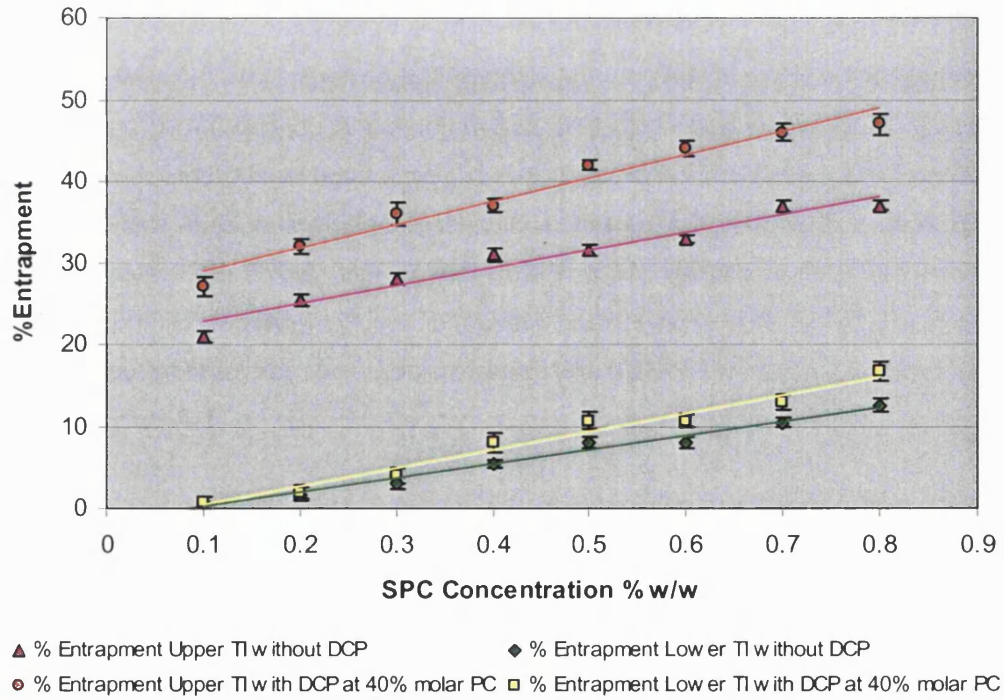


Figure 3.10 *Entrapment of salbutamol base in vesicles prepared from various concentrations of SPC with cosolvent ethanol 15% w/w (n=3 ±SD).*

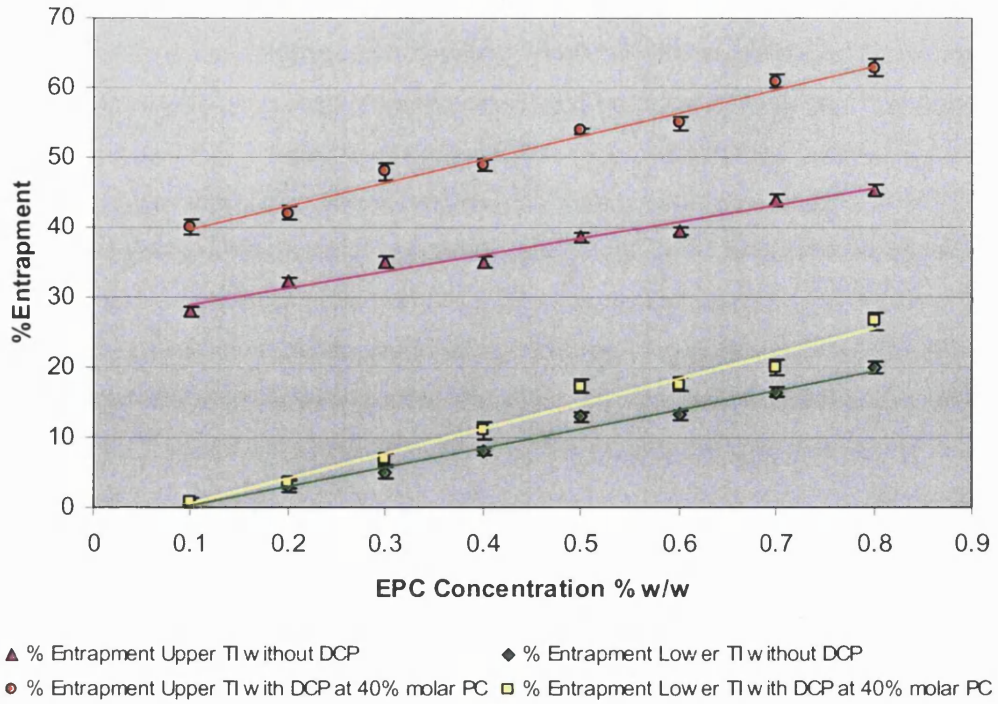


Figure 3.11 *Entrapment of budesonide in vesicles prepared from various concentrations of EPC with cosolvent ethanol 15% w/w (n=3 ±SD).*

Incorporation of negatively charged lipid, DCP at 10, 20, 30 and 40% molar concentration of PC (while keeping PC concentration constant), into SPC- and EPC-based salbutamol base and budesonide pMDI formulations, hence in SPC and EPC MLVs, increased drug entrapment. Salbutamol base entrapment increased from 12.5 ± 1.3% to 17.2 ± 1.4% and from 14.2 ± 0.8% to 16.7 ± 1.1% for SPC-based and EPC-based formulations, respectively. Budesonide entrapment increased from from 16.8 ± 1.2% to 22.5 ± 1.6% and from 19.9 ± 2.1% to 27.5 ± 2.6% for SPC-based and EPC-based formulations, respectively. Inclusion of DCP was accompanied by a significant effect on vesicle size ($P < 0.05$, analysis of variance test). Thus the increased drug entrapment when DCP was included in these formulations was probably the result of the increased vesicle size (figures 3.12-3.15). Addition of DCP at 40% molar concentration of PC to salbutamol base SPC and budesonide EPC formulations increased entrapment by 33% and 40%, respectively. The increased entrapment associated with inclusion of negative charge is not solely due to vesicle size changes.

DCP is commonly incorporated into bilayers to confer a negative charge. It is known that the inclusion of a charged lipid into the phospholipid bilayers causes electrostatic repulsion of adjacent bilayers which increases liposome size and the size of the internal aqueous compartments, and is a method by which uptake of drugs associated with aqueous volume, such as the hydrophilic drug salbutamol base, may be improved (Johnson, 1973; Alpar *et*

al., 1981). Whereas increased bilayer thickness due to increased total phospholipid content may have been responsible for direct proportionality between vesicle volume and increased entrapment of the hydrophobic drug budesonide.

A useful parameter to consider is partition coefficient (LogP), which is a measure of how hydrophilic or hydrophobic a chemical substance is. Hydrophobic drugs with high LogP values are preferentially distributed to hydrophobic compartments such as lipid bilayers while hydrophilic drugs (low LogP) preferentially are found in hydrophilic compartments (Davies *et al.*, 2003). Budesonide and salbutamol base have LogP values of 3.145 and 1.311, respectively (DrugBank, http://redpoll.pharmacy.ualberta.ca/drugbank). Salbutamol sulphate, on the other hand, has a LogP value of 0.11 (Biddlecombe and Pleasance, 1999). These values represent the ratio of concentrations of the drugs in the two phases of a mixture of two immiscible solvents at equilibrium (Leo *et al.*, 1971). Hence these coefficients are a measure of differential solubility of the compound between these two solvents. Normally one of the solvents chosen is water while the second is hydrophobic such as octanol (Sangster, 1997). Hence it can give a good indication on the partitioning of drug in our experimental system composed of water (collection medium) and formed phospholipid vesicles.

We can see from LogP values that salbutamol base is much less water-liking than salbutamol sulphate (1.311 vs. 0.11). This may indicate that the drug will preferentially partition within the formed vesicles and out of the aqueous medium in the collection compartment of the TI. The smaller LogP value of salbutamol base compared to budesonide (1.311 vs. 3.145) would also explain the lower entrapment efficiency.

The increased entrapment of drug in the presence of negatively charged lipid may also be attributed to the formation of a lipophilic ion pair between the positive centre of salbutamol base and negatively charged moiety of DCP.

Moreover, DCP-containing formulations have more phospholipid present; hence they may form more bilayers/vesicles thereby incorporating more drug.

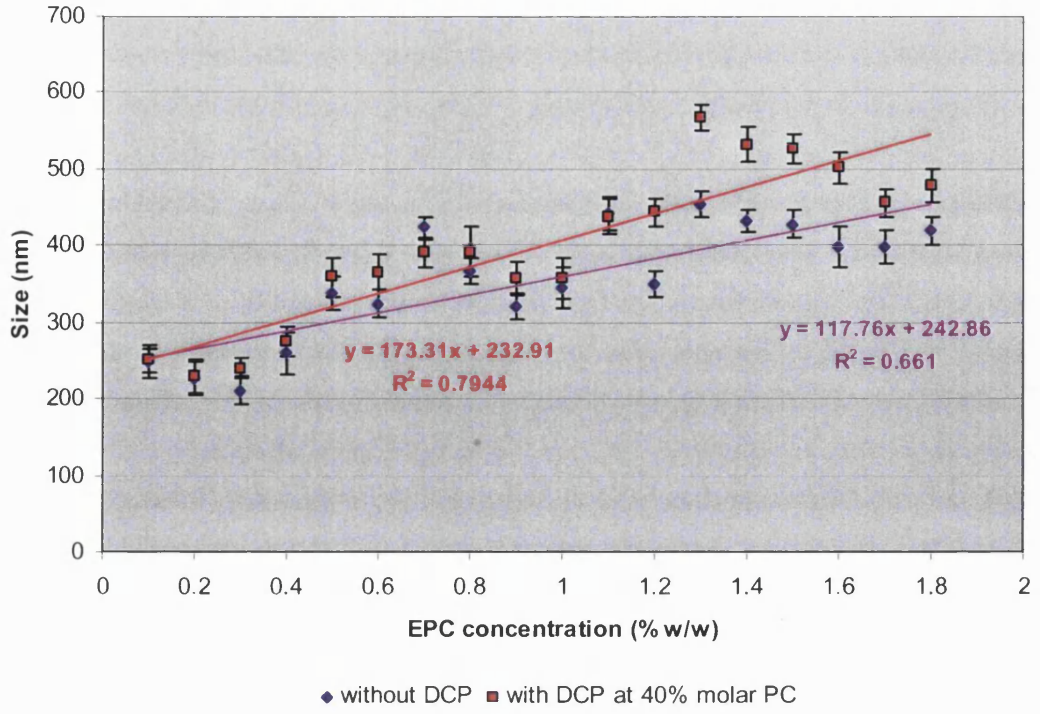


Figure 3.12 *Size of EPC vesicles in salbutamol base formulations containing DCP (n=5 ±SD).*

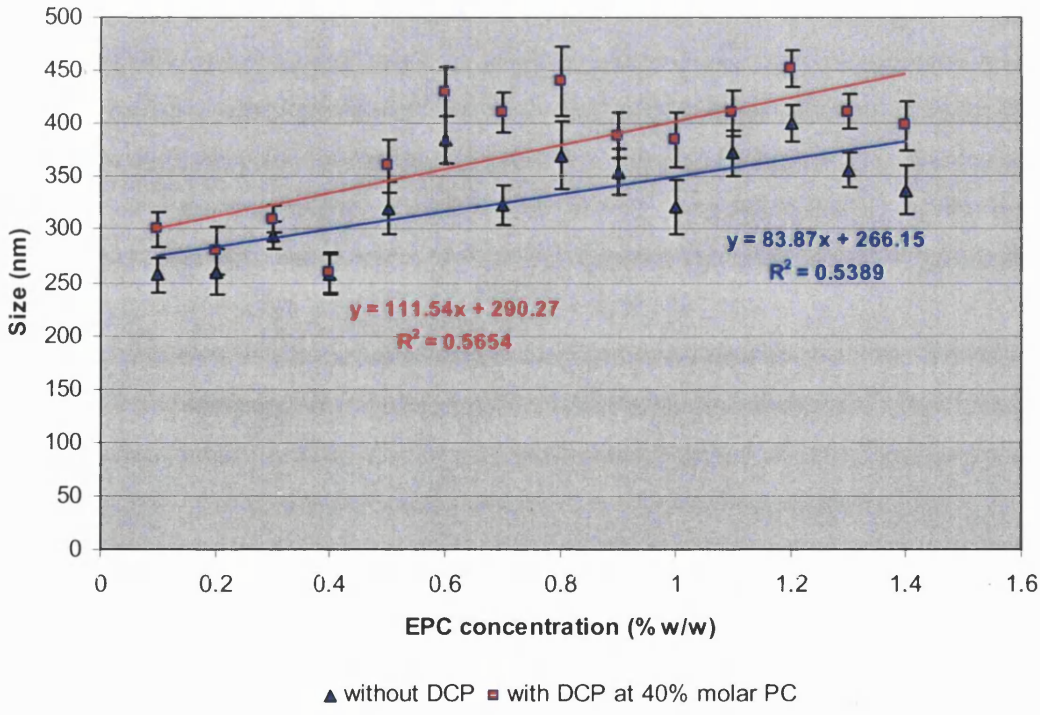


Figure 3.13 *Size of EPC vesicles in budesonide formulations containing DCP (n=5 ±SD).*

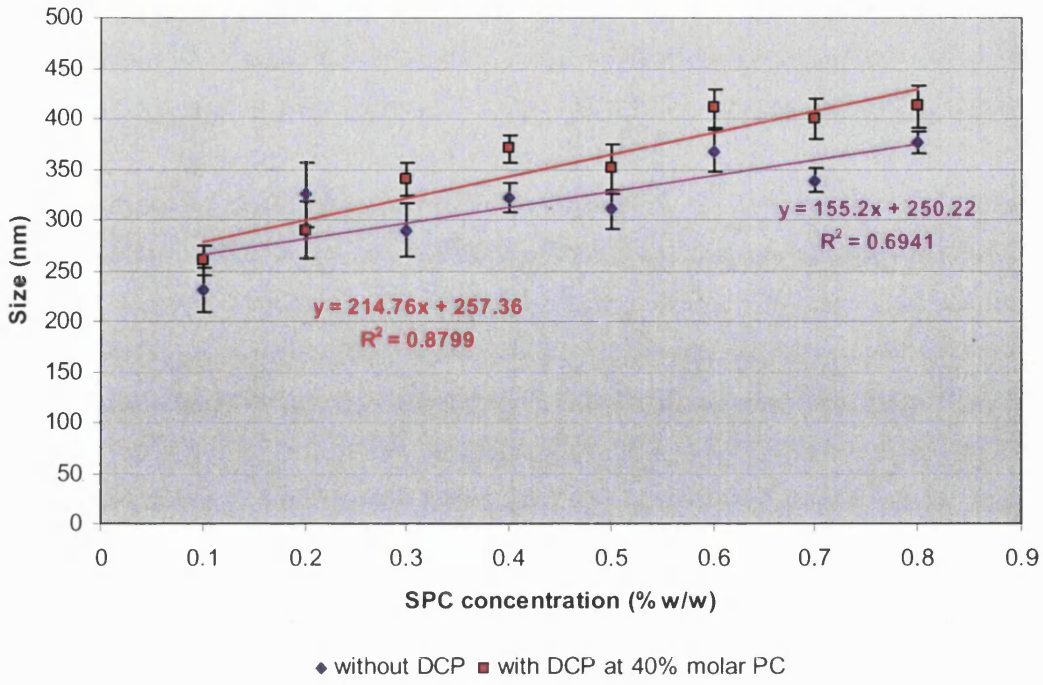


Figure 3.14 *Size of SPC vesicles in salbutamol base formulations containing DCP (n=5 ±SD).*

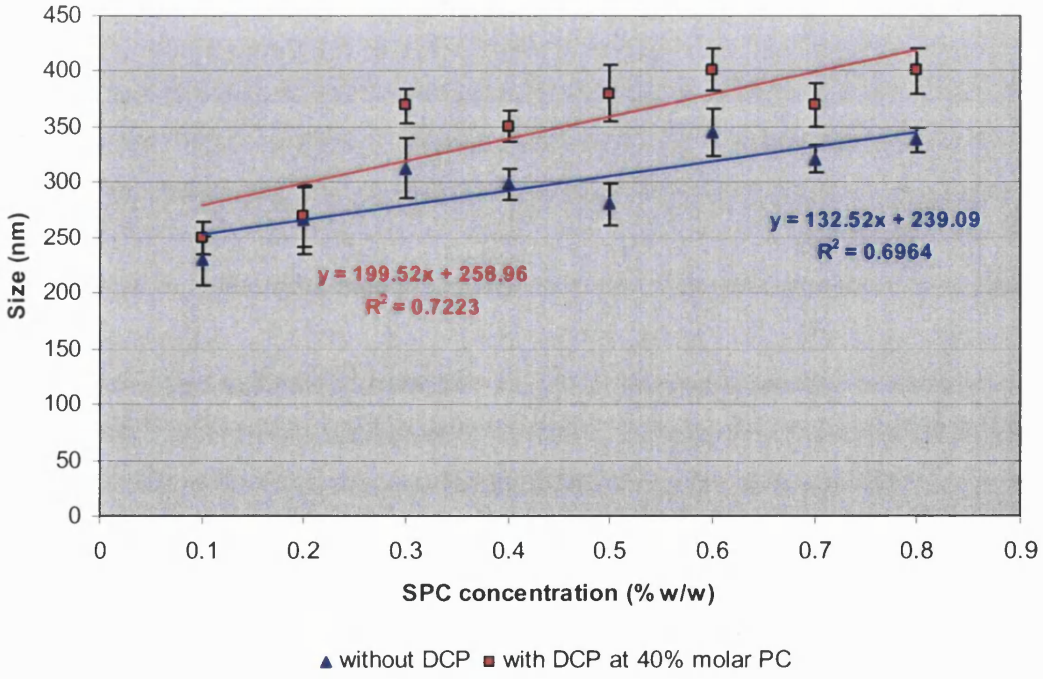


Figure 3.15 *Size of SPC vesicles in budesonide formulations containing DCP (n=5 ±SD).*

It may be assumed that the ion-pair complex formed between drug and negatively charged lipid may be hydrophobic, thereby exhibiting higher entrapment. In other words, the increased entrapment of budesonide reported previously in the presence of DCP reflects the relatively higher hydrophobicity of the complex in EPC/DPC-based liposome systems.

Overall, the data presented here show the dependence of liposomal salbutamol base and budesonide entrapments on the concentration of PC. The low entrapment of salbutamol base in 0.1% w/w SPC and 0.1% w/w EPC is typical for a relatively hydrophilic molecule since entrapment is dependent upon the volume of aqueous phase encapsulated during liposome formation. Low entrapment of budesonide in 0.1% w/w SPC and 0.1% w/w EPC is also typical for a hydrophobic molecule, which is expected to partition within the lipid bilayers (to be associated with the hydrocarbon chain region of the lipid molecules) when incorporated into PC films or bilayers, the volume of which increases with increasing PC concentrations. Increasing SPC concentration to 0.8% w/w in salbutamol base and budesonide formulations resulted in respective increases in mean vesicle diameter of 144 and 108 nm. Increasing EPC concentration from 0.1 to 1.8% w/w for salbutamol base formulations and from 0.1 to 1.4% w/w for budesonide formulations resulted in mean particle size increase of 170 and 79 nm, respectively. This corresponded to increased drug entrapments of 11.6-fold with SPC-based salbutamol base formulations and 19.0-fold with EPC-based budesonide formulations.

3.4.3 Aerodynamic particle size distribution

The evaluation of the phospholipid-based solution spray performance was carried out in terms of different formulation variables. First, the effect of the aerosol composition, i.e. solution formulation with GMO, SPC or EPC on the spray performance was analysed. Further, different amounts of cosolvent ethanol were investigated in terms of their effect on spray performance. This was followed by an assessment of the effect of drug concentration in aerosols, and finally the choice of propellant HFA 134a or HFA 227 on the aerodynamic size distribution. A more detailed definition of the different aerosol performance parameters is provided in section 4.1.1.1.

3.4.3.1 Formulation composition GMO, SPC or EPC

The effect of formulation composition on aerosol spray performance with respect to MMAD and FPF was investigated by comparing compositions containing GMO, SPC, and EPC at constant per cent w/w. Figures 3.16 and 3.17 show a comparison of the drug deposition profiles of salbutamol base and budesonide formulations for GMO, SPC and EPC aerosol samples at 0.5% w/w. The figures show the amount of drug ex valve (i.e. the amount of drug that left the pMDI inhaler) (µg) deposited on each stage. Calculated aerosol size distribution parameters, i.e. MMAD and GSD, as well as FPF and percentage recovery of each aerosol are listed in tables 3.5 and 3.6.

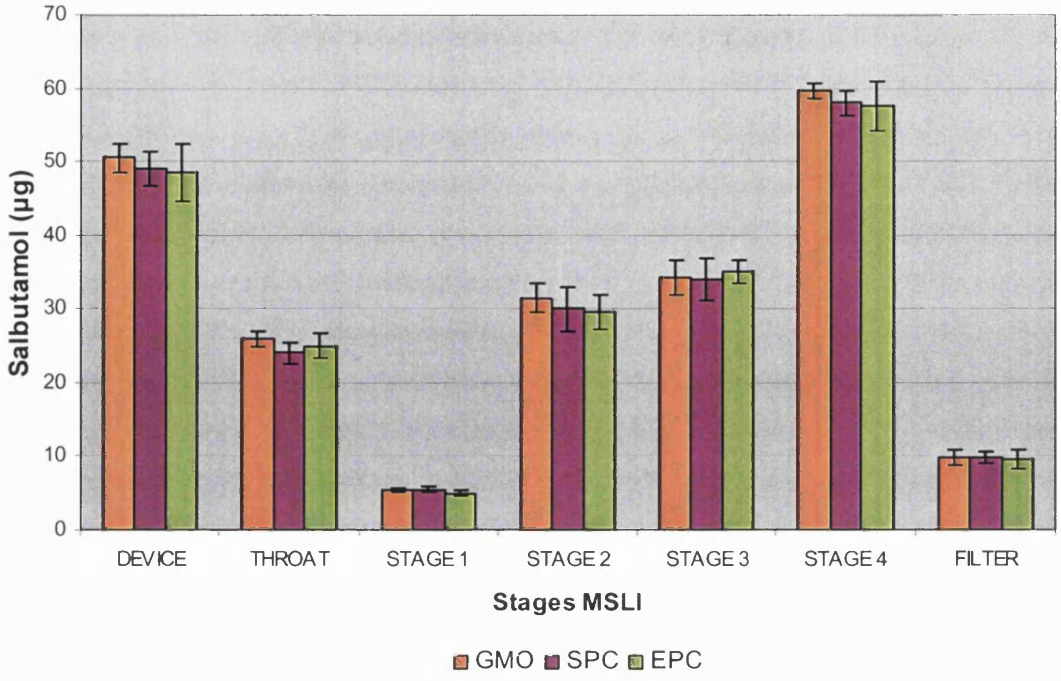


Figure 3.16 *In vitro deposition patterns of salbutamol base formulations in the MSLI. The data are for pMDI preparations containing 0.1% salbutamol base, 15% w/w ethanol and 0.5% w/w of either GMO, SPC or EPC (n=3 ±SD).*

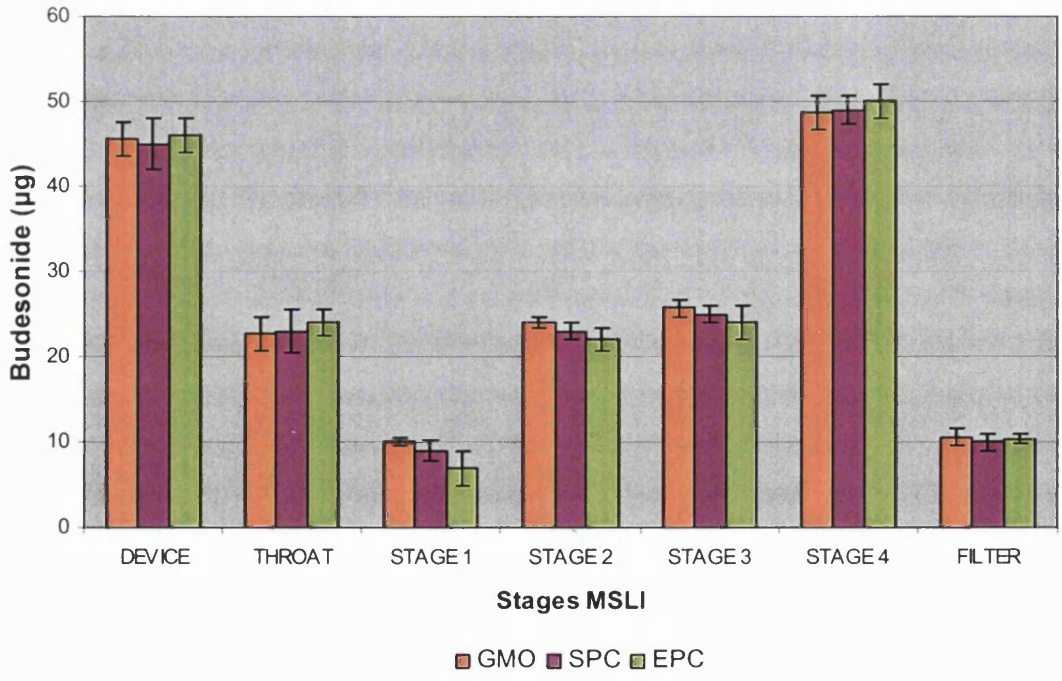


Figure 3.17 *In vitro deposition patterns of budesonide formulations in the MSLI. The data are for pMDI preparations containing 0.1% budesonide, 15% w/w ethanol and 0.5% w/w of GMO, EPC or SPC (n=3 ±SD).*

Table 3.5 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of GMO and PC-based pMDIs containing 0.1% w/w salbutamol base (n=3 ± SD).

	MMAD (±SD) (μm)	GSD (±SD)	FPF ex device (±SD)	% recovery (±SD)
Ethanol 5%				
GMO	1.9 (± 0.1)	1.8 (± 0.0)	62.5 (± 2.1)	99.8 (± 4.2)
SPC	2.0 (± 0.2)	2.0 (± 0.1)	60.2 (± 3.0)	98.3 (± 3.2)
EPC	1.9 (± 0.1)	2.0 (± 0.2)	63.0 (± 3.1)	97.8 (± 5.1)
Ethanol 10%				
GMO	2.2 (± 0.3)	2.2 (± 0.1)	57.1 (± 2.1)	99.7 (± 2.0)
SPC	2.3 (± 0.1)	2.2 (± 0.1)	58.0 (± 1.1)	100.9 (± 1.9)
EPC	2.1 (± 0.1)	2.4 (± 0.1)	57.6 (± 2.1)	101.9 (± 3.1)
Ethanol 15%				
GMO	2.5 (± 0.1)	2.3 (± 0.1)	49.3 (± 0.9)	102.2 (± 3.0)
SPC	2.6 (± 0.1)	2.4 (± 0.0)	49.0 (± 1.1)	99.8 (± 2.5)
EPC	2.6 (± 0.0)	2.3 (± 0.0)	48.9 (± 0.8)	100.2 (± 0.9)
Ethanol 20%				
GMO	3.5 (± 0.0)	2.5 (± 0.0)	42.1 (± 1.2)	98.6 (± 3.5)
SPC	3.4 (± 0.2)	2.2 (± 0.0)	40.9 (± 2.1)	99.4 (± 2.1)
EPC	4.1 (± 0.1)	2.3 (± 0.2)	41.3 (± 0.9)	101.3 (± 2.0)

Table 3.6 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of GMO- or PC-based pMDIs containing 0.1% w/w budesonide (n=3 ± SD).

	MMAD (±SD) (μm)	GSD (±SD)	FPF ex device (±SD)	% recovery (±SD)
Ethanol 5%				
GMO	2.0 (± 0.1)	2.0 (± 0.2)	61.8 (± 2.6)	100.9 (± 1.8)
SPC	2.0 (± 0.1)	2.0 (± 0.1)	59.8 (± 4.2)	99.1 (± 2.1)
EPC	2.2 (± 0.2)	2.1 (± 0.0)	63.5 (± 2.9)	98.5 (± 3.4)
Ethanol 10%				
GMO	2.4 (± 0.1)	2.3 (± 0.0)	58.4 (± 2.9)	102.8 (± 3.5)
SPC	2.5 (± 0.2)	2.2 (± 0.0)	59.0 (± 1.6)	99.9 (± 2.9)
EPC	2.4 (± 0.3)	2.3 (± 0.2)	56.6 (± 1.3)	100.5 (± 2.1)
Ethanol 15%				
GMO	2.8 (± 0.2)	2.2 (± 0.0)	47.4 (± 1.7)	99.2 (± 1.0)
SPC	2.6 (± 0.2)	2.4 (± 0.1)	46.2 (± 1.5)	102.3 (± 3.7)
EPC	2.7 (± 0.0)	2.3 (± 0.0)	46.4 (± 0.9)	97.9 (± 2.9)
Ethanol 20%				
GMO	3.6 (± 0.2)	2.5 (± 0.1)	40.8 (± 2.1)	102.7 (± 3.6)
SPC	3.5 (± 0.3)	2.3 (± 0.0)	40.7 (± 1.8)	100.7 (± 1.7)
EPC	3.8 (± 0.1)	2.3 (± 0.1)	42.3 (± 1.1)	102.0 (± 3.1)

3.4.3.2 Effect of GMO or phospholipid concentration on performance

The effect of GMO or PC concentration on the aerosol spray performance with respect to the MMAD and FPF was investigated by comparing compositions containing GMO, SPC and EPC at 0.1 to 0.5%, 0.1 to 0.8% and 0.1 to 1.8% w/w, respectively for salbutamol base formulations and compositions containing GMO, SPC and EPC at 0.1 to 1.1%, 0.1 to 0.8%, and 0.1 to 1.4% w/w, respectively for budesonide formulations. Figures 3.18 and 3.19 show the drug deposition profiles of salbutamol base and budesonide formulations for various GMO and lipid concentrations (GMO and PC in the formulation is dependent on the amount of ethanol included). The figures show the amount of drug ex valve (i.e. the amount of drug that left the pMDI inhaler) (μg) deposited on each stage. Aerosol size parameters and percentage recovery of each formulation are shown in tables 3.5 and 3.6 (note that the

formulations listed in the tables 3.5 and 3.6 contain increasing amounts of GMO and PC with increasing concentration of ethanol).

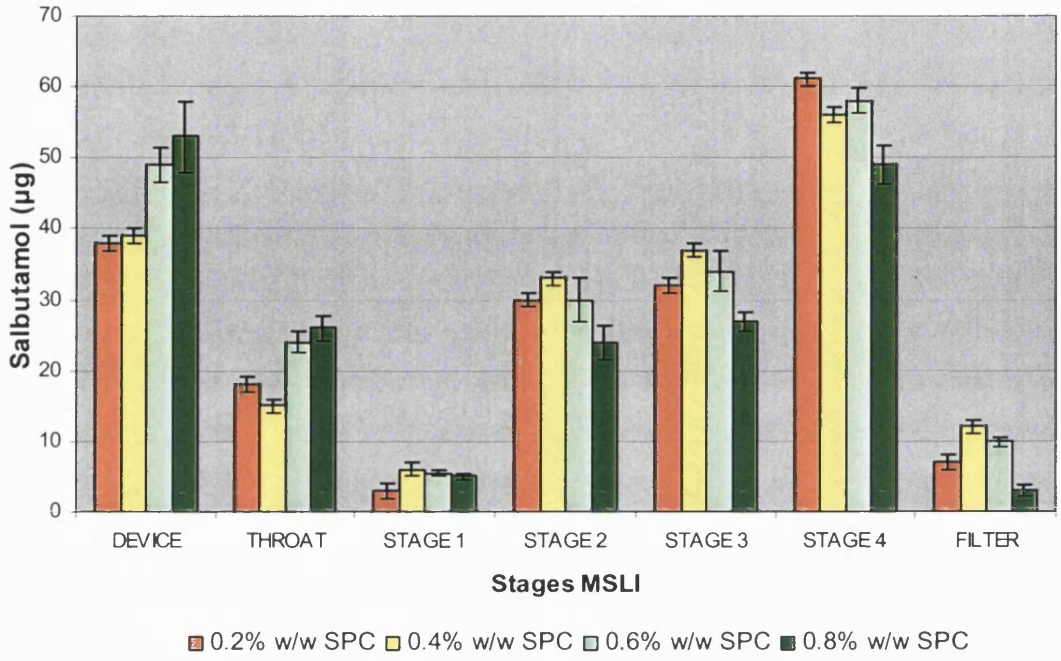


Figure 3.18 *In vitro deposition patterns of SPC based salbutamol base formulations in the MSLI. The data are for pMDI formulations containing 0.1% salbutamol base and 15% ethanol (n=3 ±SD).*

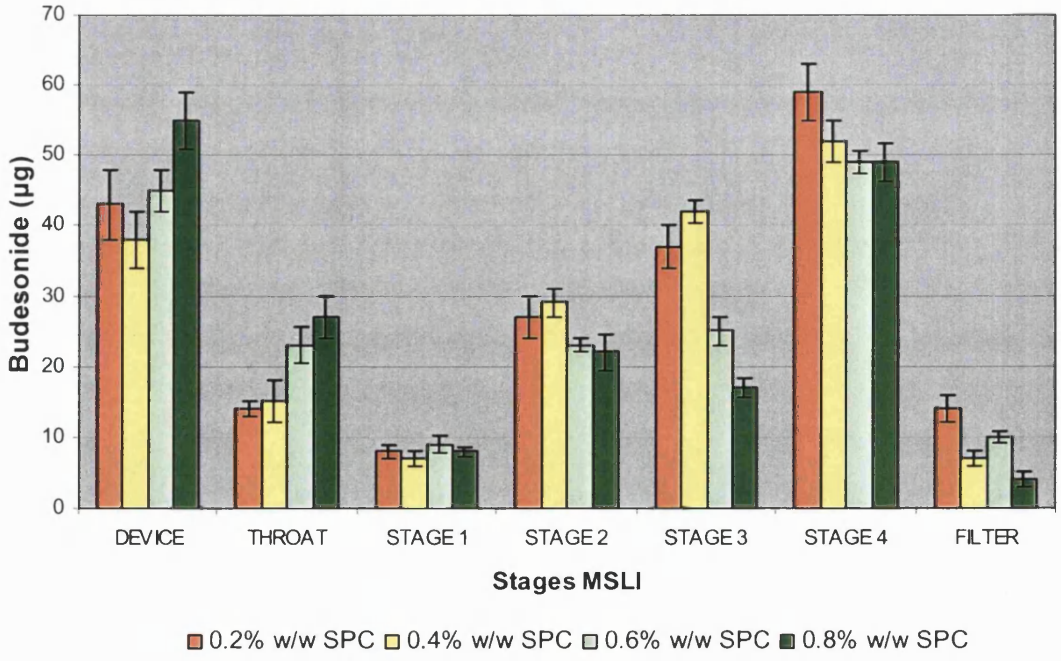


Figure 3.19 *In vitro deposition patterns of SPC based budesonide formulations in the MSLI. The data are for pMDI formulations containing 0.1% budesonide and 15% ethanol (n=3 ±SD).*

3.4.3.3 Effect of ethanol concentration on aerosol performance

The effect of ethanol concentration on the aerosol spray performance with respect to the MMAD and FPF was investigated by comparing compositions containing 5, 10, 15 and 20% w/w ethanol. Figures 3.20 and 3.21 show a comparison of the drug deposition profiles of salbutamol base and budesonide formulations for 5, 10, 15 and 20% ethanol w/w. The diagrams show the amount of drug ex valve (i.e. the amount of drug that left the pMDI inhaler) (µg) deposited on each stage. Aerosol size parameters and percentage recovery of each formulation are listed in tables 3.5 and 3.6.

Deposition within the impinger was found to be significantly influenced by ethanol concentration, but was essentially independent of drug concentrations. An increase in deposition within the impinger was observed with an increase in ethanol concentration, until a maximum was reached at an ethanol concentration of 15% w/w. Increasing the ethanol concentration from 5 to 15% resulted in a 32% and a 40% increase in deposition within the impinger for the salbutamol base and budesonide formulations, respectively.

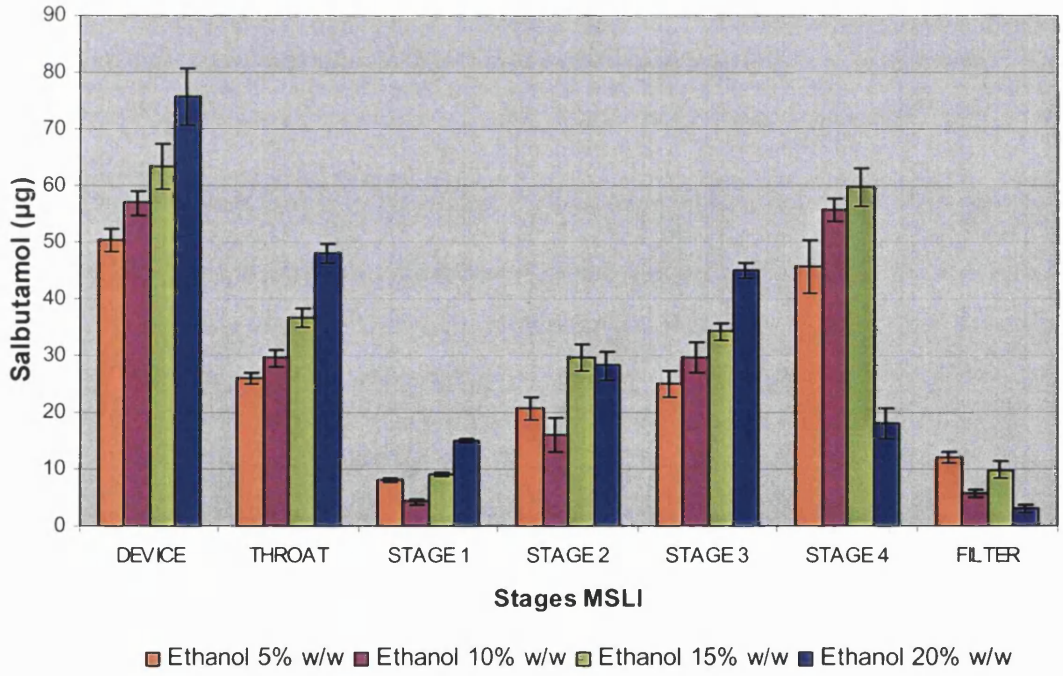


Figure 3.20 *In vitro deposition patterns of salbutamol base formulations in the MSLI. The data are for pMDI formulations containing 0.1% salbutamol base and 0.3% w/w SPC (n=3 ±SD).*

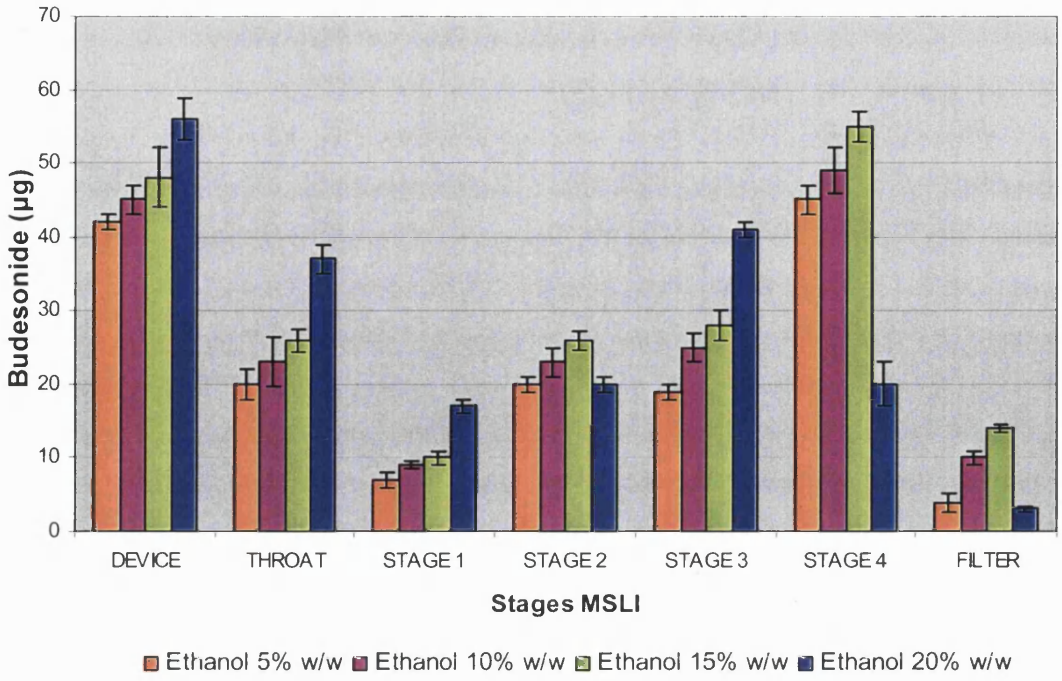


Figure 3.21 *In vitro deposition patterns of budesonide formulations in the MSLI. The data are for pMDI formulations containing 0.1% budesonide and 0.4% w/w EPC (n=3 ±SD).*

The mass median aerodynamic diameter and geometric standard deviation of various phospholipid-based pMDIs containing various concentrations of ethanol and phospholipid or GMO, and salbutamol base or budesonide, as measured by mass deposition in the MSLI, are shown in tables 3.5 and 3.6. From an inhalation standpoint, all preparations showed sizes acceptable for inhalation purposes (i.e. below 5 µm) and are suitable candidates for further release studies.

Tables 3.5 and 3.6 show the effect of ethanol on cloud characteristics of salbutamol base and budesonide formulations. In 5% w/w ethanol formulations, very fine clouds were generated and the MMADs were determined by the concentration of the drug and GMO or PC in the HFA propellant/ ethanol mixture. The addition of 10% w/w ethanol resulted in an increase of MMAD to approximately 2.2 (± 0.3), 2.3 (± 0.1) µm and 2.1 ± 0.1 µm for GMO-, SPC- and EPC-based salbutamol base formulations, respectively. It led to an increase of MMAD to 2.4 (± 0.1) µm, 2.5 (± 0.2) µm and 2.4 ± 0.3 µm for GMO-, SPC- and EPC-based budesonide formulations, respectively.

For salbutamol base, MMAD increased from 1.9 ± 0.1 µm to 3.5 ± 0.0 µm for GMO-based formulations, from 2.0 ± 0.2 µm to 3.4 ± 0.2 µm for SPC-based formulations and from 1.9 ± 0.1 µm to 4.1 ± 0.1 µm for EPC-based formulations as the ethanol concentration was increased from 5% w/w to 20% w/w. For budesonide, MMAD

increased from 2.0 ± 0.1 μm to 3.6 ± 0.2 μm for GMO-based formulations, from 2.0 ± 0.1 μm to 3.5 ± 0.3 μm for SPC-based formulations and from 2.2 ± 0.2 μm to 3.8 ± 0.1 μm for EPC-based formulations as the ethanol concentration was increased from 5% w/w to 20% w/w. The measured values for all formulations including 5-15% w/w ethanol and excluding those containing 20% w/w ethanol are smaller than those observed with marketed HFA suspension formulations which have typical values of 3.5-4.5 μm such as Becotide® (discussed in section 3.4.3.5). The addition of cosolvent ethanol depresses the fine particle dose to a degree which depends on the amount added. Corresponding data for budesonide shown in table 3.6 exhibit the same qualitative behaviour.

The measured MMAD was highly sensitive to ethanol concentration in the formulations, with the particle size increasing with increasing ethanol concentration. Furthermore, for a given ethanol concentration, an increase in MMAD was observed with increasing drug concentration but this effect was small compared to the effect of ethanol concentration.

3.4.3.4 Effect of drug concentration on aerosol performance

Considering the drug deposition profiles of solution pMDIs with increasing amount of ethanol, i.e. 5, 10, 15 and 20% w/w, at concentrations of 5% w/w ethanol, throat depositions of 26.6 μg and 20.1 μg were shown for salbutamol base and budesonide solution formulations containing SPC 0.3% w/w and EPC at 0.4% w/w, respectively, with MMADs of 2.1 and 2.0 μm. Within the respirable fraction (stage 2 to filter stage) most of the drug was deposited on stage 4 with an effective cut-off diameter of 1.7 μm. Increasing the drug concentration from 0.05 to 0.15% w/w resulted in increased MMAD of 1.8 μm and 2.0 μm for salbutamol base (3.9 μm) and budesonide (4.0 μm) aerosols, respectively. The FPF was also reduced from 60.2 to 49.7% and from 59.6 to 45.8% for salbutamol base and budesonide aerosols, respectively ($P < 0.05$), mainly as a result of device and throat deposition (tables 3.7 and 3.8).

Table 3.7 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of GMO and PC-based pMDIs at 0.5% w/w containing 15% w/w ethanol (n=3 ± SD).

	MMAD (±SD) (μm)	GSD (±SD)	FPF ex device (±SD)	% recovery (±SD)
Salbutamol base 0.05%				
GMO	2.1 (± 0.1)	2.3 (± 0.2)	60.5 (± 2.1)	97.8 (± 4.2)
SPC	2.0 (± 0.1)	2.0 (± 0.1)	60.2 (± 3.0)	99.3 (± 3.2)
EPC	2.2 (± 0.1)	2.2 (± 0.2)	62.0 (± 3.1)	98.8 (± 5.1)
Salbutamol base 0.15%				
GMO	3.9 (± 0.1)	2.4 (± 0.1)	48.7 (± 2.1)	100.7 (± 2.0)
SPC	4.0 (± 0.1)	2.2 (± 0.0)	48.0 (± 1.1)	101.9 (± 1.9)
EPC	4.2 (± 0.0)	2.3 (± 0.1)	59.4 (± 2.1)	99.9 (± 3.1)

Table 3.8 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of GMO and PC-based pMDIs at 0.5% w/w containing 15% w/w ethanol (n=3 ± SD).

	MMAD (±SD) (μm)	GSD (±SD)	FPF ex device (±SD)	% recovery (±SD)
Budesonide 0.05%				
GMO	2.2 (± 0.1)	2.2 (± 0.0)	61.8 (± 2.1)	100.6 (± 3.3)
SPC	2.1 (± 0.0)	2.1 (± 0.1)	59.2 (± 3.0)	101.3 (± 3.7)
EPC	2.0 (± 0.1)	2.3 (± 0.2)	60.0 (± 3.1)	99.0 (± 4.1)
Budesonide 0.15%				
GMO	4.1 (± 0.1)	2.3 (± 0.1)	45.8 (± 2.1)	102.5 (± 2.0)
SPC	4.2 (± 0.1)	2.3 (± 0.1)	46.0 (± 1.1)	99.6 (± 1.9)
EPC	4.0 (± 0.2)	2.1 (± 0.1)	47.2 (± 2.1)	97.9 (± 3.1)

Analysis of variance (ANOVA) was performed in order to determine whether differences between the compositions were significant. P-values calculated from ANOVA are listed in Appendix III (tables 1 and 2). Since all P-values were found to be less than 0.05, there is evidence of statistically significant differences between formulations containing different ethanol concentrations. Fisher's LSD test, however, gives more detailed information about differences between each aerosol composition (Appendix III tables 3 and 4). No statistically significant differences ($P > 0.05$) could be seen between GMO, SPC and EPC compositions when studies at constant ethanol concentration for MMAD or FPF. It can therefore be concluded that the addition of ethanol has a significant effect on the drug deposition profile of GMO and phospholipid solution pMDIs.

Ethanol addition has a significant influence ($P < 0.05$) on the FPF and MMAD. There are two possible explanations: ethanol is known to form non-ideal mixtures with HFA propellants (Tzou, 1998). HFAs have polar characteristics and hydrogen-bonding capacities. Due to the strong electron-withdrawing effect of the fluorine atoms, the positively polarised hydrogen can form a hydrogen bond with the hydrogen-bond acceptors, the fluorine atoms. When adding ethanol to HFA, it is suggested that the intermolecular forces between like molecules are stronger than between unlike molecules. It is therefore possible that ethanol in the aerosol formulation preferably adsorbs at the phospholipid surface, aiding its dissolution.

Soluble lipid or GMO components retarded the flashing and subsequent evaporation of aerosol propellants where even the addition of small amounts of GMO or phospholipid to solution-type pressurised packs led to a significant increase in MMAD and GSD of emitted aerosols. The amphiphilic nature of phospholipids would encourage the formation of a barrier of PC at the propellant droplet/air interface. This will retard the evaporation of propellant by interfering with the acquisition of heat for evaporation from the surrounding atmosphere as the droplets pass through the air. One way of quickening propellant evaporation is to increase the temperature of the collection

medium in the impinger to 37°C (Farr *et al.*, 1989). Deposition of particles in the receptor fluid at 37°C should enhance the rate of propellant evaporation with a concurrent size reduction before formation of liposomes in thermodynamic equilibrium with the aqueous environment.

Increasing phospholipid concentration is likely to enhance the barrier to evaporation. The size of aerosol droplets containing substantial amounts of lipid-entrapped propellant may be increased due to internally entrapped propellant.

Aerosol particles failing to penetrate beyond the throat stage of the MSLI are unlikely to reach the conducting airways of the respiratory tract. The results suggest therefore, that increasing phospholipid concentration leads to the production of inefficient inhalation aerosols due to the increased concentration of GMO or PC preventing the efficient disruption of the aerosol spray through delayed propellant evaporation. MMAD and GSD were calculated from plots of ECD against cumulative percentage undersize for aerosol deposition in the MSLI and showed that, generally, MMAD increased with increased lipid concentration; GSD, however, was indicative of a polydisperse system in each case.

3.4.3.5 Comparison with Qvar® and Becotide®

The *in vitro* deposition profiles of Becotide®, a micronised beclometasone dipropionate pMDI containing a blend of CFC-11 and CFC-12, and Qvar®, a beclometasone dipropionate solution pMDI containing HFA 134a were investigated using the MSLI under the same experimental conditions as for investigation of the GMO- and PC-based solution aerosols. Figures 3.22 and 3.23 compare the deposition patterns of the two commercially available products with those of EPC-based formulations. MMADs were found to be 1.2 µm (± 0.17) for Qvar® and 3.6 µm (± 0.12) for Becotide® (table 3.9) (literature values are 1.1 µm and 3.5 µm, respectively (Leach *et al.*, 1998)).

Table 3.9 Spray performance parameters for Qvar® and Becotide® determined in the MSLI (n=3 ±SD).

	MMAD (µm)	GSD	% FPF	% Recovery
Qvar®	1.2 (± 0.2)	2.2 (± 0.2)	70.4 (± 1.3)	98.7 (± 3.2)
Becotide®	3.6 (± 0.1)	1.9 (± 0.1)	36.3 (± 1.0)	100.3 (± 1.6)

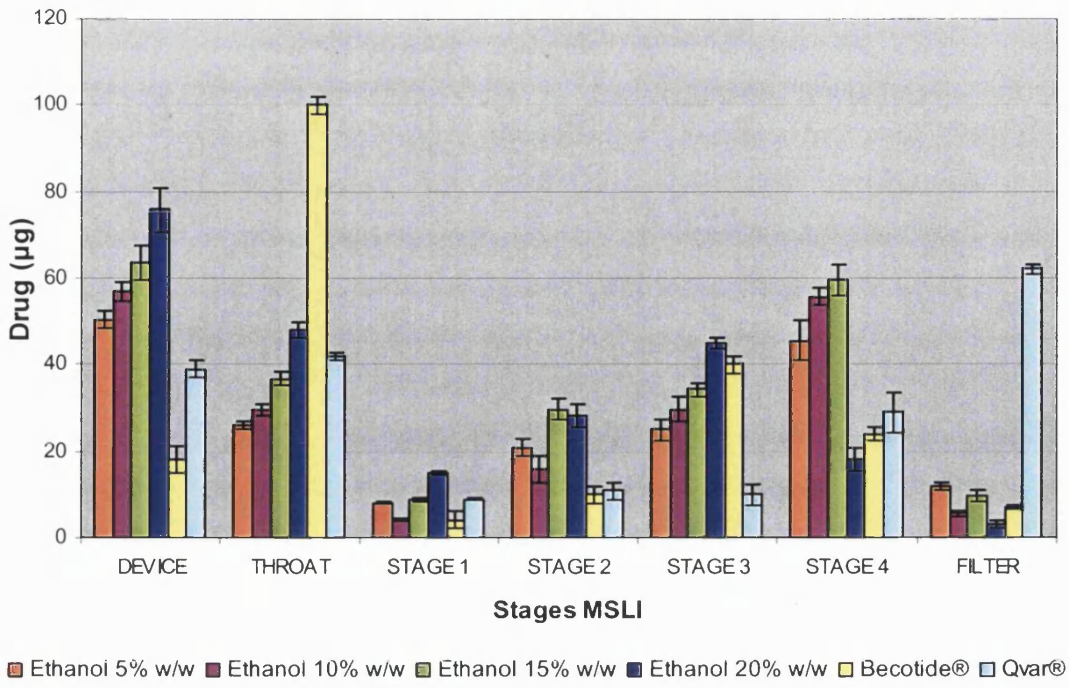


Figure 3.22 *In vitro deposition patterns of salbutamol base formulations in the MSLI. The data are for pMDI formulations containing 0.1% salbutamol base and 0.4% w/w EPC. Also shown in the graph are the in vitro depositions of Becotide® and Qvar® in the MSLI (n=3 ±SD).*

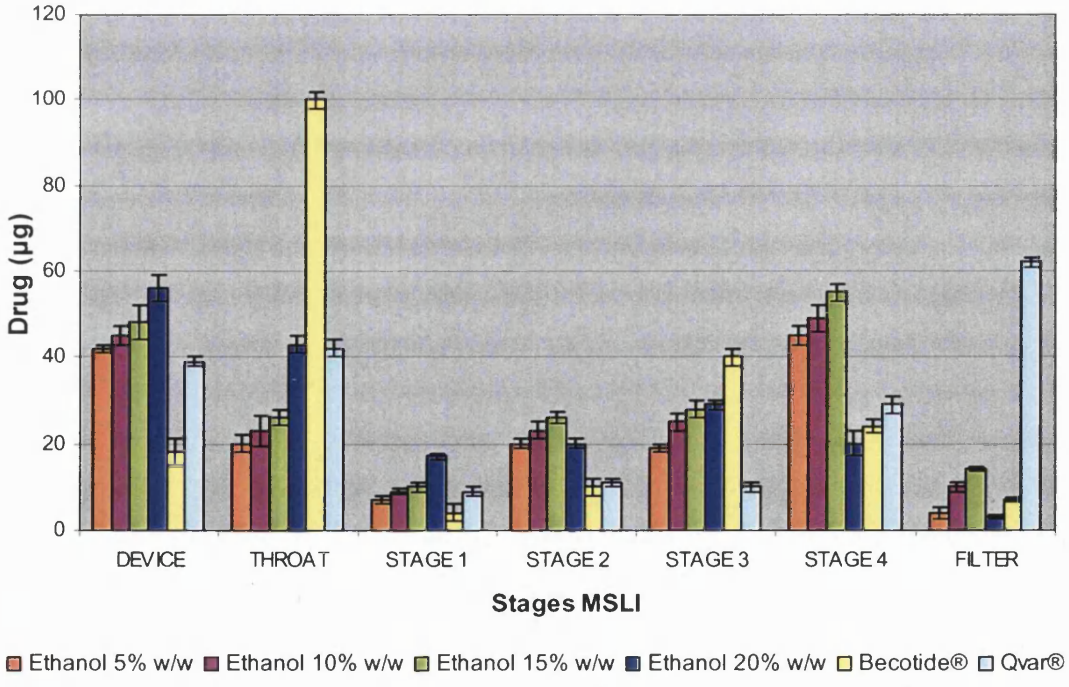


Figure 3.23 *In vitro deposition patterns of budesonide formulations in the MSLI. The data are for pMDI formulations containing 0.1% budesonide and 0.4% w/w EPC. Also shown in the graph are the in vitro depositions of Becotide® and Qvar® in the MSLI (n=3 ±SD).*

PC formulations showed a higher FPF (~40-60%) depending on ethanol concentration, compared to Becotide, which has an FPF < 40%. FPFs of about 40% were obtained with formulations containing 20% w/w ethanol. Furthermore, a shift of the main drug deposition from stage 3 (representing the upper bronchi) to stage 5 (representing the lower bronchioles and alveoli) can be achieved with the PC-based aerosols. However, there would be a predicted higher alveolar deposition (main deposition on filter stage) for Qvar®. This is also reflected in a lower MMAD of 1.1 µm compared to ~2 µm for PC formulations. The FPF for both salbutamol base and budesonide formulations is high apart from those with high ethanol content, which exhibit lower FPFs compared to Qvar®. The higher MMAD may be due to the presence of non-volatile phospholipid additives in the PC formulations (Williams and Liu, 1998). Another likely cause for the higher MMAD is the presence of ethanol in PC-based formulations. As has been shown in section 3.4.3.3, ethanol concentration has a direct effect on MMAD and FPF. Although Qvar® contains a considerable amount of the low-volatility cosolvent ethanol, it is in much smaller amounts than those used in the PC formulations. Qvar® aerosols contain a total of 0.0059 ml ethanol per puff which corresponds to a maximum of 0.59 ml (~6% w/w) per canister. Formulations developed in this work contain amounts ranging from 0.63 ml (5% w/w) to 2.54 ml (20% w/w). Factors other than vapour pressure could be contributing to the higher MMADs in PC aerosols compared to Qvar® solution pMDI. The device design is one crucial factor influencing the drug deposition profiles of pMDIs. Qvar® has an orifice diameter of 0.25 mm compared to 0.5 mm of the PC pMDIs. Hence, large aerosol droplets are expected to be generated from the PC formulation pMDIs which could result in larger aerodynamic particle sizes.

For the local drug delivery of bronchodilator drugs targeting β_2-receptors in the lung, e.g. salbutamol, an optimum bronchodilatation and minimal systemic bioavailability has been shown for particles of 2.8 µm (Howarth, 2001). The smaller particle sizes produced from solution phase aerosols are not recommended for local bronchodilatation. For the local treatment of inflammatory processes in the lung with corticosteroids, however, the delivery of small drug particles in the central and peripheral airways is desirable since it is known that steroid receptors are located throughout the lungs and therapeutic effects are evident in the tracheo-bronchial as well as in the alveolar region. Alveolar deposition, on the other hand, leads to systemic drug absorption, which can cause unwanted side effects, especially in the case of steroids. Other than the β_2-adrenoceptor stimulant drugs, the optimum particle size for maximal efficacy with minimal systemic bioavailability is still not known (Howarth, 2001; Woodcock, 2001). When formulating inhalation systems for predominantly systemic drug absorption, the higher alveolar deposition of the solution phase pMDI is certainly desirable. Systemic drug delivery via the lung may

be achieved with phospholipid-based pMDIs developed in this work (MMAD ~2 μm), whereas in terms of pulmonary corticosteroid delivery, improved or equivalent *in vitro* deposition profiles compared to the microparticle suspension CFC-pMDI Becotide® could be shown.

3.4.4 Comparing propellants HFA 134a and HFA 227

HFA 134a and HFA 227 differ significantly in vapour pressure; 570 and 390 kPa at 20°C, respectively. Addition of cosolvent and non-volatile additive lowers the propellant vapour and pack pressures, although those for HFA 134a systems remain higher than for equivalent HFA 227 systems. Drug delivery data for selected salbutamol base formulations of GMO, SPC or EPC in the two propellants are given in table 3.10. At constant GMO, SPC or EPC concentrations, an increase in vapour pressure from 390 to 570 kPa resulted in a significant higher deposition in the adaptor and MSLI with a lower fraction present in the throat (table 3.10). The EPC/ethanol/salbutamol base combination shows that the higher pack pressure HFA 134a formulation (570 kPa at 20°C) gives a cloud with an MMAD 0.9 μm smaller (2.5 μm) compared to when HFA 227 is used (3.4 μm) (pack pressure at 20°C = 390 kPa). Similar effects are obtained with formulations containing SPC and GMO. In all cases, higher vapour pressure leads to more efficient atomisation and finer sprays.

Table 3.10 Aerosol analysis of salbutamol base formulations containing 0.6% w/w EPC and 15% ethanol in HFA 134a and HFA 227 (n=3 ±SD).

Formulation	Salbutamol base in HFA 134a	Salbutamol base in HFA 227	$P<$ [2]
Vapour pressure (kPa)	570	390	-
% in adaptor[1]	33.66 ± 4.03	23.11 ± 0.99	0.01
% in throat	10.02 ± 1.54	12.26 ± 0.87	0.02
% in MSLI	56.32 ± 4.55	64.62 ± 2.23	0.01
MMAD	2.5 ± 0.2	3.4 ± 0.1	0.05
GSD	2.0 ± 0.1	2.2 ± 0.1	0.05

[1]Adaptor orifice diameter = 0.58 mm
[2]Levels of significance determined using a two-tailed Student's t-test.

An increase in propellant vapour pressure has two effects on aerosol droplets. Firstly, the droplets are subjected to greater propulsion from the nozzle of the oral adaptor, which serves to disrupt the spray at higher velocity and thus explains the greater impaction of emitted droplets within the narrow confines of the adaptor tube for the formulations exhibiting the highest vapour pressure. Secondly, high vapour pressures result in smaller initial droplets and more rapid evaporation. The greater extent of deposition in the throat for aerosols emitted from the pressure pack at 390 kPa infers that droplet size is dominant over velocity for inertial impaction within the throat (Moren, 1987).

The atomisation of liquid during the discharge of a solution pMDI is simpler than for a suspension, but the basic mechanisms are poorly understood. Clark (1991) favours aerodynamic shear thinning rather than flash atomisation as the mechanism of dispersion. Figure 3.24 illustrates his analysis of discharge from a valve through the aperture to the atmosphere, shown part way through the process, at a point where flow into and out of the expansion chamber are equal and the pressure is at a maximum. It is clear that vapour pressure controls the generation of the two phases in the expansion chamber, and that the orifice diameter will influence rate of efflux. He concluded that the amount of fine particles in the spray would be inversely proportional to the orifice diameter and proportional to the square root of the pressure.

Use of the higher pressure HFA 134a propellant will promote greater shear thinning with the generation of a finer cloud whereas coarser clouds are generated by the lower pressure resulting from the use of HFA 227.

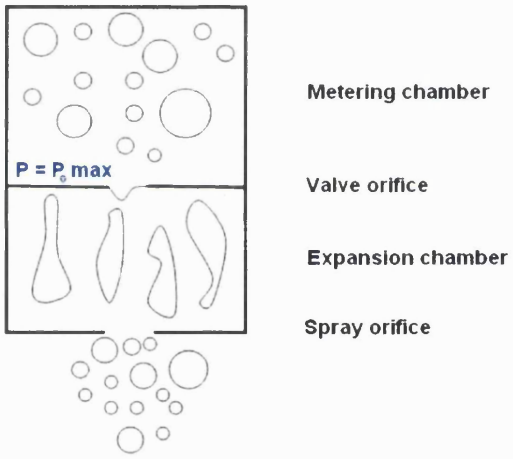


Figure 3.24 *Schematic for metered discharge from a solution pMDI (adapted from Clark, 1991).*

Aerosols that enter the MSLI are considered equivalent to the effective dose delivered to the respiratory tract, particularly those capable of penetration into the lower stages (stage 3 and 4). Owing to the ECD of stage 2, such droplets are accepted as the most useful in therapeutic terms, i.e. the fine particle fraction (FPF). Decreasing vapour pressure reduced FPF as a result of a combination of greater impaction within the adaptor and throat and an increase in MMAD of aerosol entering the impinger.

The data obtained from solubility experiments and those obtained from *in vitro* characterisation combine to create a fundamental limit of different excipient in the solution pMDIs. Depending on the drug solubility, significant amounts of a cosolvent such as ethanol may be needed in order to solubilise sufficient drug to obtain a therapeutic

effect (figures 3.25-3.27). However, adding the cosolvent leads to reduced efficiency of the pMDI. Thus, these competing effects combine to provide an upper limit to the respirable dose that can be delivered from a solution pMDI. This limit will be highly sensitive to drug solubility and the volatility of the cosolvent selected. This provides insight into the net benefit obtained by increasing the ethanol level in solution pMDIs.

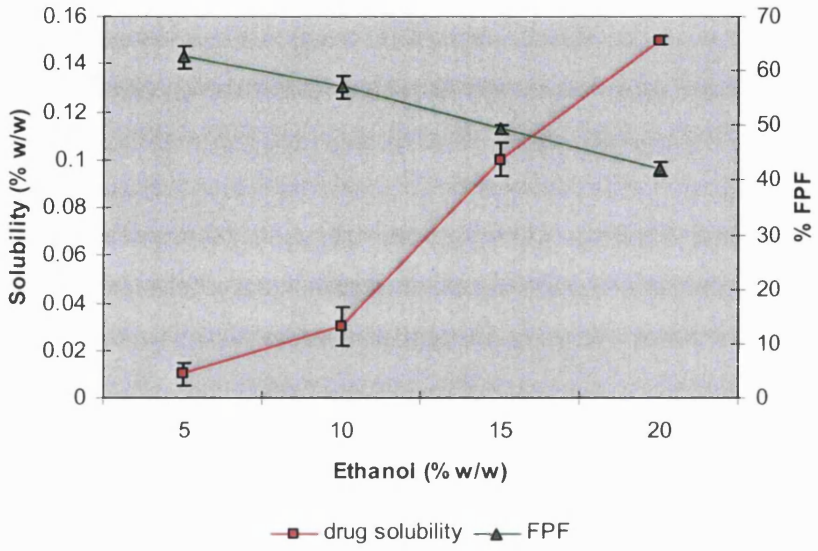


Figure 3.25 *Salbutamol base solubility and FPF as a function of ethanol concentration for GMO-based (0.3% w/w) formulations (n≥3 ± SD).*

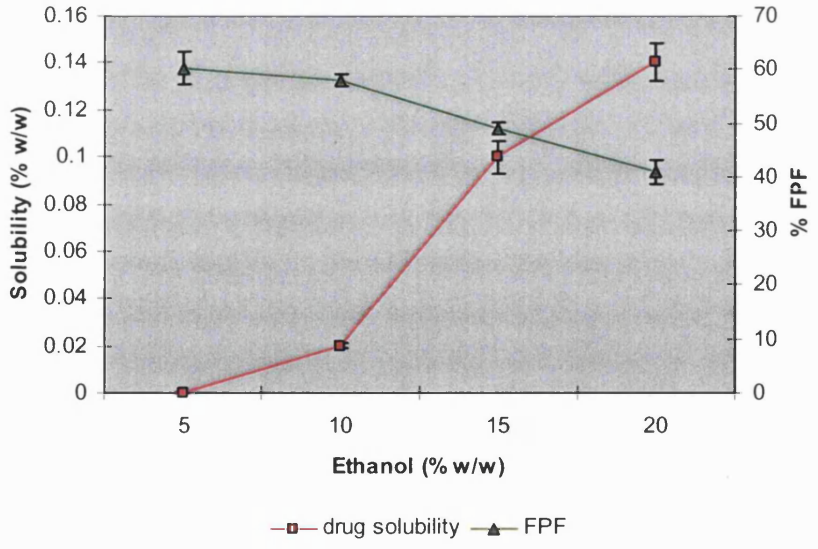


Figure 3.26 *Salbutamol base solubility and FPF as a function of ethanol concentration for SPC-based (0.4% w/w) formulations (n≥3 ± SD).*

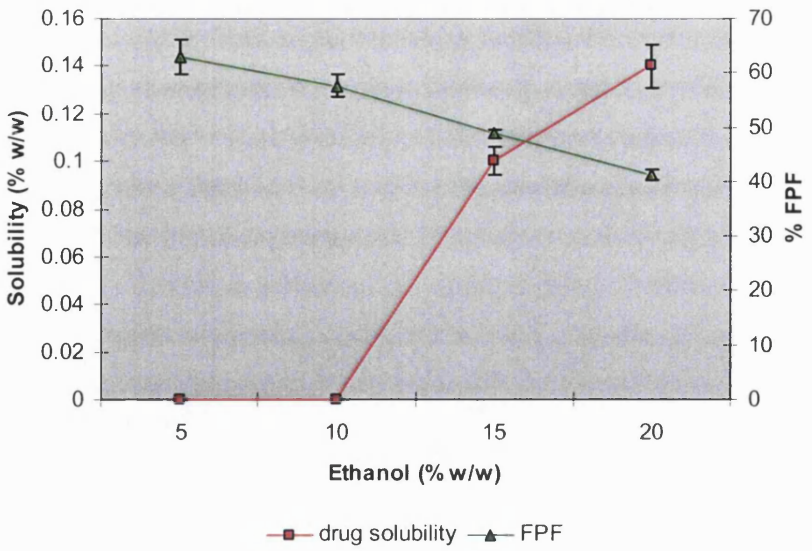


Figure 3.27 *Salbutamol base solubility and FPF as a function of ethanol concentration for EPC-based (0.4% w/w) formulations (n≥3 ± SD).*

At ethanol concentrations <15% w/w, the increased drug solubility more than compensates for the decreased respirable deposition as ethanol concentration increases. However, the benefit of increased drug solubility above 15% ethanol is offset by the decreased respirable deposition. The total dose increases with increasing ethanol levels in the formulation due to the increase in drug solubility. Thus while more drug is delivered with increasing ethanol concentration, the benefit of this additional drug delivery is marginal. In fact, the additional drug delivery at high ethanol levels may be detrimental as it may predominantly deposit in the mouth and throat of the patient potentially leading to undesirable side-effects.

In the present study, the effects of formulation composition, drug concentration and cosolvent on product performance characteristics have been evaluated. Addition of cosolvent resulted in an increase in throat deposition, increase in deposition within the impinger, and decrease in respirable deposition, and a slight increase in the MMAD. Following actuation of the solution pMDI, the droplets released from the device contain the drug, the phospholipid or GMO along with the cosolvent ethanol and the propellant. The release of a high cosolvent volume per actuation may retard the evaporation of the propellant-cosolvent-drug-phospholipid droplets. As a consequence, there is a greater chance for the droplets released to impact on the USP throat or the extension. A significant effect of ethanol concentration on the MMAD was observed. with the increasing ethanol concentration, large droplets released from the pMDI are lost by impaction on the upper stages, mainly in the throat, which manifests itself as an increase in the throat deposition, decrease in the respirable deposition, larger droplets containing higher amounts of ethanol increase the stage deposition and MMAD (tables 3.5 and 3.6; figures 3.28 and 3.29).

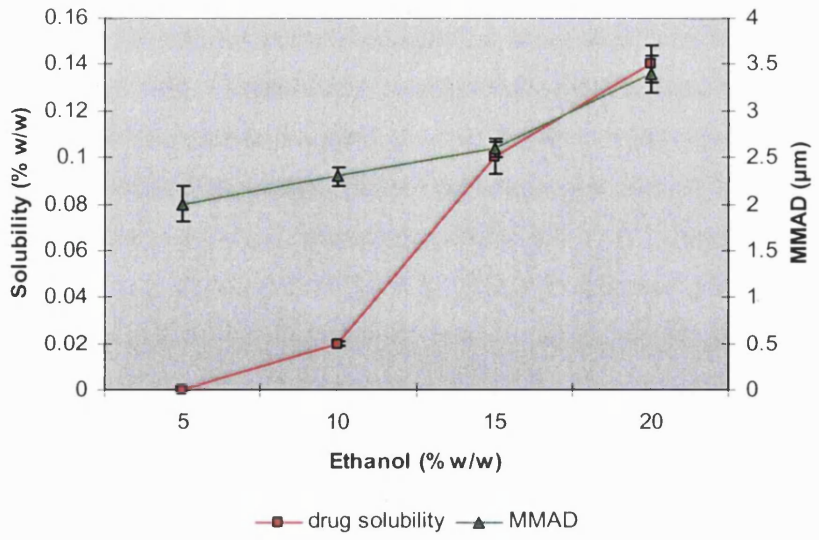


Figure 3.28 *Salbutamol base solubility and MMAD as a function of ethanol concentration for SPC-based (0.4% w/w) formulations (n≥3 ± SD).*

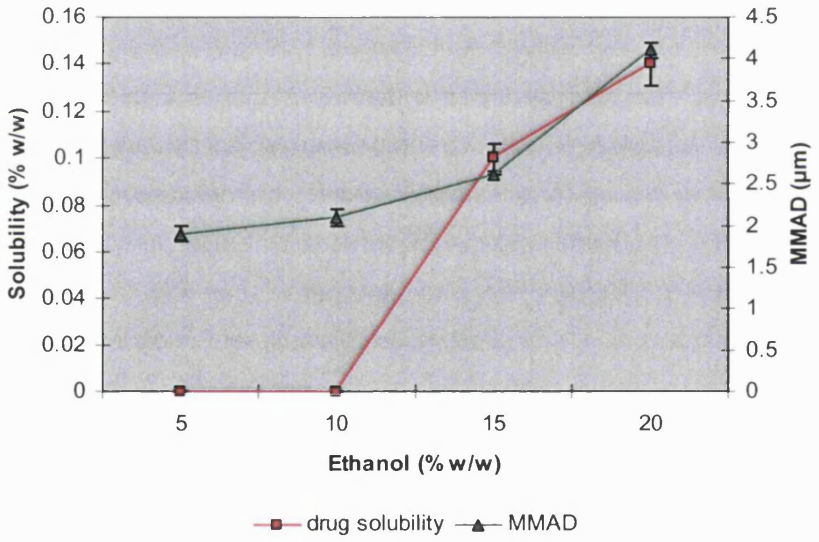


Figure 3.29 *Salbutamol base solubility and MMAD as a function of ethanol concentration for EPC-based (0.4% w/w) formulations (n≥3 ± SD).*

From a formulator's point of view, the optimal cosolvent concentration needs to be defined and selected in order to balance respirable mass and throat deposition. Increasing cosolvent concentration leads to an increase in the drug solubility but is accompanied by a decrease in the respirable deposition and an increase in impinger deposition as seen in this study. Pre-formulation knowledge needs to be defined in order to be able to design solution pMDIs with optimum therapeutic response. Since the therapeutic effect depends on the small fraction of aerosol that gets into the lungs, the various formulation parameters, especially the cosolvent content, should be balanced to optimise drug delivery from the solution pMDI.

3.4.5 Aerosol cloud sizing

All formulations developed produced aerosol clouds having a small droplet size. The droplets produced upon actuation of the device were in the respirable range as determined by the Spraytec. The Spraytec 10th, 50th and 90th percentile (Dv10, Dv50, and Dv90) values for HFA salbutamol base pMDIs were 0.243 ± 0.021, 0.534 ± 0.041 and 5.967 ± 0.012 µm, respectively for formulations containing 0.1% w/w salbutamol base and 0.5% GMO, SPC or EPC (n=3). Particle size distributions calculated for the entire pMDI aerosol for budesonide formulation containing GMO, SPC and EPC as measured with the Malvern Spraytec are shown in Figures 3.30-3.32. There was no statistical difference between formulations containing GMO, SPC and EPC at a constant per cent w/w ($P > 0.05$).

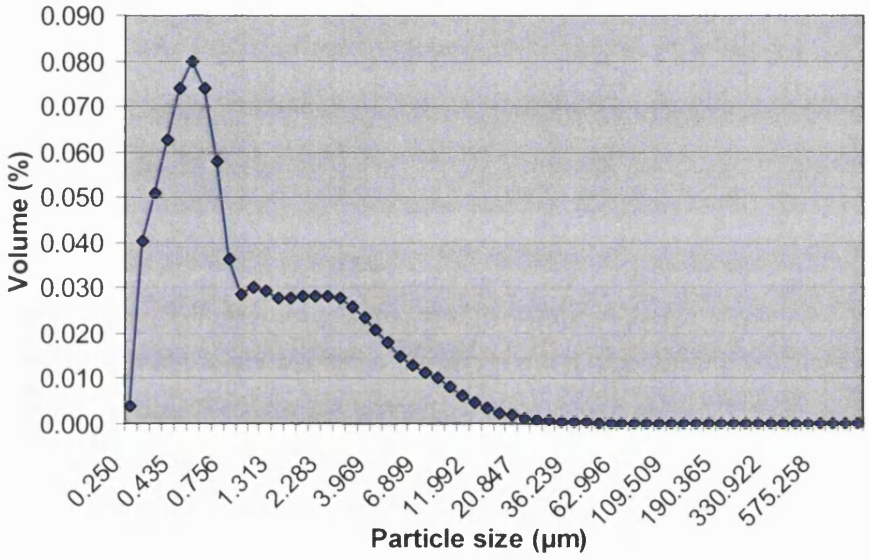


Figure 3.30 *Aerosol particle size distribution for a budesonide formulation containing 0.1% w/w budesonide, 0.5% w/w GMO and 15% w/w ethanol, as measured with the Malvern Spraytec (n=3).*

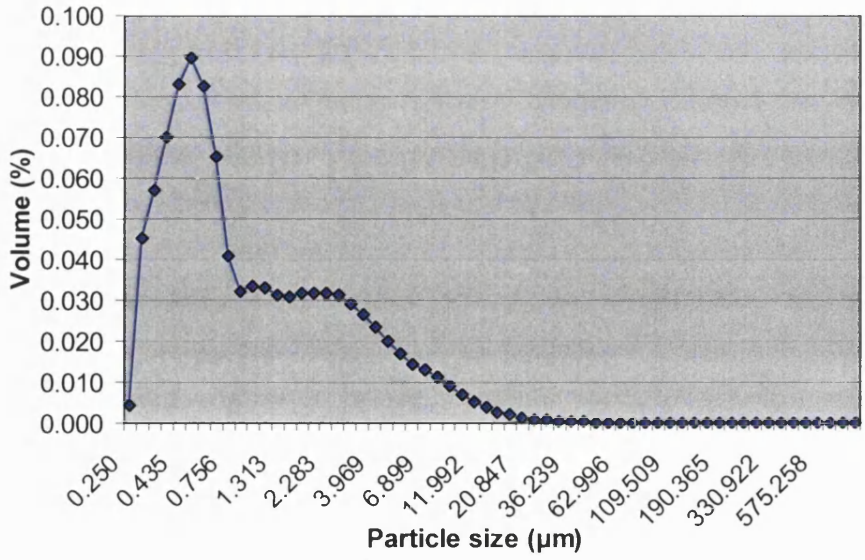


Figure 3.31 *Aerosol particle size distribution for a budesonide formulation containing 0.1% w/w budesonide, 0.5% w/w SPC and 15% w/w ethanol, as measured with the Malvern Spraytec (n=3).*

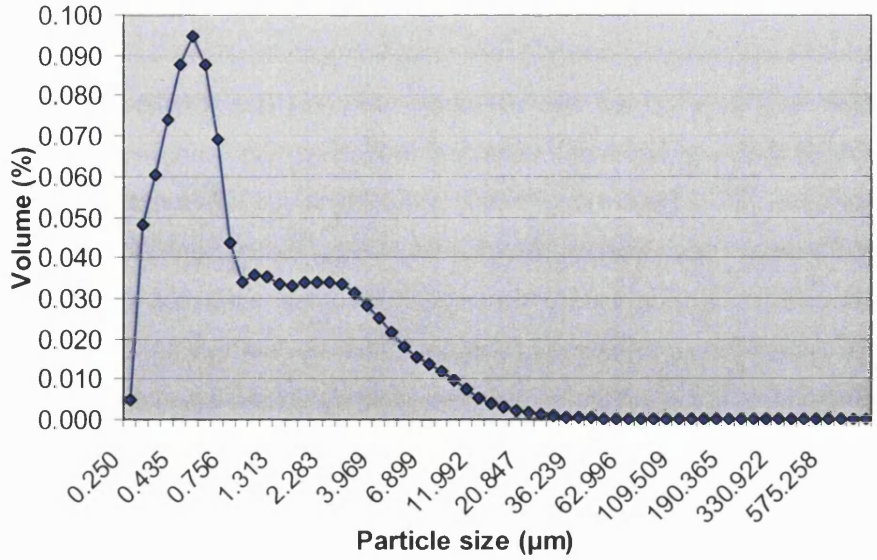


Figure 3.32 *Aerosol particle size distribution for a budesonide formulation containing 0.1% w/w budesonide, 0.8% w/w EPC and 15% w/w ethanol, as measured with the Malvern Spraytec (n=3).*

3.4.6 Drug release from vesicles

Figures 3.33 and 3.34 show drug release from liposomes deposited within the TI. Incorporation of DCP into EPC-based pMDIs increased drug retention within vesicles (figures 3.35-3.41). Total loss of encapsulated drug occurred from EPC liposomes within 480 min (97.5 ± 1.47, 97.5 ± 2.35 and 91.6 ± 2.30 for salbutamol base aerosols at 0.1, 1.0 and 1.8% w/w EPC, respectively and 97.8 ± 1.03, 93.0 ± 1.89 and 82.3 ± 2.08 for budesonide aerosols at 0.1, 1.0 and 1.8% w/w EPC, respectively). Efflux half-lives for preparations containing DCP at 0, 20 and 40% molar concentration of PC are shown in table 3.11.

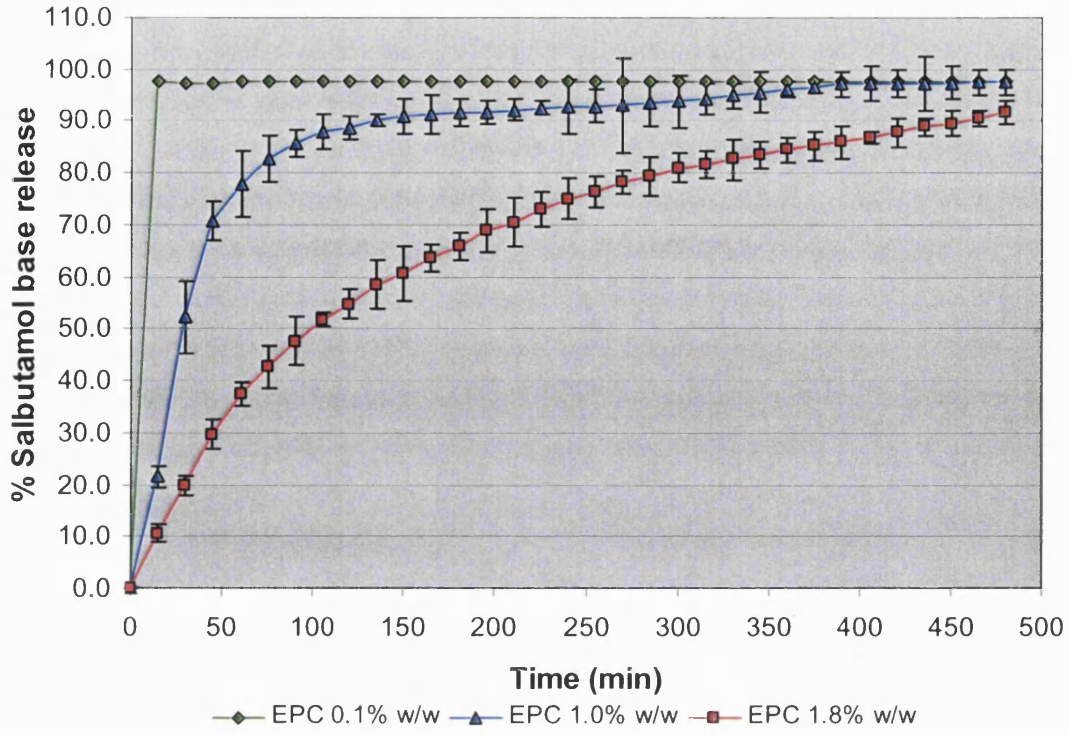


Figure 3.33 *Salbutamol base release into Triton X-100 (0.5%, w/v) for HFA 134a pMDI solution formulations containing EPC at 0.1, 1.0 and 1.8% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

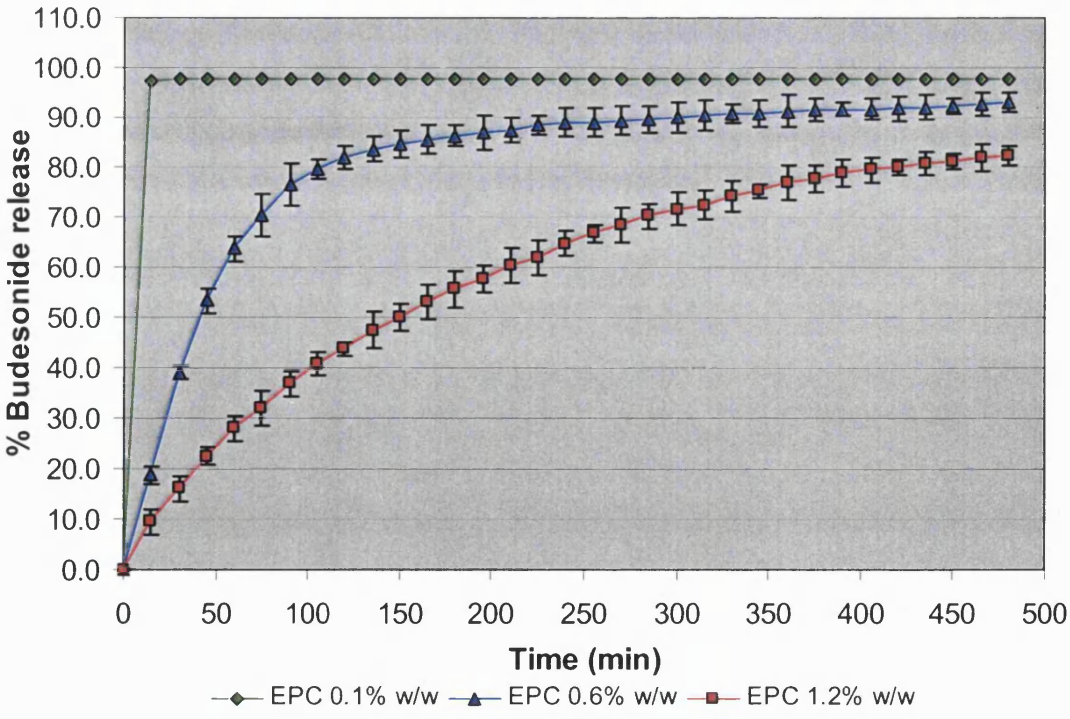


Figure 3.34 *Budesonide release into Triton X-100 (0.5%, w/v) for HFA 134a pMDI solution formulations containing EPC at 0.1, 0.6 and 1.2% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

As evident from figures 3.33 and 3.34, the shift in the release profile to the right of the release curve as the concentration of PC is increased indicated a sustained release effect. The half-time, t_{50}, represents the time required to release 50% of the initial drug loading and can be used to calculated the first order rate constant, k (equation 3.4).

$$t_{50} = \frac{\ln 2}{k} \qquad \textit{Equation 3.4}$$

Increasing PC concentration decreased the release rate for drug from the liposomes. This was expected as drug entrapment increased at higher PC concentrations, and an increase in vesicle material relative to drug provided more barrier to release since it resulted in higher entrapment values. Release was complete at approximately 8 h.

Drug retention was prolonged by the inclusion of DCP, such that the efflux half-life for salbutamol base was increased from 28.8 to 33.3 h (1% EPC), 39.0 to 52.3 h (1.3% EPC), 51.8 to 76.1 h (1.5% EPC) and from 97.4 to 148.0 h (1.8% EPC) when DCP concentration was increased from 0 to 40% molar concentration of EPC for salbutamol base formulations (table 3.11).

These studies highlighted the ability of PC-based formulations, to retard the release of salbutamol base and budesonide.

Inclusion of DCP in the formulation delayed the release of drug ($P < 0.05$) at PC concentrations higher than 0.5% w/w, which is attributed to increased vesicle size and higher encapsulation efficiencies.

Table 3.11 T_{50} and k values for formulations containing DCP at 0, 20 and 40% molar concentration of EPC, salbutamol base, ethanol and EPC.[1]

		EPC (% w/w)			
		1.0	**1.3**	**1.5**	**1.8**
DCP (0% molar concentration of EPC)	**t_{50} (min)**	28.8	39.0	51.8	97.4
	k (min^{-1})	0.024	0.018	0.013	0.007
DCP (20% molar concentration of EPC)	**T_{50} (min)**	30.5	44.1	61.3	120.0
	k (min^{-1})	0.023	0.016	0.011	0.006
DCP (40% molar concentration of EPC)	**t_{50} (min)**	33.3	52.3	76.1	148.0
	k (min^{-1})	0.021	0.013	0.009	0.005

[1]For exact amounts refer to table 3.1.

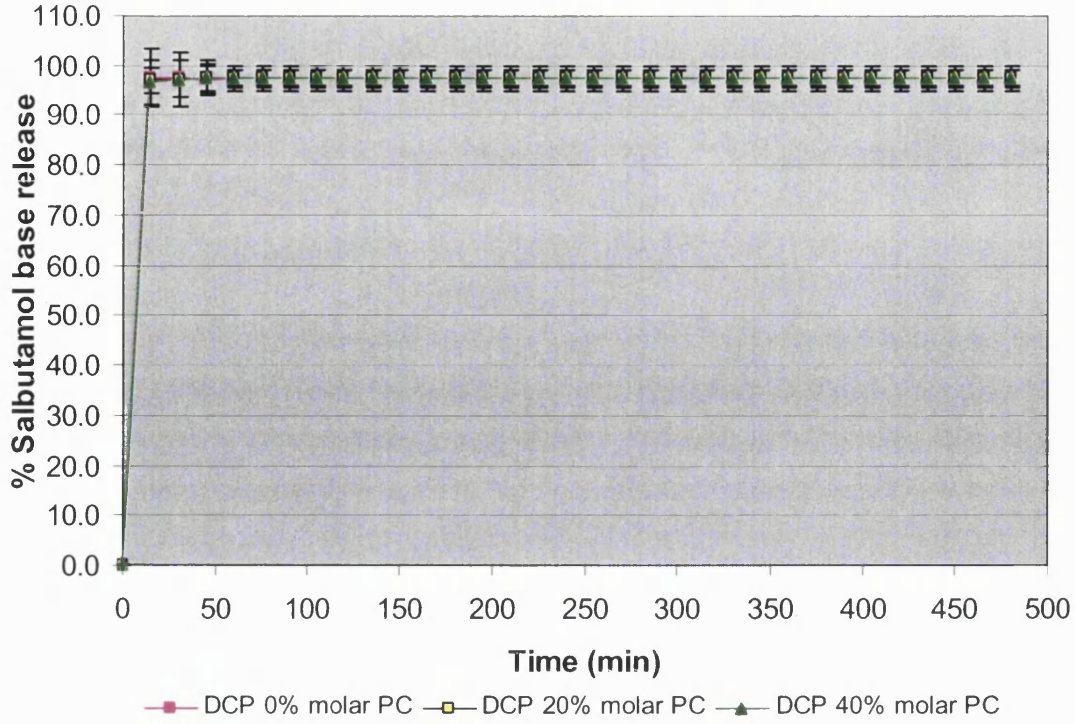


Figure 3.35 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 0.1% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

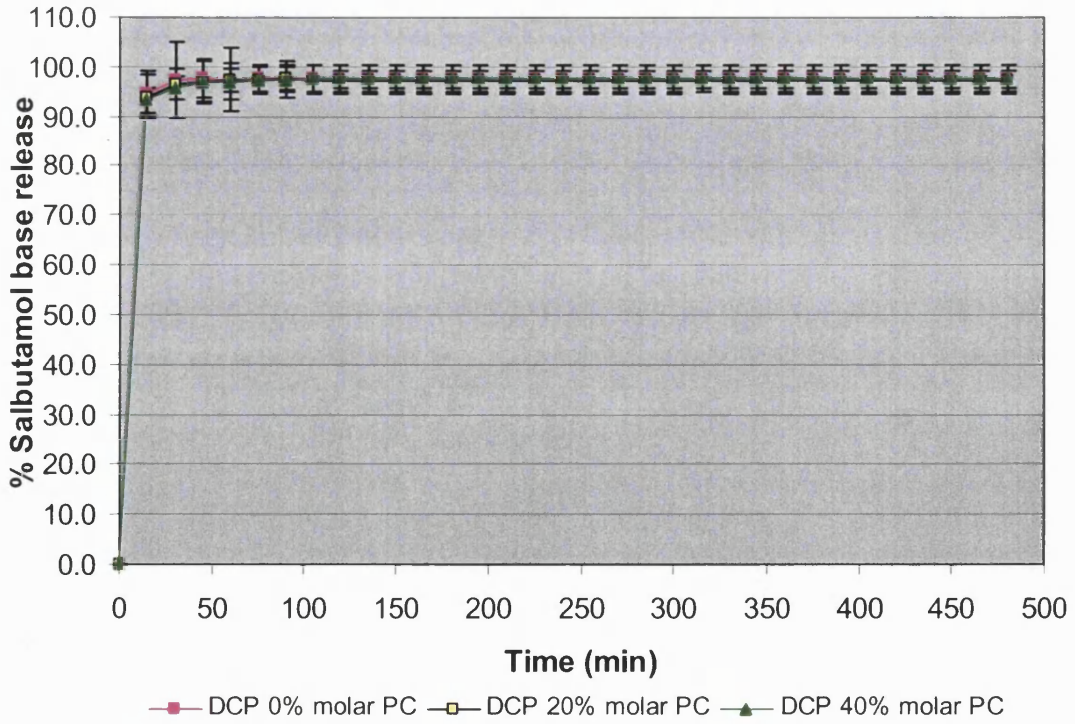


Figure 3.36 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 0.5% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

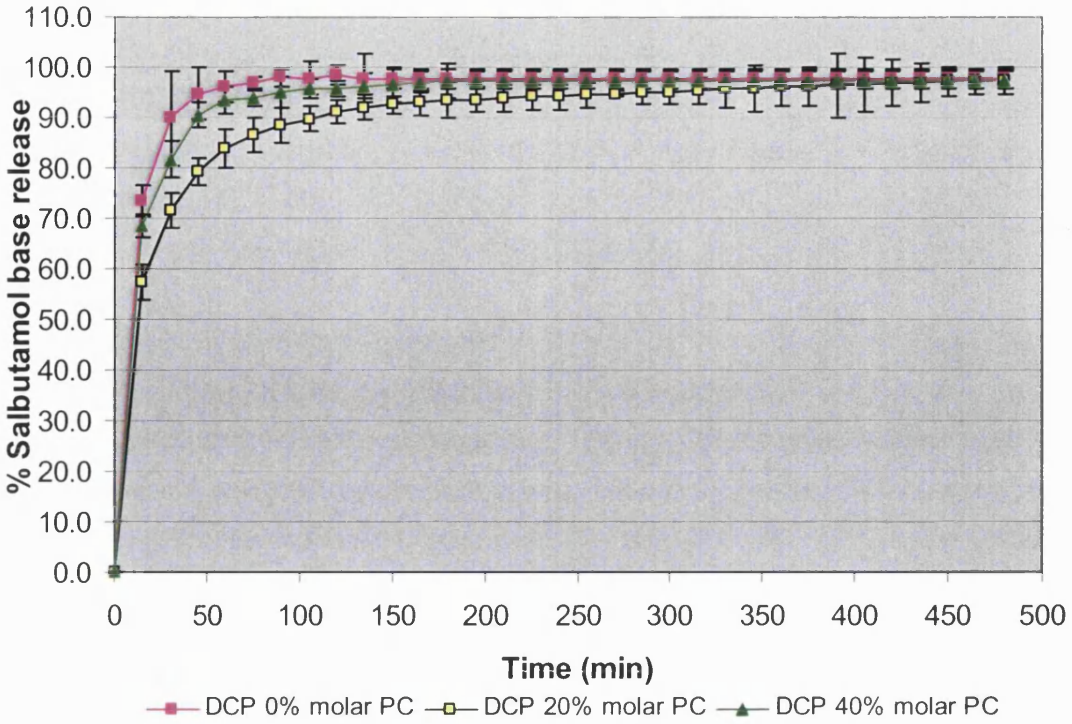


Figure 3.37 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 0.7% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

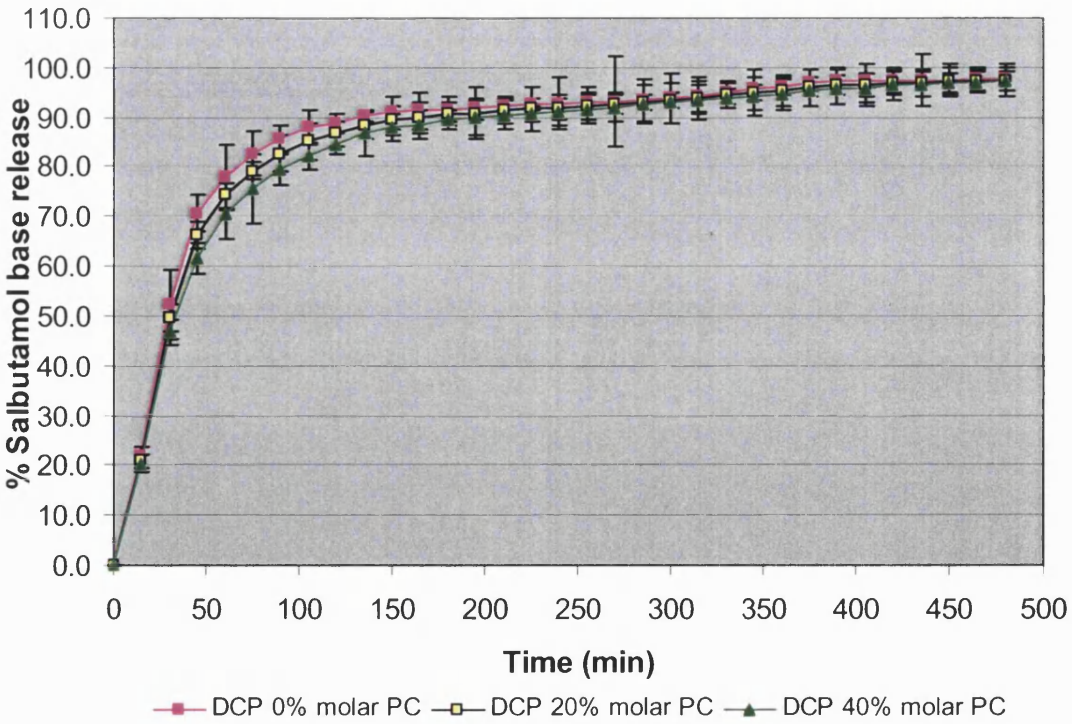


Figure 3.38 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 1.0% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

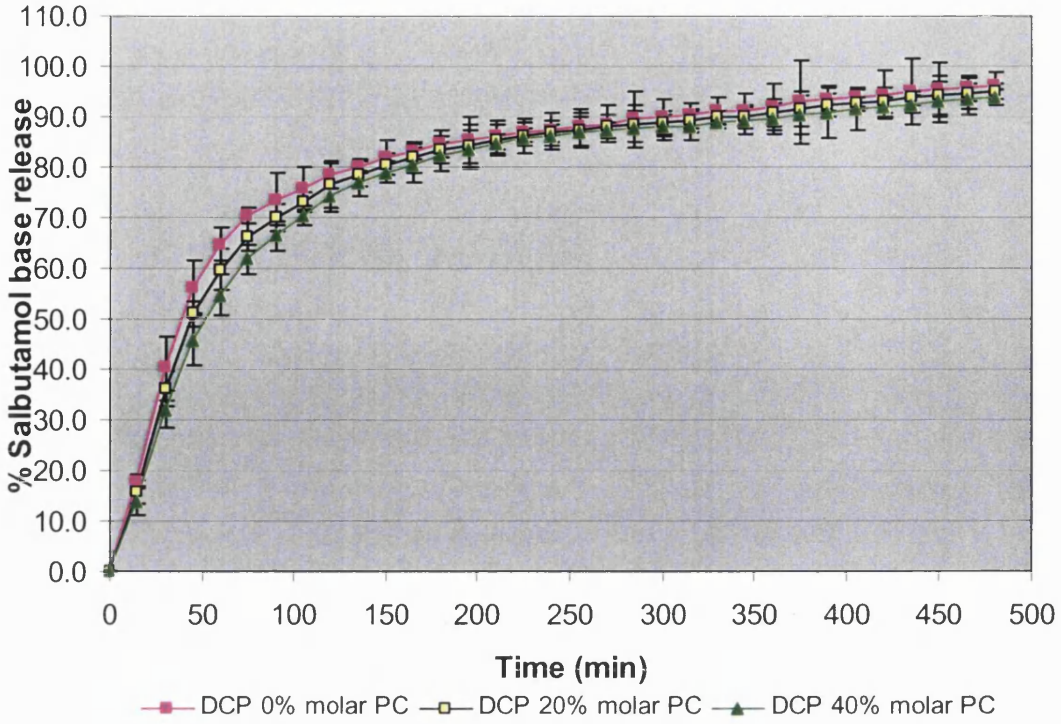


Figure 3.39 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 1.3% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

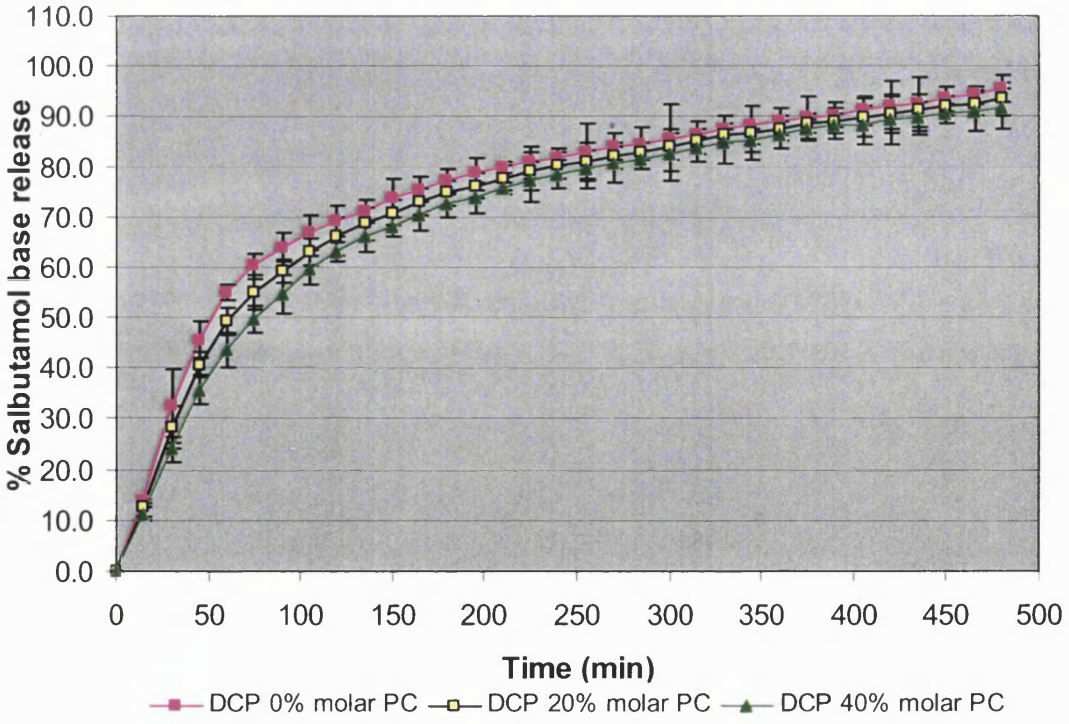


Figure 3.40 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 1.5% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

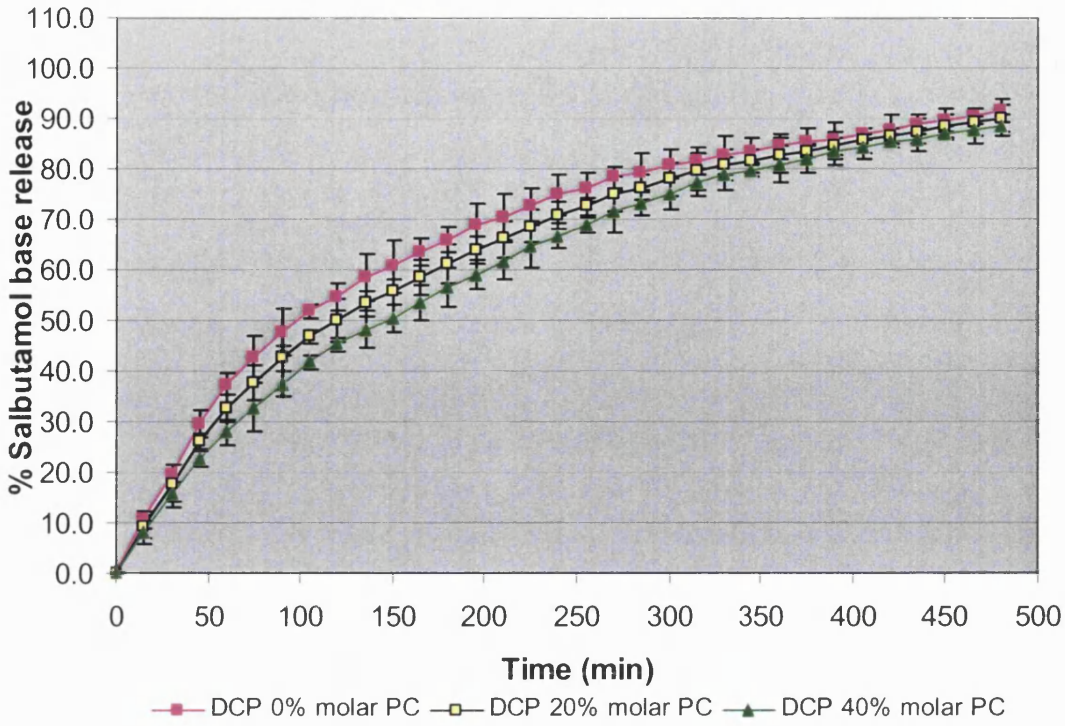


Figure 3.41 *Effect of DCP on salbutamol base release vs. time for pMDI solution formulations containing EPC 1.8% w/w and cosolvent ethanol at 15% w/w (n=3 ±SD).*

3.5 Conclusion

The concept of spontaneous formation of vesicles on dispersion of various phospholipid/drug-containing HFA-based pMDI solution formulations into the TI or MSLI containing deionised water has been demonstrated. The liquid present as the collection medium in each stage of the TI or MSLI provides the appropriate environment for liposome formation from deposited phospholipid aerosols. The instrument, therefore, forms the basis of a reasonable model for assessing partitioning and release of drugs from liposomal systems produced from the respirable fraction of the aerosol clouds. Clearly, the ability of this novel approach to prolong the residence times of relevant drugs within the lung will be a complex function of other factors such as pulmonary clearance and the integrity of liposomal structure within respiratory secretions. Nevertheless, such *in vitro* studies will provide a mechanism through which prospective aerosol formulations may be evaluated.

Developed formulations comprise of phospholipids in a solution in HFA 134a based pMDIs containing cosolvent ethanol and model drug salbutamol base or budesonide. Salbutamol sulphate, a hydrophilic drug, did not form a solution system in the three-component system (HFA 134a-ethanol-GMO, SPC or EPC), thus it was not investigated in terms of its controlled delivery potential. This novel approach of

delivering liposomes in solution pMDI form was advantageous to ensure delivery of solubilised phospholipid to the site of action for liposome formation and to avoid possible leakage of the encapsulated drug. The encapsulation efficiency was dependent on the concentration of the phospholipid. SPC and EPC-based pMDIs containing salbutamol base or budesonide successfully delivered phospholipid to a TI and an MSLI. GMO-based pMDIs failed to form vesicles in the impinger and showed erratic aerosol droplet size measurements indicating the unsuitability of this formulation for generating vesicles using this approach. Furthermore, the ability to form vesicles from compounds endogenous to pulmonary surfactant is a major advantage over other colloidal systems. Compared to a conventional salbutamol or budesonide solution preparation, delivery of a drug in a liposomal formulation from pMDIs may be more advantageous and liposomes formed in this approach were shown to successfully extend drug release.

It was possible to increase encapsulation of salbutamol base, a slightly hydrophilic drug within vesicles, through ionic complexation with a negatively charged lipid. Inclusion of the negatively charged compound DCP into vesicle bilayers increased drug encapsulation, thereby slowing its release. The improved entrapment of salbutamol base could be due to the formation of a lipophilic ion-pair between the positive centre of salbutamol and the negative moiety of DCP. Another possible mechanism of increased entrapment is that the charged lipid increased liposome size through electrostatic separation of the bilayers. Therefore, this study has indicated that liposomal entrapment can markedly prolong the release of drug under *in vitro* test conditions. Moreover, the rate of drug release can be modified by alteration of the liposomal lipid composition.

It has been shown that formulation variables exert significant influence on the aerosol dose available to the respiratory tract. The preceding data will prove useful in the future design of pressure packs, allowing optimum pulmonary delivery of phospholipid/drug mixes. HFA 134a pMDIs showed better performance than HFA 227, probably because of the higher vapour pressure of HFA 134a, which led to more efficient atomisation and finer sprays. Moreover, from the results it is evident that the solubility of salbutamol base increased with increasing ethanol concentration but at the same time the respirable deposition decreased. The maximum solubility benefit above a certain ethanol concentration is negated by lower therapeutic efficiency since the gain in solubility by increasing ethanol concentration is opposed by a decrease in respirable deposition. Although there is more drug per unit in the aerosol plume, it may not reach the desired location in the lung, as seen *in vitro*. The reduced amount of

drug that is able to reach the deep lung not only reduces the pharmacological effect, but increases the drug concentration in the oropharyngeal cavity as well, which may lead to local and systemic side-effects. The deposition of the therapeutically active agent in the optimum quantity, at a desired location within the respiratory tract is the principal aim of inhalation therapy. As shown, this can be achieved with the appropriate cosolvent concentration, which in turn increases the drug solubility, without compromising the respirable deposition and the particle size. Thus, a balance has to be created between the various formulation components of a solution pMDI in order to achieve the deposition efficiency. Improved deposition profiles compared to Becotide® were shown for all PC-based solution formulations, especially with respect to the FPF. The MMAD is strongly dependent on the concentration of the various components of the formulation. PC-based pMDIs did not show comparable MMADs and FPFs with the solution phase pMDI Qvar® probably due to the presence of phospholipids which are non volatile and higher concentrations of cosolvent compared to the commercial aerosol. These were shown to have a direct effect on MMAD and FPF.

The results obtained from the current investigation can serve as a model for the approach that can be used to optimise pMDI formulations, thus enabling formulation of a therapeutically effective solution pMDIs. Although offering a positive result *in vitro,* the usefulness of such systems has yet to be established *in vivo* in disease states that may benefit from such formulations.

CHAPTER 4

ASSESSMENT OF SPRAY PERFORMANCE AND STABILITY KINETICS OF HFA 134a SUSPENSIONS PREPARED WITH 1H,1H,2H,2H-PERFLUOROOCTANOL AND PEG-PHOSPHOLIPIDS

4.1 Introduction

Due to differences in physicochemical characteristics between CFCs and HFAs, new formulations for pMDIs need to be devised (Vervaet and Byron, 1999). Most drugs and surfactants are insoluble in HFAs (Leach, 1995; Elveerog, 1997; Krafft et al., 1998; Dickinson et al., 2000) because these fluorinated compounds are simultaneously hydrophobic and lipophobic. Suspensions of solid drugs in HFAs lead to emission of non-homogeneous, hence non-reproducible amounts of drugs from the aerosol can when the valve is actuated. In order to overcome this problem, stable suspensions of fine and narrowly dispersed drug particles in a continuous fluorocarbon phase are formulated.

The physical stability of non-polar pharmaceutical suspensions (e.g. pMDIs) is critical to their performance because it often not only dictates the dosing reproducibility of the formulation but, as they are 'self-propelled', it can also determine the site of delivery of the suspended particulates (Heyder *et al.*, 1980). The principles of the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory are often used to explain colloid stability in both aqueous and non-aqueous (non-polar) suspensions. The DLVO theory states that the stability of the dispersed colloids is governed mainly by electrostatic forces exceeding the attractive van der Waals forces between particles (Verwey and Overbeek, 1948; Derjaguin and Landau, 1941). However, there is still debate over the contribution that electrostatic forces make to suspension stabilisation in non-polar media, which contain very few charged species (Hiestand, 1964; Farr *et al.*, 1994).

In this chapter, the spray performance of 1H,1H,2H,2H-perfluorooctan-1-ol and PEG-phospholipid suspension pMDIs containing the drugs budesonide, formoterol fumarate and terbutaline sulphate suspended in HFA 134a is investigated and compared to commercially available pMDIs.

4.1.1 Assessing pMDI spray performance

4.1.1.1 Measures of the performance of pMDIs

In order to measure the respiratory drug delivery performance of pMDIs, both *in vivo* and *in vitro* tests are carried out. *In vivo* tests include correlating measured plasma drug/physiological marker concentrations to clinical efficacy and toxicity. Clinical efficacy is determined by spirometry, measuring peak expiratory flow (PEF), bronchoprovocation testing, and measurement of inflammation markers or tissue biopsy (Hickey and Thompson, 1992; Howarth, 2001). Scintigraphic studies, on the other hand, allow visualisation of drug deposition profiles by incorporating gamma-radiating nuclides into the formulation (Newman, 1998). However, due to the high costs implicated with these measures, as well as being time-intensive, *in vitro* measurements are mostly used to

assess pMDI performance. It is generally accepted that for *in vitro* pMDI testing, aerodynamic particle size is the single most important parameter for pulmonary drug delivery and can be used as a determining factor of aerosol deposition efficacy. Particle size measurements represent a simple measure to compare inhalation formulations (Barnes and Nash, 1996; Barry and O'Callaghan, 1997). Laser diffraction or microscopic investigations are generally used to evaluate the particle size distribution in pMDI formulations; however, this size distribution is not always representative of the aerodynamic particle size distribution emitted from the aerosol cloud (Polli *et al.*, 1969; Gonda, 1985). The aerodynamic size distribution describes the way in which an aerosol deposits during inhalation, whereas the aerodynamic diameter is described as the diameter of a spherical particle with unit density that settles at the same rate as the particle in question (Taylor and Kellaway, 2001). The MMAD is considered the most important parameter defining particle size, hence drug deposition. It is described by the diameter that divides the particle size distribution into two halves with respect to the mass, i.e. 50% of the mass lies in particles above and below the MMAD (figure 4.1). The GSD represents a measure of the polydispersity of the aerodynamic particle size distribution and is given by equation 4.1 (USP24/NF19, 1999). A monodisperse aerosol will exhibit a GSD of 1.0, theoretically. In practice however, a GSD of ≤1.22 is considered as monodisperse (Fuchs and Sutugin, 1966; Hickey, 1992a). According to the United States Pharmacopoeia (USP29/NF24, 2006), the MMAD and the GSD may be used to characterise the aerodynamic size distribution.

$$GSD = \sqrt{(size\ X\ /\ size\ Y)} \qquad \textbf{Equation 4.1}$$

where; X is the particle size at the 84.13% cumulative undersize value, Y is the particle size at the 15.87% cumulative undersize value.

Commonly, inertial impaction is the principle most methods characterising aerodynamic particle size distribution from pharmaceutical aerosols operate on, whereby particle inertia is the basis of separating aerosol particles from a moving airstream. As the airstream direction changes, the particles suspended in the airstream continue to move in the original direction until complete loss of inertia as a result of friction and collision with the molecules in the surrounding medium. An inertial impaction device comprises a collection medium that is either liquid (e.g. an impinger) or solid (e.g. a cascade impactor) and is placed in the path of the airstream original direction. Small particles follow the new direction of the airflow without impacting on the surface of the collection medium, whereas large particles impact on the surface. A number of jets of decreasing orifice diameters are placed in the airstream, producing increased airstream velocity and increased inertia. Smaller particles impact on the collection medium as a consequence of the increased airstream velocity (Hickey, 1992a).

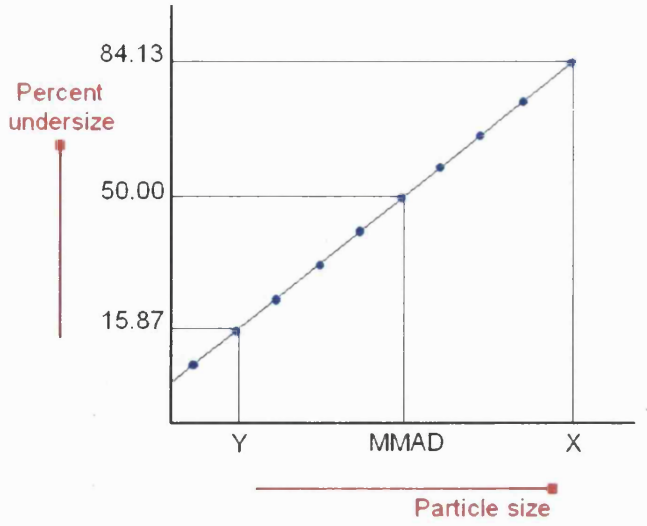


Figure 4.1 *Plot of cumulative percentage of mass less than stated aerodynamic diameter versus aerodynamic diameter (adapted from USP24/NF19, 1999).*

Three inertial impaction apparatuses are described in the United States Pharmacopoeia (USP29/NF24, 2006): the Andersen Cascade Impactor (ACI), the Marple-Miller Impactor (MMI), and the multi-stage liquid impinger (MSLI). However, according to the USP Advisory Panel on Aerosols (1994), the use of the ACI for official purposes only is proposed for the determination of aerodynamic particle size distributions (LeBelle *et al.*, 1997). The ACI provides greatest resolution in particle size distribution because of its eight stage design compared to five stages for the MMI and four stages for the MSLI. The ACI consists of eight stages (stage 0-7) following an induction port onto which a mouthpiece adaptor is placed, and the filter stage. Each stage has its associated collecting plate. Appropriate airflow through the apparatus is generated by a vacuum pump that is connected to the apparatus.

An operational airflow of 28.3 l/min is specified in the United States Pharmacopoeia (USP24/NF19, 1999). Figure 4.2 illustrates the principle of operation of the ACI. Particles generated from the aerosol are suspended in the airstream and follow its direction into the apparatus. Particles having sufficient momentum (product of mass and velocity) will impact onto one of the eight collection plates (stages 0–7) or onto the filter. The particle velocity on each stage is defined by the jet diameter, the larger the jet diameter the lower the particle velocity, thus the smaller the inertia. As the jet diameter decreases with each stage, larger particles will deposit onto upper stages, whereas smaller particles will travel further down and will be deposited on lower stages.

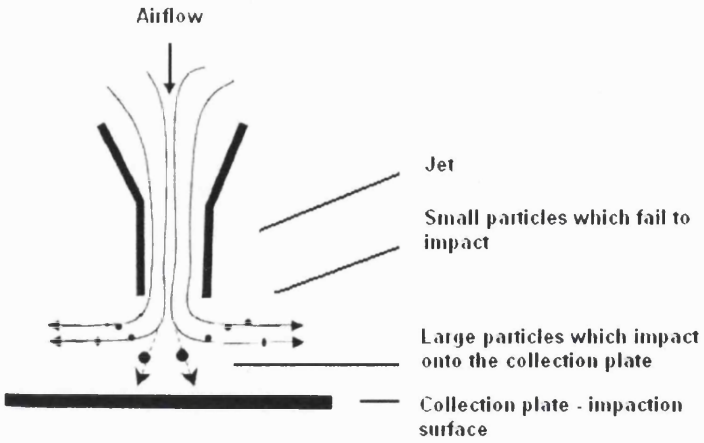


Figure 4.2 *Schematic representation of the principle of operation of the ACI (adapted from LeBelle et al., 1997).*

Each stage of the ACI apparatus is designed to represent an area in the lung since the apparatus is considered to stimulate the airways in the lung. Deposition in the induction port (also referred to as 'throat') down to stage 1 is considered as the oropharyngeal deposition (mouth down to larynx). The amount of drug on stage 2 and below, i.e. <5.8 µm, represents the respirable fraction (RF) or fine particle fraction (FPF) as this is the fraction that reaches the lung. Deposition on stages 2-5 refers to the tracheo-bronchial deposition and particle sizes are between 5.8 µm and 2.1 µm. Further down, the deposition on stage 6 to filter stage, represents the alveolar deposition for particles less than 2.1 µm. The relationship between the deposition pattern in the lung and in the apparatus with corresponding cut-off diameters for each stage is illustrated in figure 4.3.

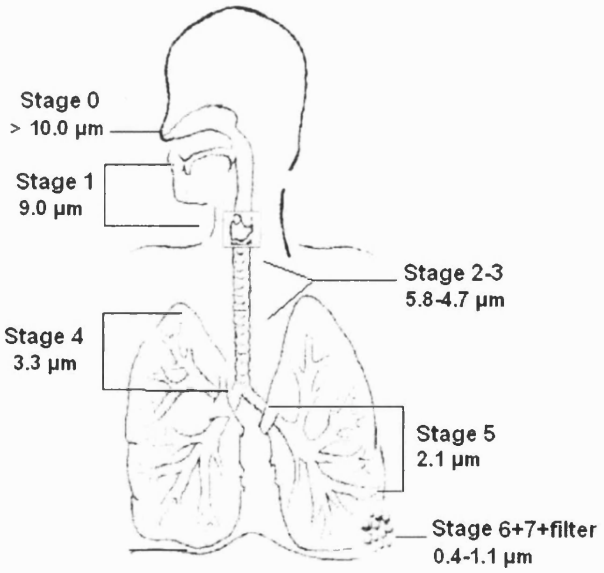


Figure 4.3 *Schematic representation of particle deposition in the respiratory tract and the ACI (adapted from http://www.copleyscientific.co.uk).*

4.1.1.2 Suspension pMDI stability

Suspension stability in pMDIs is critical to their performance. Instability of suspensions may lead to unpredictability of dose emitted and size characteristics, leading to poor therapy. Dispersibility and particle growth are the two major properties which control the reproducibility of dosing from suspension type pMDIs (Byron, 1992). Particle growth due to crystallisation is considered one of the most destabilising processes in drug suspensions. Rapid and non-redispersible sedimentation or caking may be the result of growth processes, which change the aerodynamic size distribution and deposition pattern of an aerosol. There are various conditions that promote crystal growth in pMDIs, such as temperature fluctuations (Carless and Foster, 1966; Tzou *et al.*, 1997), water permeation (Phillips, 1990), amorphous drug content (Phillips, 1992; Mullin, 1993; Phillips and Byron, 1994; Steckel *et al.*, 2003), presence of solubilising surfactants (Phillips, 1992; Phillips and Byron, 1994), addition of cosolvents (Tzou *et al.*, 1997) and polydispersity of solid drug particles inducing Ostwald ripening (Simonelli *et al.*, 1970; Buckton and Beezer, 1992; Mullin, 1993; Phillips and Byron, 1994; Steckel and Müller, 1998). Most of these conditions induce drug dissolution and supersaturation, which stimulates crystallisation processes (Metha *et al.*, 1970; Simonelli *et al.*, 1970). Drug solubility in the propellant is related to crystal growth in pMDIs, and as a rule of thumb, a suspension will not be stable if the solubility of the solid in the propellant can be measured by the most sensitive method (Phillips, 1990; Tzou *et al.*, 1997). The crystallisation process involves three steps: (1) the creation of a supersaturated system, (2) the formation of stable nuclei of critical size – either spontaneously or crystal induced (the nucleation process involves the diffusion of molecules through the bulk of the solution, collision with each other and formation of a nuclei of a critical size), and (3) the crystal growth process (Mullin, 1993; Ohtaki, 1998; Raghavan *et al.*, 2001). The overall Gibbs free energy of formation of the cluster nucleus, ΔG_t, equals the sum of the decrease in the Gibbs free energy of formation of the cluster lattice, ΔG_l, and the surface excess energy, ΔG_s, (figure 4.4, equation 4.2).

$$\Delta G_t = \Delta G_l + \Delta G_s \qquad \textit{Equation 4.2}$$

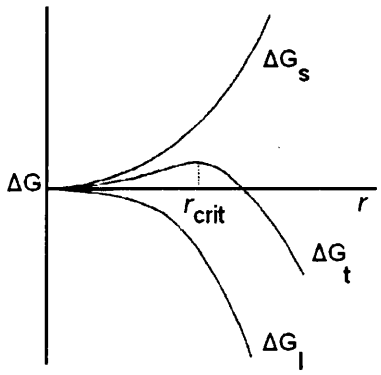


Figure 4.4 *The formation of a cluster as a function of the radius of the cluster (r) (adapted from Ohtaki, 1998).*

The aim of this study was to establish a novel formulation for suspension pMDIs in liquefied propellants to obtain physically stable suspensions. The method is based on using PEG phospholipids, various drugs and perfluorooctanol to stabilise the suspension. Different types and concentrations of excipients and drugs were investigated. Different concentrations of the fluorinated alcohol were investigated to assess their influence on the physical stability of the suspension. In addition, the performance of MDI units containing the novel formulations was assessed by twin impinger (TI), Andersen cascade impactor (ACI) and aerosol particle sizing (APS).

4.2 Materials

4.2.1 Chemicals

All chemicals were used as received. The propellant 1,1,1,2-tetrafluoroethane (Solkane 134a pharma) was purchased from Solvay Fluor und Derivate GmbH (Hannover, Germany). Sodium hexane sulphonate, orthophosphoric acid, sodium dihydrogen orthophosphate dehydrate and potassium dihydrogen orthophosphate were purchased from Sigma-Aldrich, UK. Ethanol absolute (99%-100%) and glacial acetic acid (99-100%) were purchased from VWR International Limited, UK. Water, methanol and acetonitrile used in HPLC assay were all HPLC-grade and purchased from Fisher Scientific Limited, UK. Budesonide, formoterol fumarate, terbutaline sulphate, 1H,1H,2H,2H-perfluorooctan-1-ol, 4,4,4-trifluorobutan-1ol, 1H,1H,2H,3H,3H-perfluoro-1,2-nonandiol, 1H,1H-perfluorononan-1-ol, n-butyl-pentafluropropionate and PEG-phospholipids: methyl-poly(ethylene) glycol-1,2-dimyristoyl-sn-glycero-3-phosphatidylethanolamine (methoxy-PEG-DMPE or mPEG-DMPE) (MW 5,000), methyl-poly(ethylene) glycol-1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (methoxy-PEG-DSPE or mPEG-DSPE) (MW 5,000) and 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine-methyl-poly(ethylene) glycol-1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (DSPE-PEG-DSPE) (MW 5,000), Becotide® beclometasone dipropionate (Allen & Hanbury's), metering valves with 63 µl per actuation spray volume and 20 mm valve length (Valois DF60 MK42; Valois, France), aluminium aerosol cans coated with a fluoropolymer and actuators with an orifice diameter of 0.5 mm were supplied by AstraZeneca (Charnwood, Loughborough, UK).

4.2.2 Equipment

Manual bottle crimper:	Model 3000-B, Aero-Tech Laboratory Company, Maryland, USA.
Pressure filling equipment:	Pressure burette, Aero-Tech Laboratory Equipment, Maryland, USA.
Sonicating bath:	XB6 Ultrasonic Bath, Grant Instruments Limited, Royston, Hertfordshire, UK.
Twin Impinger:	Copley Instruments, Copley Scientific Limited, Nottingham, UK.
Andersen Cascade Impactor:	Copley Instruments, Copley Scientific Limited, Nottingham, UK.
TSI:	Model 3306 Impactor Inlet, TSI Instruments Limited, Buckinghamshire, UK.
Aerodynamic Particle Sizer:	Spectrometer Model 3321, TSI Instruments Limited, Buckinghamshire, UK.
Malvern Spraytec:	Malvern Instruments Limited, Worcestershire, UK.
HPLC:	Instrument HP 1050 Series HPLC with a UV-Visible detector, Hewlett-Packard Co., USA.
Turbiscan MA 2000:	Malvern Instruments Limited, Malvern, Worcestershire, UK.

4.3 Methods

4.3.1 Selection of novel formulation combinations

Preliminary work was carried out in order to select novel formulation combinations. A number of fluorinated compounds were assessed for their potential use in the HFA pMDI units. Their miscibility in propellant HFA 134a was investigated as this is a pre-requisite for the fluorinated additive to play a suitable role in the formulation. Subsequently, the solubility of 3 excipients; mPEG-DMPE MW 5000, mPEG-DSPE MW 5000 and DSPE-PEG-DSPE MW 5000 was tested in the fluorinated liquid that was miscible in the propellant.

4.3.1.1 Miscibility of fluorinated molecules in HFA 134a

The fluorinated compound must be miscible in propellant HFA 134a at the concentration required in order to be useful in the novel HFA formulations. The fluorinated compound (1H,1H,2H,2H-Perfluorooctan-1-ol, 4,4,4-Trifluorobutan-1ol, 1H,1H,2H,3H,3H-Perfluoro-1,2-nonandiol, 1H,1H-Perfluorononan-1-ol or n-Butyl-pentafluoropropionate) was weighed in a clear aerosol vial. The vial was then crimped, and subsequently pressure filled with propellant 134a until the desired total weight was reached.

4.3.1.2 Solubility of PEG-phospholipids in perfluorooctanol

The solubility of the excipients (mPEG-DMPE MW 5000, mPEG-DSPE MW 5000 and DSPE-PEG-DSPE MW 5000) in the fluorinated liquid perfluorooctanol (PFOH) was determined. The excipients were weighed in glass vials with a screw-on plastic cap. PFOH was added by weight at the required concentration, and the vial sealed with Teflon tape and the screw-on cap. The samples were sonicated for 10 min and heated to 35°C to quicken the dissolution of the excipients. The vials were then allowed to cool to room temperature. Visual observations were subsequently made on the cold samples to asses their solubility.

4.3.2 Preparation of pMDI units

A series of suspension aerosols containing 5, 10, or 15% w/w fluorinated cosolvent PFOH, 0.1-0.2% w/w of either of the three drugs budesonide, formoterol fumarate or terbutaline sulphate and 0.1-0.2% of one of three PEG-phospholipids mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE were prepared. An appropriate amount of the drug, PEG-phospholipid and cosolvent was added to an aluminium aerosol can. The can was then cooled in a dry ice/acetone bath before the liquefied propellant was added by weight using a cold filling technique (Tunnicliff Engineering Co Limited, UK). The cold filling technique was employed since only a maximum pressure of 100 psig could be applied to the pressure burette, which is insufficient for pressure filling through metering valves. Once the required weight of 14 g (=10 ml) of propellant was filled into the can, a metering valve (Valois DF60 MK42; Valois, France) was immediately crimped (Manual bottle crimper 3000, Aero-Tech Laboratory Equipment Company, USA) onto the can in order to avoid evaporation of propellant. The suspension pMDIs were then sonicated (XB6 Grant Instruments Limited, UK) for approximately 15 min and stored. Investigations were performed on each aerosol unit within 2 h of preparation. Three cans of each aerosol composition were prepared. Samples for adhesion, creaming and sedimentation analysis tests were prepared in clear aerosol vials fitted with a continuous valve. The excipient and fluorinated compound were mixed and the drug was weighed into the vial. The mixture of fluorinated compound and excipient was then added to the drug. Once the continuous valve was manually crimped (Manual bottle crimper 3000, Aero-Tech Laboratory Equipment Company, USA), the propellant was transferred through the valve under pressure to the desired weight (Aero-Tech Laboratory Equipment Company, Maryland, USA). The samples were sonicated for at least 15 min (XB6 Grant Instruments Limited, UK), and left to stand for equilibration for up to 2 h, before observations were made. The samples were then assessed and kept under standard laboratory conditions at room temperature (21 ± 2°C).

4.3.3 Assessment of formulations

The novel formulations were tested in terms of their ability to reduce drug adhesion to the can walls, reduce phase separation times and keep the suspension finely dispersed. Three tests were performed: assessment of can wall adhesion, evaluation of creaming or sedimentation rates and sizing of the dispersion. The results were compared with the characteristics of selected control samples (detailed later).

4.3.3.1 *In vitro* characterisation

4.3.3.1.1 Twin Impinger analysis

The TI was used to determine the deposition characteristics of the suspensions after release from the pMDIs in order to determine the physical stability of drug particles within the HFA indirectly. The TI apparatus was set up and run using a flow rate of 60 l/min. The pMDIs were primed prior to use by discharging approximately 10 shots into a fume cupboard. A total of 20 actuations were sprayed into the apparatus from each inhaler. After completion of each run the impinger was taken apart and the drug assayed according to the amounts deposited on the device, stage 1 and stage 2. The device was washed with 50 ml of liquid and both stages 1 and 2 with 100 ml of washing solution. HPLC mobile phase specific to each drug was used both as the impinger solvent and as the washing solution. HPLC analysis (described below) was used to quantify the drug within the washing solutions. The quantities of the therapeutic agents delivered by the pMDI formulations on each stage of the impinger were determined as a percentage of the total quantity of drug recovered from the device and the impinger. For example, in order to calculate the stage 1 % deposition, equation 4.3 was used.

$$\%\ \text{Stage}\ 1 = \frac{Q_{s1}}{Q_m + Q_{s1} + Q_{s2}} \times 100 \qquad \textit{Equation 4.3}$$

where; % stage 1 is the percentage of the drug on stage 1 of the impinger, Q_m is quantity of the drug on the mouthpiece, Q_{s1} is the quantity of the drug on stage 1 of the impinger, Q_{s2} is the quantity of the drug on stage 2 of the impinger. The fine particle fraction (FPF) in the TI was defined as the % of the drug on stage 2 of the impinger, i.e. the % of particles <6.4 µm. A Becotide® pMDI was tested in triplicate using an identical TI method and the quantity of beclometasone dipropionate (BDP) recovered (*%REC*) from the apparatus was compared to the label dose using equation 4.4.

$$\%REC = \frac{T_m + T_{s1} + T_{s2}}{LD} \times 100 \qquad \textit{Equation 4.4}$$

where; T_m is the quantity of drug per shot deposited on the mouthpiece, T_{s1} is the quantity of drug per shot deposited in the TI stage 1 and T_{s2} is the quantity of drug per shot deposited in the TI stage 2, LD is the label dose.

4.3.3.1.2 Andersen Cascade Impactor analysis

Cascade Impaction (Andersen Cascade Impactor, Copley Scientific Limited, UK) was used to determine the aerodynamic particle size distribution of the emitted dose of each aerosol prepared. The ACI was assembled according to USP 24 (USP24/NF19, 1999) and fitted with an aluminium USP Induction port. A filter (Whatman Int. Limited, Maidstone, UK) was placed on the filter stage. A custom made rubber mouthpiece adapter, which was purchased from Copley (Copley Scientific Limited, UK), was fitted onto the induction port. The investigations were carried out at airflow of 28.3 l/min. The airflow was calibrated by attaching an electronic digital flowmeter (model DFM, Copley Scientific Limited, UK) at the beginning of each run. The aerosol can was inserted into the actuator then shaken for 5 s, actuated 5 times to waste and subsequently inserted into the mouthpiece adapter such that the device was aligned along the horizontal axis (in accordance with the procedure described in the USP 24 (USP24/NF19, 1999)). One dose was then fired into the cascade impactor and the inhaler removed after 10 s. This procedure was repeated for a total of 10 actuations. After the last dose had been discharged, the ACI was disassembled and the drug deposited in the cascade impactor was recovered employing the following washing procedure: the cascade impactor was divided into 11 washing units, which were washing 1: the device (actuator and valve), washings 2-11: the throat and mouthpiece, each stage (stage 0-7) and its associated collection plate, the filter stage and filter. Each unit was washed with 25 ml of appropriate diluent. In order not to lose any drug during the washing, a 25 ml volumetric flask was placed beneath a funnel. Each impactor unit was held above the funnel and washed with diluent from a squeezable plastic bottle. The volumetric flasks were filled up to volume with the diluent. The washings were immediately analysed employing a different HPLC assay for the determination of each drug as described later. The aerodynamic size distribution of each aerosol composition was tested in triplicate. Calculations of the MMAD and GSD according to USP 24 (USP24/NF19, 1999) were carried out using a calculation model kindly supplied by AstraZeneca. Calculations were made on the basis of stages 2-5. The FPF was calculated as the fraction ex device of drug deposited on stage 2 to filter stage, i.e. < 5.8 µm.

4.3.3.1.3 Aerodynamic Particle Sizing (APS) analysis

Aerosol cloud size analysis was assessed using the APS impactor inlet in conjunction with the Aerodynamic Particle Sizer (APS). Each vial was shaken then primed by actuating three times to waste. Five actuations were delivered for each test, and 3 tests were conducted for each vial (n = 3). The MMAD was calculated using the TSI software, Aerosol Instrument Manager, Rev B (2002). Actuators of the same orifice diameter were used for all samples. The TSI Model 3306/3321 System (section 3.3.4.1) was operated at a flow rate of 28.3 l/min. For this study, the target cut-point of the impactor stage was 4.7 µm,

thereby allowing for the collection of a fine particle mass less than 4.7 µm ($FPF_{4.7\mu m}$) from the filter. The larger aerosol particles that do not flow through to the filter are collected on the impaction plate. The plate and filter were assayed via HPLC, as for the ACI.

4.3.3.2 High Performance Liquid Chromatography (HPLC)[1]

The chemical analysis of drug was performed using an HPLC system (HP 1050 Series HPLC with a UV-Visible detector, Hewlett-Packard Co., USA). A validated method developed at AstraZeneca R&D Charnwood was used for the reverse-phase, isocratic assay of each drug. Chromatography conditions were constant throughout all experiments and are summarised in table 4.1.

4.3.3.3 Aerosol cloud analysis

pMDIs containing different blends of budesonide, formoterol fumarate or terbutaline sulphate added to PEG-phospholipids mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE, PFOH and HFA 134a were investigated. Aerosol cloud size analysis was assessed using the APS Impactor Inlet in conjunction with APS Aerodynamic Particle Sizer (APS) and the Malvern Spraytec. Each vial was shaken then primed by actuating three times to waste. Three tests of five actuations each were conducted for each vial. Actuators of the same orifice diameter, 50 µm, were used for all samples.

4.3.3.4 Statistical analysis

For the comparison of two pMDIs or more than two pMDIs, refer to section 3.3.7.

4.3.3.5 Stability of HFA suspension pMDIs

4.3.3.5.1 Turbiscan experiments

Micronised drug suspension formulations were prepared using mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE (concentration ranges 0.062 to 0.470, 0.052 to 0.700 and 0.048 to 0.708% w/w, respectively) and cosolvent PFOH (at levels of 5 to 15% w/w) in HFA 134a. Three micronised drugs were used: budesonide, formoterol fumarate and terbutaline sulphate. Optical characterisation of the stability was performed using the Turbiscan MA 2000 at room temperature (21 ± 2°C). The detection head scanned the entire height of the sample (50 mm), acquiring transmission (T) and backscattering (BS) data during 30 or 60 min, every min, in steps of 40 µm.

[1]Brief details disclosed. For specific HPLC method, please contact AstraZeneca R&D Charnwood, who will release test method on a case-by-case consideration.

Table 4.1 A summary of HPLC run conditions for budesonide, formoterol fumarate and terbutaline sulphate.

Budesonide	
Column	Supelcosil LC-Si C18
Mobile phase	2.3 mM phosphate buffer solution in 29:71 v/v acetonitrile:water
Mobile phase filter	0.2 µm cellulose nitrate
Flow Rate	1.5 ml/min
Injection Volume	250 µL
Column Temperature	Ambient
Detection Wavelength	248 nm
Run Time	10 min
Formoterol fumarate	
Column	Supelcosil LC-Si C18
Mobile phase	2.3 mM phosphate buffer solution in 29:71 v/v acetonitrile:water
Mobile phase filter	0.2 µm cellulose nitrate
Flow Rate	1.5 ml/min
Injection Volume	250 µL
Column Temperature	Ambient
Detection Wavelength	214 nm
Run Time	17 min
Terbutaline sulphate	
Column	Kromasil C18
Mobile phase	1.0 M phosphate buffer in deionised water, orthophosphoric acid/ethanol
Mobile phase filter	0.2 µm cellulose nitrate
Flow Rate	1.5 ml/min
Injection Volume	100 µl
Column Temperature	45°C
Detection Wavelength	210 nm
Run Time	5 min

4.3.3.5.1.1 Colloidal systems

A system is called colloidal when it is composed of at least two non-miscible phases, one of which is dispersed as < 1 µm particles in the other. The particles constitute the dispersed phase and the dispersing phase is called continuous. Liquid dispersions are thermodynamically unstable. Usually, two main types of destabilisation processes appear: (a) particle migration phenomena, particles migrate either to the top (creaming), or to the bottom (sedimentation) without size variation. These phenomena generate only a local

particle concentration variation; and (b) size particle variation phenomena, either coalescence when the particle size is increasing, or flocculation when the particles are aggregating. These destabilisation phenomena always lead to a separation phase. Flocculation and sedimentation of suspended solids in a liquid phase represent one of the most central problems in colloidal science (Balastre *et al.*, 2002).

4.3.3.5.1.2 The Turbiscan MA 2000

The Turbiscan MA 2000 is a macroscopic analyser of colloidal systems physical stability used to semi-quantify sedimentation and creaming rates. It allows the investigation of the homogeneity and stability of liquid mixtures from turbidity versus height measurements. The sample is held in a flat bottomed cylindrical cell made of a borosilicate glass tube (Turbiscan tube) (12 mm diameter, 140 mm height), which is closed at the bottom by a plug made of black Teflon that absorbs the light.

It consists of a reading head that moves along a flat-bottomed cylindrical cell to scan the entire sample length. The reading head comprises of a pulsed near infrared light source (λ = 850 nm) and two synchronous detectors of small aperture and rectangular cross section: (1) the transmission detector monitors light transmitted through the product (detection angle θ = 0°); and (2) the backscattering detector receives multiple backscattered light flux by the product (detection angle θ = 135°) (figure 4.5).

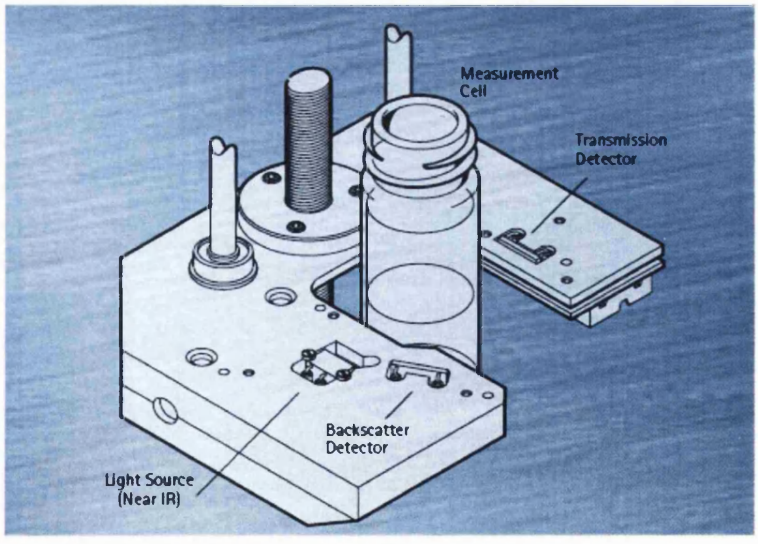


Figure 4.5 *The Turbiscan reading head. Courtesy of Malvern Inc.*

The optical reading head using a near infrared light (850 nm) moves vertically along the cell scanning a height of 65 mm and acquires transmission and backscattering data every 40 µm along the sample tube for a maximum length of 80 mm. The optical signals are then

translated into electrical signals, which are converted to numerical data. The integrated microprocessor software (Turbisoft) handles data acquisition. Basic profiles of transmission (transmitted light) and backscattering (backscattered light) are then obtained with data processing treatment (figure 4.6). The profiles display the macroscopic fingerprint of the sample at time, t. This fingerprint of the product characterises its homogeneity, particle concentration, and mean particle diameter. Results are represented on the software screen by a curve showing the percentage of backscattered or transmitted light as a function of the sample tube length (in mm).

The acquisition scan is then repeated many times with a programmable frequency and results are superimposed on a time scan graph. The stability or instability of the product can then be determined by analysing the time graph. If the scans at different times overlap, the product is stable. The zero time corresponds to the end of the transfer of the suspension into the tube, when the first scanning starts. For pressurised systems a cell capable of handling pressurised samples is required; such a cell was used for the evaluations of these HFA formulations.

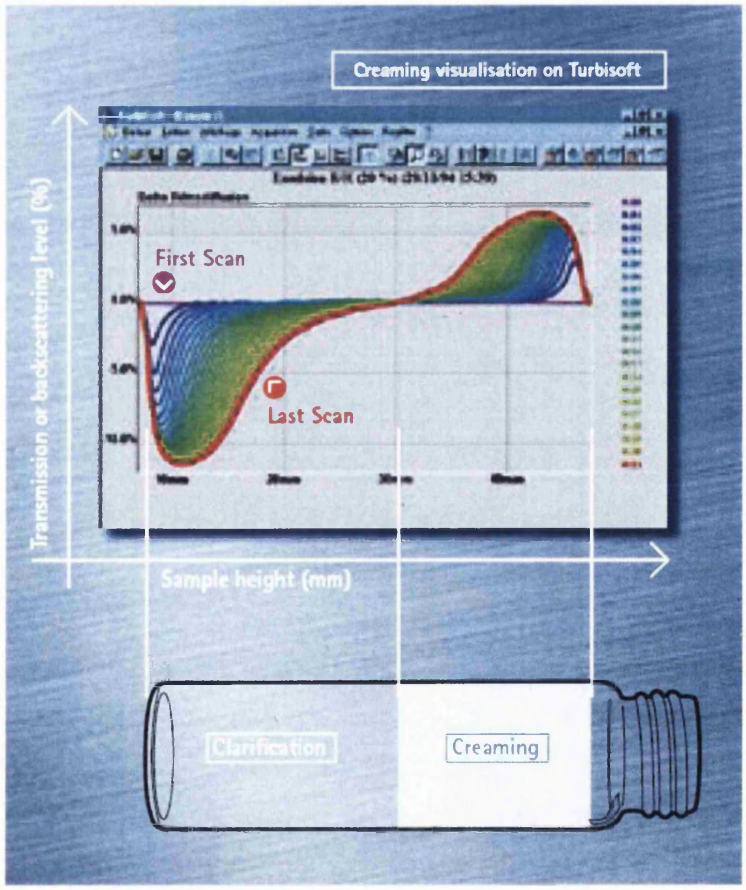


Figure 4.6 *Principle of the Turbiscan MA 2000 (creaming kinetics as analysed with the instrument). Courtesy of Malvern Inc.*

Theoretically, we have to obtain a maximum in transmission and a minimum in backscattering for transparent systems, and the converse for opaque systems. For a turbid medium, we obtain an intermediate response in transmission and backscattering: the mean levels of transmission and backscattering depend on the particle concentration, the particle size, and the refractive index of the dispersion. Concentrated systems (> 0.1%) have maximum backscattering and minimum transmission. Backscattering is a maximum for particle size about 0.3 µm. The relative refractive index (n) can be calculated from equation 4.5 (Mengual *et al.*, 1999; Bordes *et al.*, 2002; Lemarchand *et al.*, 2003).

$n = n_1/n_2$ ***Equation 4.5***

where; n_1 is the dispersed phase refractive index; n_2 is the continuous phase refractive index.

4.3.3.5.1.3 Physical parameters

The light scattering method quantifies the interaction of a focused light beam with particles randomly distributed in a fluid (Bordes *et al.*, 2002). Before being absorbed, backscattered or transmitted through the sample, incident photons undergo multiple scattering events. In randomly distributed dispersed media, when there is no interference between the waves scattered by each particle of the medium, light scattering may be considered as independent and the photon mean path length $\ell(\Phi,d)$ is greater than the wavelength λ of the incident radiation (equation 4.6) (Mengual *et al.*, 1999).

$$\ell(\phi,d) = \frac{1}{n(\pi d^2/4)Q_e} = \frac{2d}{3\varphi Q_e} \quad and \quad \varphi = n\frac{\pi d^3}{6} \qquad \textit{Equation 4.6}$$

where; n is the particle density, Φ the particle volume fraction, Q_e the extinction efficiency factor for scattering and absorption phenomena (ratio of the extinction cross-section to the geometrical cross-section). It is true for particles larger than the wavelength ($d > \lambda = 0.85$ µm).

The characteristic lengths involved in conservative multiple light scattering (no light absorption phenomena) are the photon mean path ℓ, and the transport mean path, ℓ^*, depending on the physical properties of the scattering medium (refractive index n_f of suspending fluid, refractive index n_p of particles, particle mean diameter d and particle volume fraction Φ). The photon mean path, ℓ, is the average distance between two scattering events in the medium. All information about the initial direction of a photon is further lost over the transport mean path, ℓ^*, representing a 'decorrelation' length

between the direction of photon propagation and the incident direction. The scattering medium geometry and the transport mean path, ℓ^*, determines the photon path length distribution in the medium particles (Bordes *et al.*, 2002).

4.3.3.5.2 Intrinsic stability

The intrinsic stability and stability under storage for all formulations were investigated. For each formulation, three samples were prepared and stored vertically at room temperature (21°C ± 2) and observed every minute for 2 h after sonication, then at time 3 h; 4 h; 5 h; 6 h; 7 h; 8 h and 24 h. Any change was recorded; separation, sedimentation or creaming and the corresponding heights (easily transformed into volumes). In another experiment, nine samples were prepared for each formulation. Three were stored vertically at room temperature (21 ± 2°C), three in a hot air oven at 45°C ± 2, and three at 4 ± 2°C. Observations were made every minute for 2 h after sonication then at time 3 h; 4 h; 5 h; 6 h; 7 h; 8 h and 24 h.

4.4 Results and Discussion

4.4.1 Selection of novel formulation combinations

The miscibility in HFA 134a of five chosen fluorinated compounds (PFOH, 4,4,4-Trifluorobutan-1ol, 1H,1H,2H,3H,3H-Perfluoro-1,2-nonandiol, 1H,1H-Perfluorononan-1-ol and n-butyl-pentafluropropionate) is shown in table 4.2. The concentration values listed in the second column indicate the upper limit at which the test was done such that the fluorinated compounds were not necessarily miscible at higher concentrations. Systems at concentrations equal to or below these limits are therefore monophasic.

Table 4.2 Miscibility of selected fluorinated molecules in HFA 134a.

Fluorinated molecule	Maximum concentration (% w/w)
1H,1H,2H,2H-Perfluorooctan-1-ol	C ≤21.7
4,4,4-Trifluorobutan-1ol	C ≤4.72
1H,1H,2H,3H,3H-Perfluoro-1,2-nonandiol	C ≤5.21
1H,1H-Perfluorononan-1-ol	C ≤4.87
n-Butyl-pentafluropropionate	C ≤11.43

PFOH was found to have the highest miscibility in HFA 134a (21.7% w/w) and was chosen as the fluorinated alcohol for further experimental work. The solubility of PEG-phospholipids in PFOH is listed in table 4.3. The concentration values listed in the second column indicate the maximum amount of each PEG-phospholipid that could be dissolved in the perfluoroalcohol.

Table 4.3 Solubility of selected PEG-phospholipids in PFOH.

Excipient	Maximum solubility (% w/w)
mPEG-DMPE MW 5000	5.12
mPEG-DSPE MW 5000	4.16
DSPE-PEG-DSPE MW 5000	4.06

From these results (tables 4.2 and 4.3), it was possible to devise suitable excipient combinations that form the novel formulations. The following nine combinations were assessed in this work (all drug material used was micronised):

1. budesonide with mPEG-DMPE MW 5000 and PFOH in HFA 134a;
2. formoterol fumarate with mPEG-DMPE MW 5000 and PFOH in HFA 134a;
3. terbutaline sulphate with mPEG-DMPE MW 5000 and PFOH in HFA 134a;
4. budesonide with mPEG-DSPE MW 5000 and PFOH in HFA 134a;
5. formoterol fumarate with mPEG-DSPE MW 5000 and PFOH in HFA 134a;
6. terbutaline sulphate with mPEG-DSPE MW 5000 and PFOH in HFA 134a;
7. budesonide with DSPE-PEG-DSPE MW 5000 and PFOH in HFA 134a;
8. formoterol fumarate with DSPE-PEG-DSPE MW 5000 and PFOH in HFA 134a;
9. terbutaline sulphate with DSPE-PEG-DSPE MW 5000 and PFOH in HFA 134a.

A range of control samples containing the drug only in HFA 134a with no additives was prepared to compare directly with the novel formulations (all drug material used was micronised): (1) budesonide in HFA 134a; (2) formoterol fumarate in HFA 134a; and (3) terbutaline sulphate in HFA 134a. Formulations 1-9 were prepared at 3 different concentrations of PFOH to study the influence of the fluorinated alcohol concentration. The aerosols prepared were coded as in table 4.4.

Table 4.4 Aerosol compositions of HFA suspension formulations.

Formulation*	Drug (% w/w)	PEG-phospholipid (% w/w)	Perfluorooctanol (% w/w)	HFA 134a (% w/w)
1-A	0.1	0.1	5	94.8
1-B	0.2	0.1	10	89.7
1-C	0.2	0.1	15	84.7
2-A	0.1	0.1	5	94.8
2-B	0.2	0.1	10	89.7
2-C	0.2	0.1	15	84.7
3-A	0.1	0.1	5	94.8
3-B	0.2	0.1	10	89.7
3-C	0.2	0.1	15	84.7

*Formulations 1-A to 1-C, 2-A to 2-C and 3-A to 3-C contain mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSP, respectively.

4.4.2 Assessment of formulations

4.4.2.1 Assessment of the extent of drug adhesion

The adhesion of drugs to the can walls was assessed visually. The level of drug adhesion was seen on the ring across the vial (adhesion at the propellant-gas interface). In the absence of adhesion, no ring was observed. The aerosol vials were placed against a dark background for visual assessment. The samples prepared in clear aerosol vials were observed after 2 h storage at room temperature (21 ± 2°C). They were shaken to enable re-dispersion of the creamed or sedimented layer. It was observed that budesonide samples creamed and showed some drug adhesion; whereas samples prepared with formoterol fumarate and terbutaline sulphate tended to sediment, thus showing very little adhesion in the head space due to clarification of the top layer. This was mostly due to density difference. Propellant HFA 134a, budesonide, formoterol fumarate and terbutaline sulphate have respective densities of 1.21 g/ml (Byron *et al.*, 1994), 1.24 g/ml (De Boer, 2005), 1.3 g/ml (Slowey *et al.*, 2005), and 1.28 g/ml (Trofast, 1999). Since all three drugs have higher densities than HFA 134a, they are expected to sediment in the propellant medium. It has been reported previously that budesonide particles cream when suspended in HFA 134a. The observed creaming of budesonide however could be due to a slightly lower density for the batch used in these experiments (ρ < 1.24 g/ml) than that reported in literature (De Boer, 2005). As stated earlier, compounds were used as received, with no further micronisation work or sizing measurements. The true density of budesonide powder was determined at a later stage by buoyancy method (Low and Richards, 1952). Powder samples (1-2 mg) were placed in a density gradient liquid and centrifuged at 3500 rpm and 5°C for 30 min. The particle density was equal to the liquid density when the particles remained stationary in the liquid after centrifugation and was found to be 1.19 g/ml (direct communication with AstraZeneca, Charnwood). This is in agreement with results obtained in this study.

Drug formulations in PFOH systems were compared with controls containing drug only in HFA 134a (with no added PEG-phospholipids or PFOH). In all cases, there was virtually no drug on the can wall for the PFOH-based formulation samples. These systems showed a significantly improved performance over their respective control samples by reducing the amount of drug adhesion to the wall of the can. Drug adhesion was observed in all the control samples; two types of which were present: (1) head space adhesion, where the particles are spread in the whole head space area; (2) adhesion at the propellant-gas interface, also known as ring adhesion. In the fluoroalcohol-based formulations however, the first kind of adhesion was not present. The ring adhesion did exist in some of the budesonide samples, but was very faint. There was no drug adhesion observed in all fluoroalcohol-based formoterol fumarate and terbutaline sulphate samples. Since these

formulations showed a tendency to sediment, the absence of drug adhesion could be due to the fact that the particles were not at the interface, and remained wetted in the liquid, hence they were not likely to adhere in the head space and form a dry ring or surface coating similar to those observed in budesonide samples.

4.4.2.2 Assessment of the phase separation kinetics of the novel formulations

PEG-phospholipids were insoluble in HFA 134a even at concentrations as low as 0.05% w/w and separated very rapidly due to their unfavourable thermodynamic state. PFOH enhanced their solubility and led to the formation of a clear one-phase system. When drug was added to the ternary PEG-phospholipid/ PFOH / propellant system, budesonide formulations creamed whereas formoterol fumarate and terbutaline sulphate formulations sedimented due to the density difference between the particles and the propellant. Control drug suspensions with no added stabilisers had much reduced stability and took a few seconds to a few minutes to be fully destabilised. The novel PFOH formulations, however, took much longer to separate. It took 4-8 h to form a separate solid phase layer with 10% w/w PFOH. This is an advantage of the use of PFOH as a stabiliser for HFA suspension formulations. The level of stability was dependent on the concentration of PFOH in the formulation. In all PFOH-containing systems, the onset of detectable creaming for budesonide samples or sedimentation for formoterol fumarate and terbutaline sulphate formulations was in excess of 8 h, 24 h and 24 h respectively, at 15% w/w PFOH. This is beyond the time scale observed in the control samples, where creaming or sedimentation occurred within a few seconds to a few minutes after shaking.

As well as assessing the phase separation kinetics of the PFOH formulations visually, measurements were carried out using the Turbiscan to study slow phase separation kinetics. Throughout the experiments, all fluoroalcohol-containing suspension formulations were opaque and milky (T and BS profiles at t=0 were subtracted from all the others to better visualise the signal modifications as a function of time), indicating finely dispersed and stable formulations. Figures 4.7-4.11 show the Turbiscan MA 2000 screen page illustrating transmission (T) and backscattering (BS) profiles as a function of the height of the sample (30 selected scans, one every min) for budesonide, formoterol fumarate and terbutaline sulphate suspensions stabilised with PFOH and mPEG-DMPE or mPEG-DSPE in HFA 134a. The upper Y-axis and the lower Y-axis represent the transmission and backscattering intensities in per cent relative to an external standard (suspension of polystyrene latex beads: particle diameter d = 0.3 μm and volume fraction Φ = 10%), respectively. The X-axis represents the sample height in mm (h = 0 mm corresponds to the measurement cell bottom). So as to visualise modifications occurring as a function of time

the first profile was deducted from all the others. Therefore, at time t = 0, the first profile is on the line BS = 0%. The influence of PFOH was investigated at constant PEG-phospholipid/drug volume fraction: 0.655% w/w. PFOH successfully stabilised the drug suspension, the lowest concentration used was 5% w/w. T and BS signals remained close to zero and 30-50%, respectively. The phase separation observed with the drugs used was creaming or sedimentation after 1 h, at 5% w/w PFOH, the rate of which decreased with increasing cosolvent concentration. The drug particles in formoterol fumarate and terbutaline sulphate formulations migrate from the top to the bottom of the sample which induces a progressive fall in concentration at the sample top (clarification) and therefore a fall in backscattering in good agreement with the theoretical model (BS decreases when Φ decreases). Furthermore, it induces a progressive increase in concentration at the sample bottom and therefore a backscattering increase that is characteristic of the sediment formation. Budesonide drug particles migrate from the bottom to the top of the sample which induces a progressive fall in concentration at the sample bottom and therefore a fall in backscattering and a progressive increase in concentration at the sample top and therefore a backscattering increase that is characteristic of creaming phenomenon.

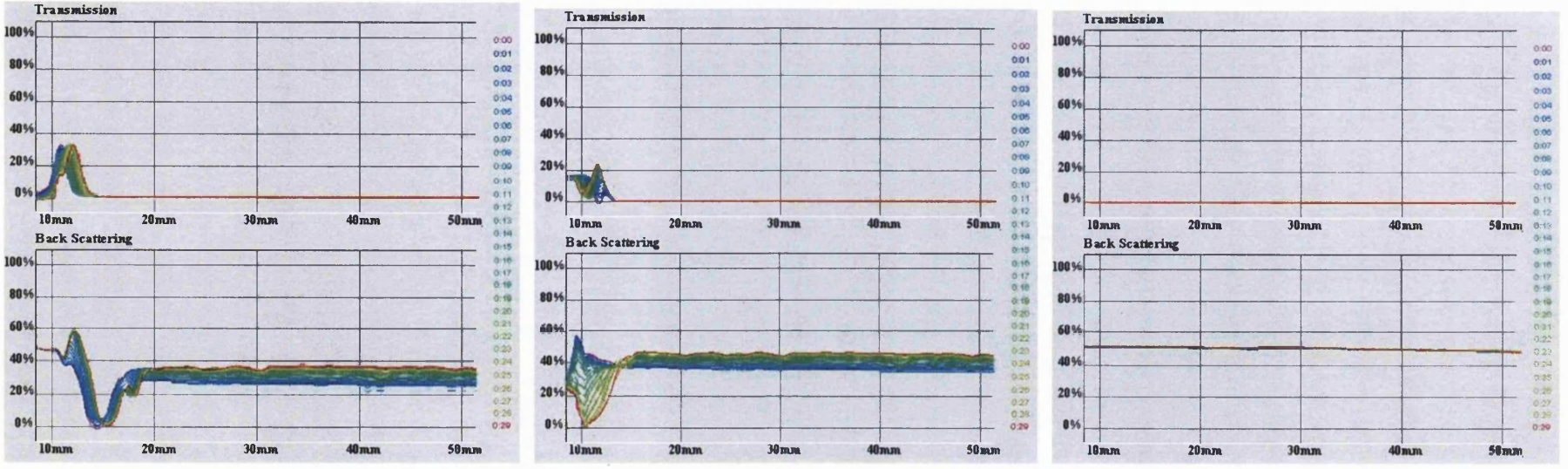


Figure 4.7 *Transmission and backscattering data for an HFA 134a pMDI suspension formulation containing: (a) 0.1% w/w mPEG-DMPE, 0.2% w/w budesonide and 5% w/w PFOH; (b) 0.1% w/w mPEG-DMPE, 0.2% w/w budesonide and 10% w/w PFOH; and (c) 0.1% w/w mPEG-DMPE, 0.2% w/w budesonide and 15% w/w PFOH.*

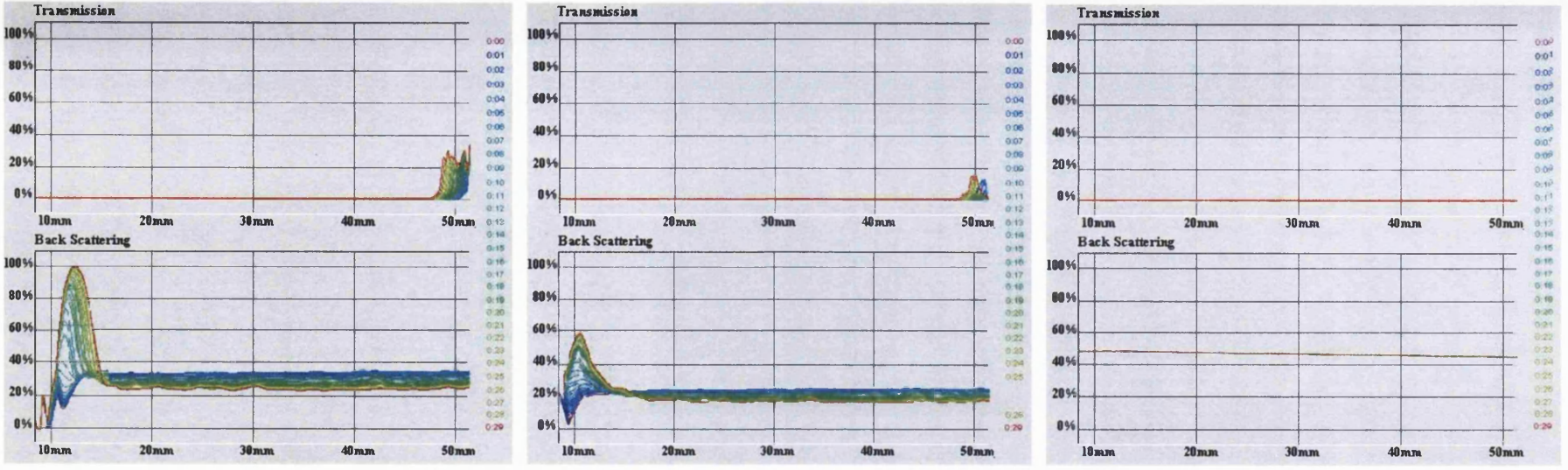


Figure 4.8 *Transmission and backscattering data for an HFA 134a pMDI suspension formulation containing: (a) 0.1% w/w mPEG-DMPE, 0.2% w/w formoterol fumarate and 5% w/w PFOH; (b) 0.1% w/w mPEG-DMPE, 0.2% w/w formoterol fumarate and 10% w/w PFOH; and (c) 0.1% w/w mPEG-DMPE, 0.2% w/w formoterol fumarate and 15% w/w PFOH.*

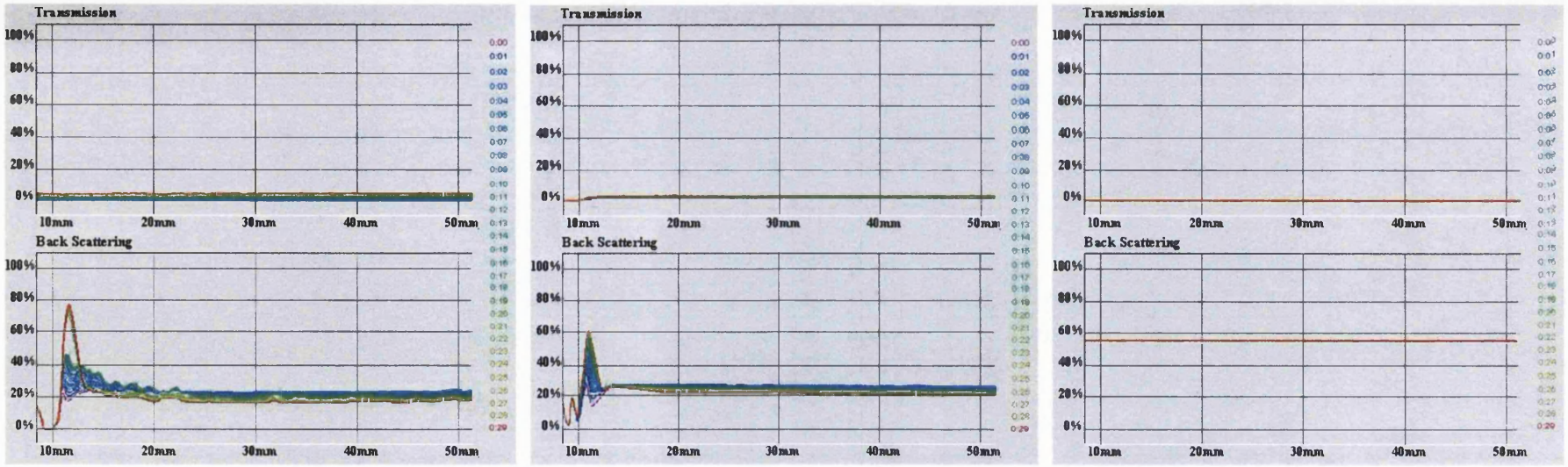


Figure 4.9 *Transmission and backscattering data for an HFA 134a pMDI suspension formulation containing: (a) 0.1% w/w mPEG-DMPE, 0.2% w/w terbutaline sulphate and 5% w/w PFOH; (b) 0.1% w/w mPEG-DMPE, 0.2% w/w terbutaline sulphate and 10% w/w PFOH; and (c) 0.1% w/w mPEG-DMPE, 0.2% w/w terbutaline sulphate and 15% w/w PFOH.*

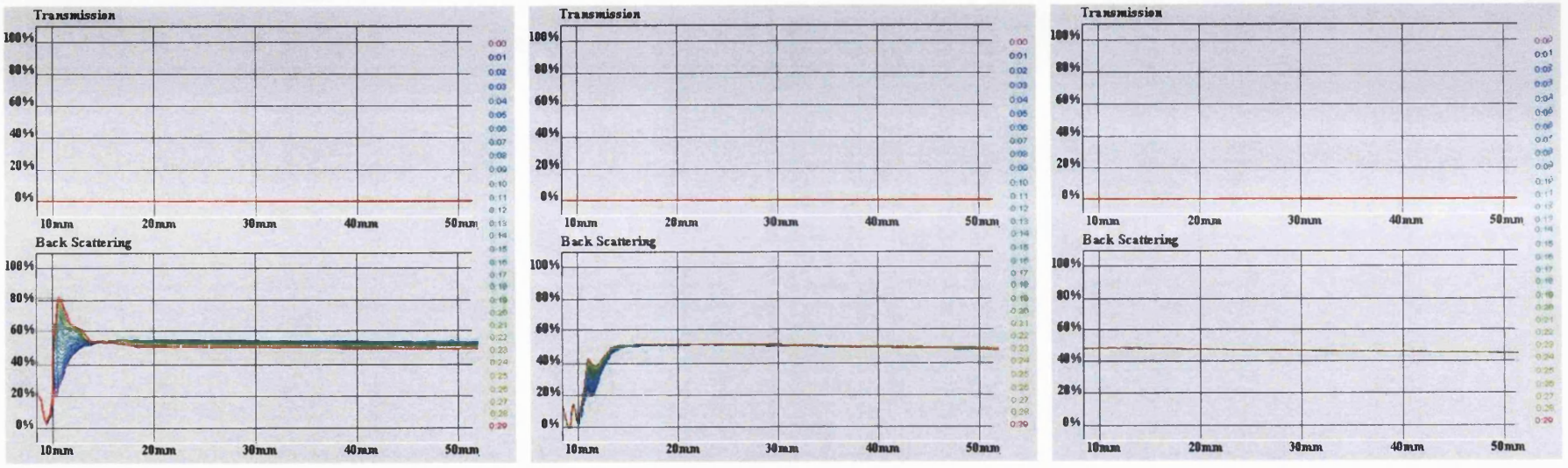


Figure 4.10 *Transmission and backscattering data for an HFA 134a pMDI suspension formulation containing: (a) 0.1% w/w mPEG-DSPE, 0.2% w/w formoterol fumarate and 5% w/w PFOH; (b) 0.1% w/w mPEG-DSPE, 0.2% w/w formoterol fumarate and 10% w/w PFOH; and (c) 0.1% w/w mPEG-DSPE, 0.2% w/w formoterol fumarate and 15% w/w PFOH.*

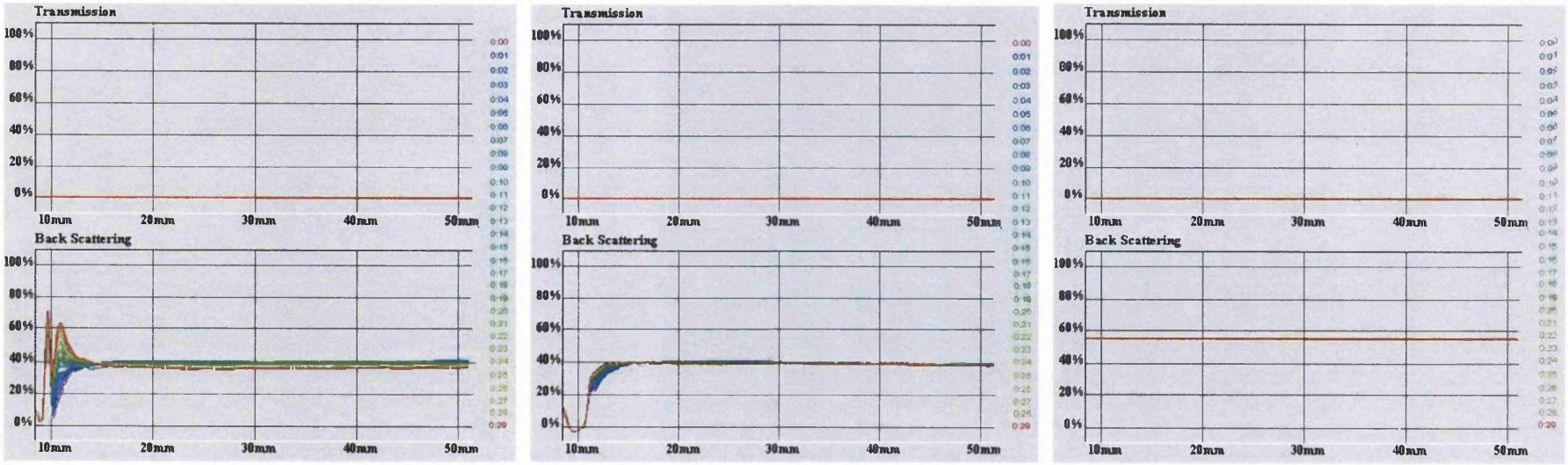


Figure 4.11 *Transmission and backscattering data for an HFA 134a pMDI suspension formulation containing: (a) 0.1% w/w mPEG-DSPE, 0.2% w/w terbutaline sulphate and 5% w/w PFOH; (b) 0.1% w/w mPEG-DSPE, 0.2% w/w terbutaline sulphate and 10% w/w PFOH; and (c) 0.1% w/w mPEG-DSPE, 0.2% w/w terbutaline sulphate and 15% w/w PFOH.*

The enhanced stability of HFA suspensions conferred by the combination of PFOH, PEG-phospholipids and HFA could be due to a change in the density of the formulations due to addition of the fluoroalcohol. The possibility of matching the density of propellant/cosolvent blends with that of micronised drugs could improve pMDI delivery efficiency. All formulations exhibited a tendency to sediment except for budesonide. Stability of budesonide formulations is reflected by the value of the creaming percentage used to assess formulations (Roland *et al.*, 2003). The value of the creaming volume percentage was calculated for each formulation using equation 4.8. According to this equation, it is worth noticing that a larger value of the parameter C is an indication of a more stable formulation.

$$C = 100\,\frac{(V_t - V_l)}{V_t} \qquad \textbf{Equation 4.8}$$

where; C is the creaming volume percentage, V_t (ml) is the total volume of the sample, V_l (ml) is the volume of the lower phase layer.

For the purpose of this work, another factor will be defined, S, which refers to the sedimentation volume percentage, calculated for formoterol fumarate and terbutaline sulphate formulations according to equation 4.9. Again, a large value of the parameter S is an indication of a more stable formulation.

$$S = 100\,\frac{(V_t - V_u)}{V_t} \qquad \textbf{Equation 4.9}$$

where; S is the sedimentation volume percentage, V_t (ml) is the total volume of the sample, V_u (ml) is the volume of the upper phase layer.

The creaming volume percentage as a function of time for budesonide formulations is shown in figure 4.12. The sedimentation volume percentage as a function of time for formoterol fumarate and terbutaline sulphate formulations is shown in figures 4.13 and 4.14. No change can be macroscopically observed in formoterol fumarate and terbutaline sulphate formulations over 1 h, 4 h and 24 h at 5, 10 and 15% w/w PFOH, respectively. Budesonide formulations showed no decrease in creaming volume percentage over 1h, 4h and 8h at 5, 10 and 15% w/w PFOH, respectively. Unchanged creaming or sedimentation volume percentage (100%) reflects system physical stability.

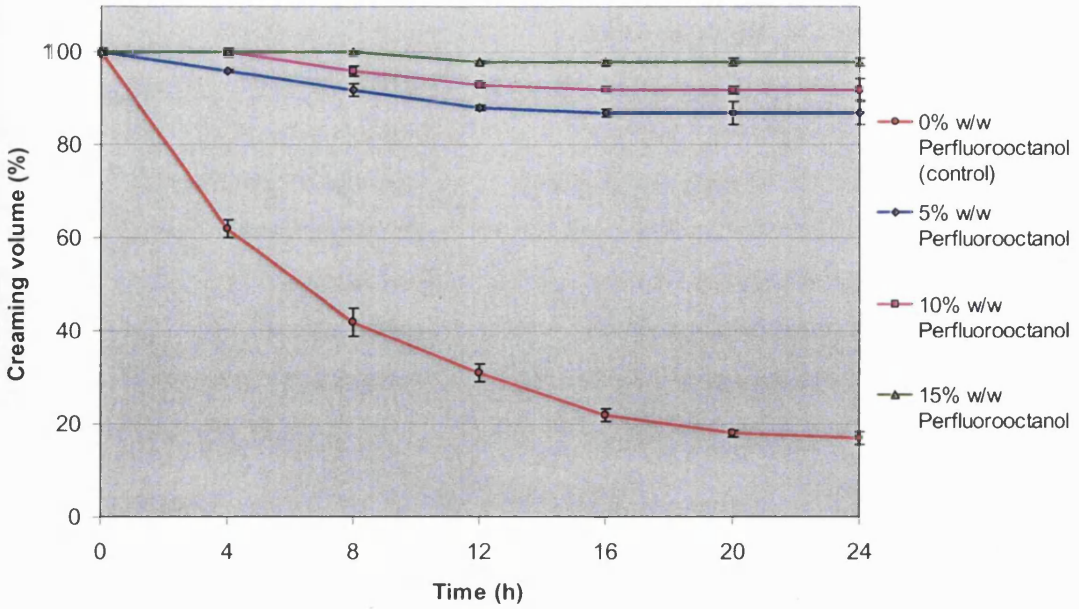


Figure 4.12 *Creaming volume percentage determined macroscopically for budesonide formulations at 0 (control), 5, 10 and 15% w/w PFOH over 24 h at room temperature (21 ± 2°C). Perfluoroalcohol-based formulations contain mPEG-DMPE at 0.1% w/w; control formulations contain HFA 134a and no other excipients (n=3 ±SD).*

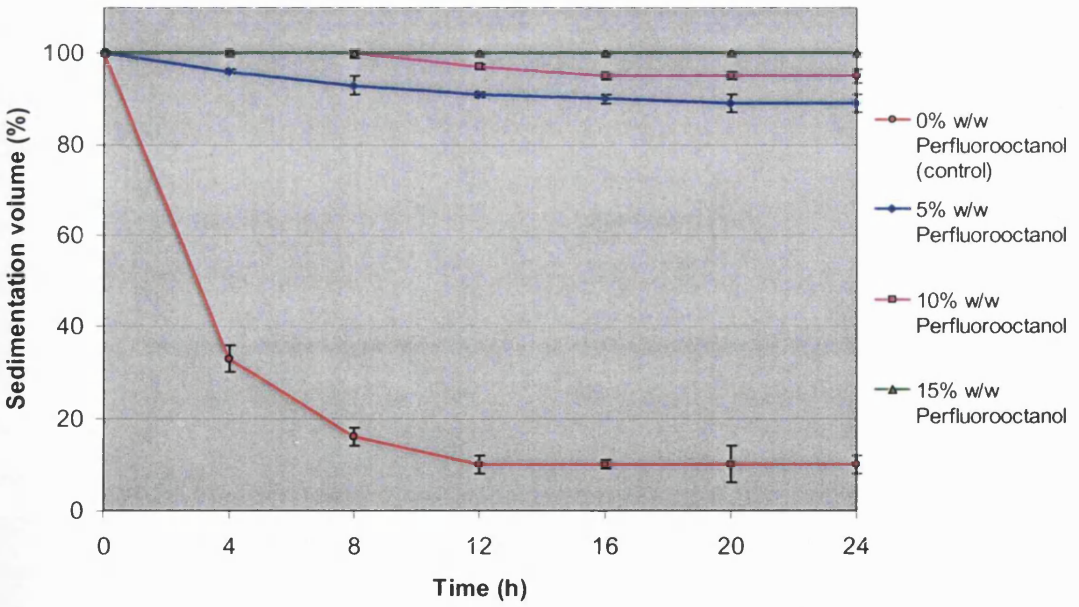


Figure 4.13 *Sedimentation volume percentage determined macroscopically for formoterol fumarate formulations at 0 (control), 5, 10 and 15% w/w PFOH over 24 h at room temperature (21 ± 2°C). Perfluoroalcohol-based formulations contain mPEG-DMPE at 0.1% w/w; control formulations contain HFA 134a and no other excipients (n=3 ±SD).*

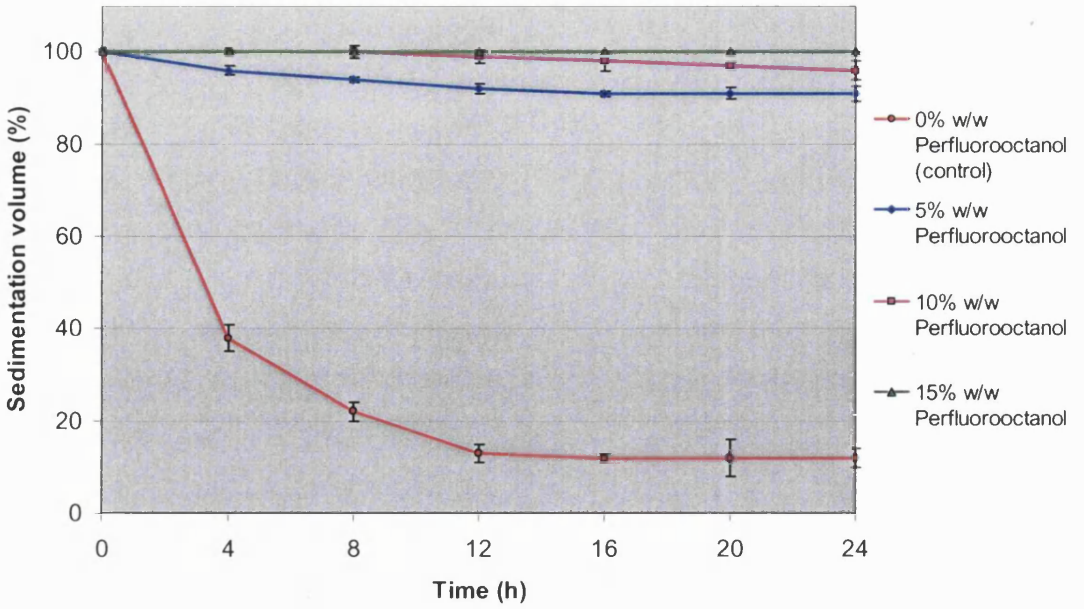


Figure 4.14 *Sedimentation volume percentage determined macroscopically for terbutaline sulphate formulations at 0 (control), 5, 10 and 15% w/w PFOH over 24 h at room temperature (21 ± 2°C). Perfluoroalcohol-based formulations contain mPEG-DMPE at 0.1% w/w; control formulations contain HFA 134a and no other excipients (n=3 ±SD).*

Creaming volume percentages were compiled for each storage condition: room temperature (21 ± 2°C), 4°C and 45°C over 24 h; and are shown in figures 4.15-4.17.

Creaming and sedimentation were observed only after 1-2 h at 4°C and 45°C for all formulations studied. If the formulation is intended to be stored at, or exposed to, high temperatures (storage in tropical countries, transport, etc) this test is critical. A whole analysis should be carried out again after storage of the formulation in the representative conditions (temperature, length of time). Storage at 4°C may be of importance in the case of formulations containing active substances likely to crystallise. This experiment is useful as it may reveal crystallisation or other phenomena likely to disrupt the stability of the system. The results suggest that the fluoroalcohol stabilising effects are not temperature-dependent since there was no great effect of temperature on stability over the temperature range investigated. The lack of temperature dependence can be interpreted as an indication of low enthalpy of dispersion in propellant HFA 134a, i.e. the enthalpy of particle aggregation is nearly compensated by that due to mixing the particles, PFOH and propellant. Therefore, the dispersion process must be driven to a significant extent by gains in entropy. An advantage for this is that the problem of crystal growth during temperature-cycled storage may be diminished with the fluorinated cosolvent due to the reduced dependence of stability on temperature over the range usually considered.

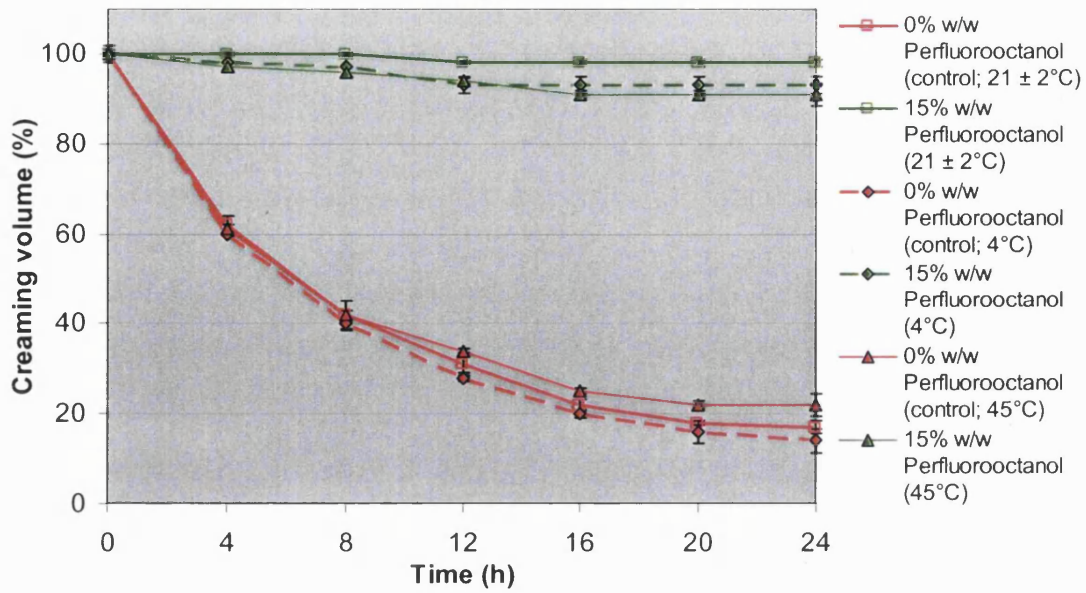


Figure 4.15 *Stability at room temperature (21 ± 2°C), 4°C and 45°C for budesonide formulations (n=3 ±SD).*

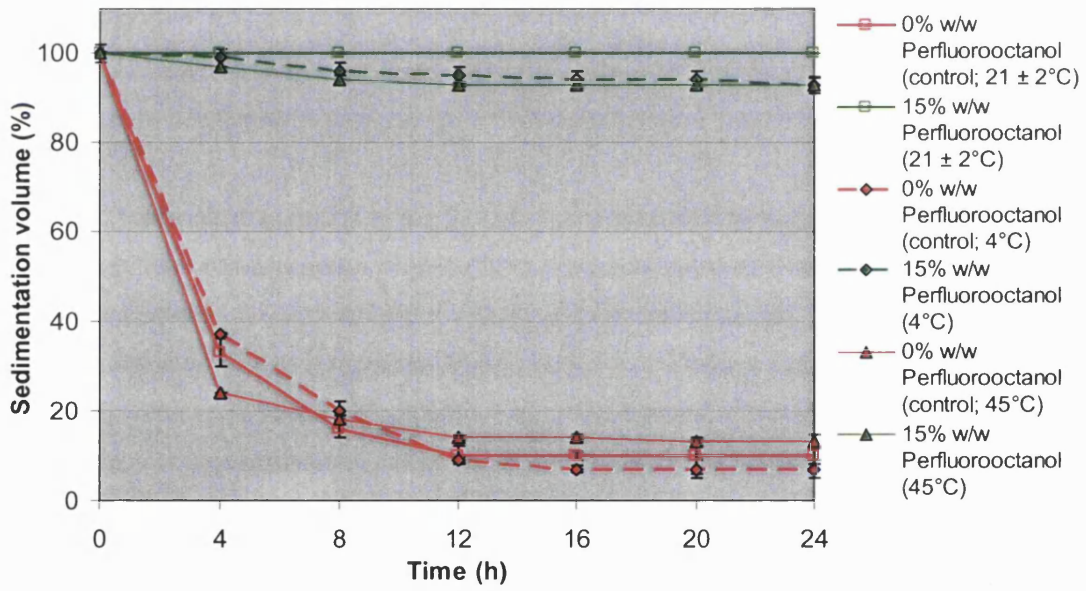


Figure 4.16 *Stability at room temperature (21 ± 2°C), 4°C and 45°C for formoterol fumarate formulations (n=3 ±SD).*

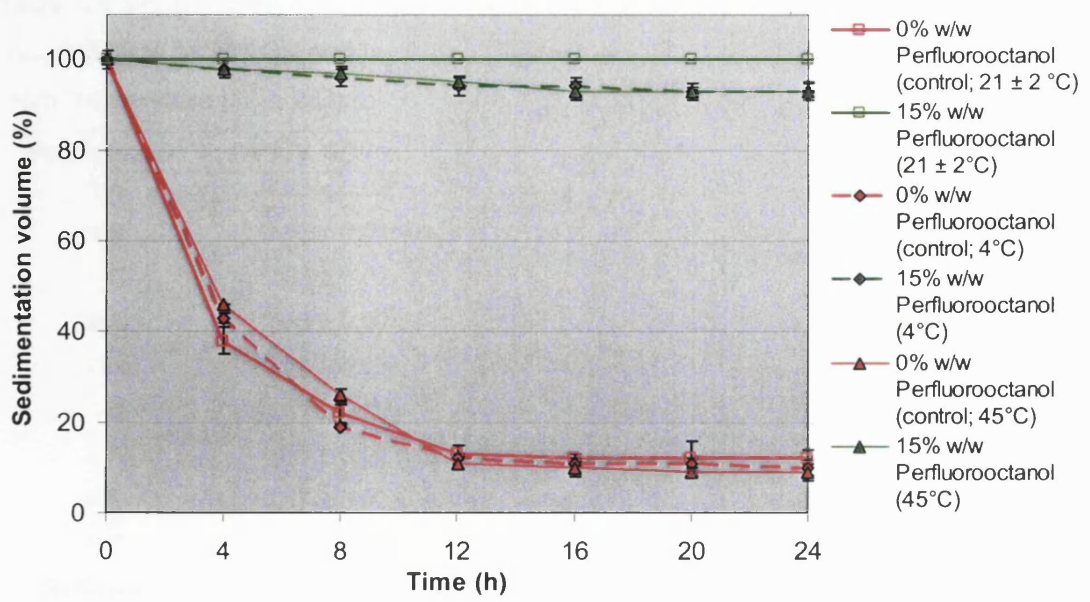


Figure 4.17 *Stability at room temperature (21 ± 2°C), 4°C and 45°C for terbutaline sulphate formulations (n=3 ±SD).*

4.4.2.3 Assessment of the particle size of novel formulations

Selected novel formulations were sized with a Malvern Spraytec to demonstrate the absence of flocculation. Each vial was shaken then primed by actuating three times to waste. Three tests of five actuations each were conducted for each vial. Budesonide, formoterol fumarate and terbutaline sulphate-based formulations were studied. All drugs were micronised. The systems were formulated with mPEG-DMPE MW 5000, mPEG-DSPE MW 5000 and DSPE-PEG-DSPE MW 5000 in HFA 134a. The results could then be compared with sizing results of the same drugs in reference HFA formulations. The sizing results have been summarised in tables 4.5-4.7. It was observed that the fluoroalcohol-based formulations had a milky appearance, i.e. they were fine suspensions, compared to the controls that were coarser (particles were easily seen in the samples).

Briefly, budesonide, formoterol fumarate and terbutaline sulphate were sized in 9 (3x3) formulations each. Each formulation containing a PEG-phospholipid; mPEG-DMPE MW 5000, mPEG-DSPE MW 5000 or DSPE-PEG-DSPE MW 5000 as well as PFOH at 5, 10 or 15% w/w. The HFA formulation used as a reference was based on the drug dispersed in HFA 134a alone. The experimental concentrations are listed in table 4.4 and the sizing results in tables 4.5-4.7. Span is calculated as [Dv90 – Dv10]/Dv50.

Table 4.5 Spraytec results for budesonide suspension pMDI formulations containing PEG-phospholipids and PFOH and budesonide in HFA 134a suspension after 4 days storage at room temperature (21 ± 2°C) (n=3 ±SD).

Formulation[1]	Dv10 ± SD (µm)	Dv50 ± SD (µm)	Dv90 ± SD (µm)	Span ± SD (µm)
1-A	0.53 ± 0.02	2.63 ± 0.04	5.53 ± 0.06	1.90 ± 0.02
1-B	0.56 ± 0.02	2.99 ± 0.03	6.67 ± 0.05	2.04 ± 0.02
1-C	0.60 ± 0.02	3.56 ± 0.04	7.85 ± 0.03	2.04 ± 0.02
2-A	0.55 ± 0.02	2.66 ± 0.04	5.45 ± 0.04	1.84 ± 0.02
2-B	0.58 ± 0.02	2.89 ± 0.03	6.85 ± 0.03	2.17 ± 0.02
2-C	0.61 ± 0.02	3.37 ± 0.04	7.98 ± 0.05	2.19 ± 0.02
3-A	0.54 ± 0.02	2.64 ± 0.04	5.53 ± 0.06	1.89 ± 0.02
3-B	0.57 ± 0.02	3.00 ± 0.04	6.61 ± 0.03	2.01 ± 0.02
3-C	0.60 ± 0.02	3.49 ± 0.04	7.92 ± 0.02	2.10 ± 0.02
Reference	7.64 ± 0.02	38.12 ± 0.04	93.73 ± 0.09	2.26 ± 0.02

[1] Compositions shown in table 4.4.

The results show that micronised budesonide formulated with PEG-phospholipids and PFOH had a narrower size distribution than the reference HFA formulation, and the particles have a smaller measured average size. This is due to the fact that the novel formulations are finely dispersed in which particles do not aggregate and exist as individual particles rather than clusters. This has some effect on the performance of the pMDI and the ex-valve dose is expected to be finer as well. A finely dispersed suspension is a good indicator of efficient suspending agents. The suspensions are well stabilised by the added excipients.

Table 4.6 Spraytec results for formoterol fumarate suspension pMDI formulations containing PEG-phospholipids and PFOH and formoterol fumarate in HFA 134a suspension at after 4 days storage at room temperature (21 ± 2°C) (n=3 ±SD).

Formulation[1]	Dv10 ± SD (µm)	Dv50 ± SD (µm)	Dv90 ± SD (µm)	Span ± SD (µm)
1-A	0.66 ± 0.02	2.32 ± 0.03	8.33 ± 0.06	3.30 ± 0.03
1-B	0.70 ± 0.02	2.68 ± 0.04	9.67 ± 0.09	3.35 ± 0.02
1-C	0.73 ± 0.02	3.01 ± 0.04	11.58 ± 0.09	3.61 ± 0.02
2-A	0.68 ± 0.02	2.45 ± 0.03	8.65 ± 0.08	3.26 ± 0.02
2-B	0.70 ± 0.02	2.70 ± 0.04	9.84 ± 0.08	3.39 ± 0.02
2-C	0.73 ± 0.02	3.08 ± 0.04	11.88 ± 0.10	3.61 ± 0.02
3-A	0.68 ± 0.02	2.54 ± 0.03	8.61 ± 0.07	3.23 ± 0.02
3-B	0.70 ± 0.02	2.70 ± 0.04	9.40 ± 0.08	3.22 ± 0.02
3-C	0.73 ± 0.02	3.00 ± 0.04	11.64 ± 0.10	3.63 ± 0.02
Reference	4.04 ± 0.01	11.31 ± 0.03	135.21 ± 0.02	11.59 ± 0.02

[1] Compositions shown in table 4.4.

As for budesonide, the sizing results show that micronised formoterol fumarate in the new formulation has a narrower size distribution than the reference formulation, with the particles having a smaller mean size.

Table 4.7 Spraytec results for terbutaline sulphate suspension pMDI formulations containing PEG-phospholipids and PFOH and terbutaline sulphate in HFA 134a suspension after 4 days storage at room temperature (21 ± 2°C) (n=3 ±SD).

Formulation[1]	Dv10 ± SD (µm)	Dv50 ± SD (µm)	Dv90 ± SD (µm)	Span ± SD (µm)
1-A	1.43 ± 0.02	3.74 ± 0.03	7.12 ± 0.05	1.52 ± 0.02
1-B	1.49 ± 0.02	3.97 ± 0.03	8.67 ± 0.06	1.81 ± 0.02
1-C	1.54 ± 0.02	4.36 ± 0.03	10.55 ± 0.03	2.07 ± 0.02
2-A	1.46 ± 0.02	3.75 ± 0.03	7.15 ± 0.11	1.52 ± 0.02
2-B	1.50 ± 0.02	3.94 ± 0.03	8.99 ± 0.03	1.90 ± 0.02
2-C	1.55 ± 0.02	4.76 ± 0.03	11.11 ± 0.09	2.01 ± 0.02
3-A	1.48 ± 0.02	3.77 ± 0.03	7.14 ± 0.05	1.50 ± 0.02
3-B	1.51 ± 0.02	4.02 ± 0.04	9.01 ± 0.08	1.87 ± 0.02
3-C	1.55 ± 0.02	4.57 ± 0.03	10.73 ± 0.09	2.01 ± 0.02
Reference	5.47 ± 0.02	16.60 ± 0.04	56.34 ± 0.12	7.82 ± 0.02

[1] Compositions shown in table 4.4.

The sizing results show that micronised terbutaline sulphate in the PEG-phospholipid formulations has a narrower size distribution than the reference formulation, with the particle distribution centred on a smaller mean size.

For all drug suspensions investigated, the effect of adding PFOH resulted in a slight shift in particle size distribution to larger sizes (figures 4.18-4.20). For budesonide formulations, volume median diameter (VMD) increased from 2.63 ± 0.04 µm to 3.56 ± 0.04 µm ($P < 0.05$), from 2.66 ± 0.04 µm to 3.37 ± 0.04 µm ($P < 0.05$) and from 2.64 ± 0.04 µm to 3.49 ± 0.04 µm ($P < 0.05$) for mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively. For formoterol fumarate formulations, VMD increased from 2.32 ± 0.03 µm to 3.01 ± 0.04 µm, from 2.45 ± 0.03 µm to 3.08 ± 0.04 µm and from 2.54 ± 0.03 µm to 3.00 ± 0.04 µm for mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively. For terbutaline sulphate formulations, VMD increased from 3.74 ± 0.03 µm to 4.36 ± 0.03 µm, from 3.75 ± 0.03 µm to 4.76 ± 0.03 µm and from 3.77 ± 0.03 µm to 4.57 ± 0.03 µm for mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively. Whereas budesonide, formoterol fumarate and terbutaline sulphate control suspensions had respective VMD values of 38.12 ± 0.04 µm, 11.31 ± 0.03 µm and 16.60 ± 0.04 µm. This is due to lower vapour pressure resulting from addition of cosolvent, which in turn leads to larger droplet size distributions. Alcohols are known to have an effect on vapour pressure and emitted droplet size of pMDIs (Vervaet

and Byron, 1999). It may not be possible to detect this effect below certain concentrations (1-5% w/w) as particle diameters can be smaller for HFA 134a formulations containing smaller amounts of PFOH compared to those with high concentrations of cosolvent. Other reasons put forward for aerosol modifications include the modification of the dynamic surface tension of the HFAs by the kinetics of absorption of the excipients (Stein and Myrdal 2004). Incomplete evaporation of PFOH is another possible explanation.

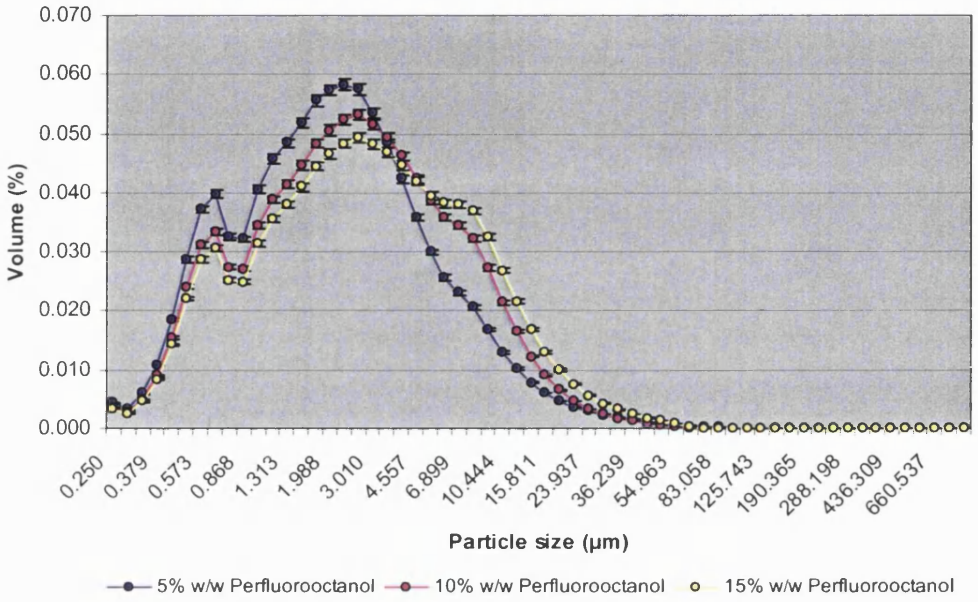


Figure 4.18 *Particle size distribution for 0.2% w/w budesonide suspension pMDIs containing: (1) 0.1% w/w mPEG-DSPE and 5% w/w PFOH; (2) 0.1% w/w mPEG-DSPE and 10% w/w PFOH; and (3) 0.1% w/w mPEG-DSPE and 15% w/w PFOH (n=3 ±SD).*

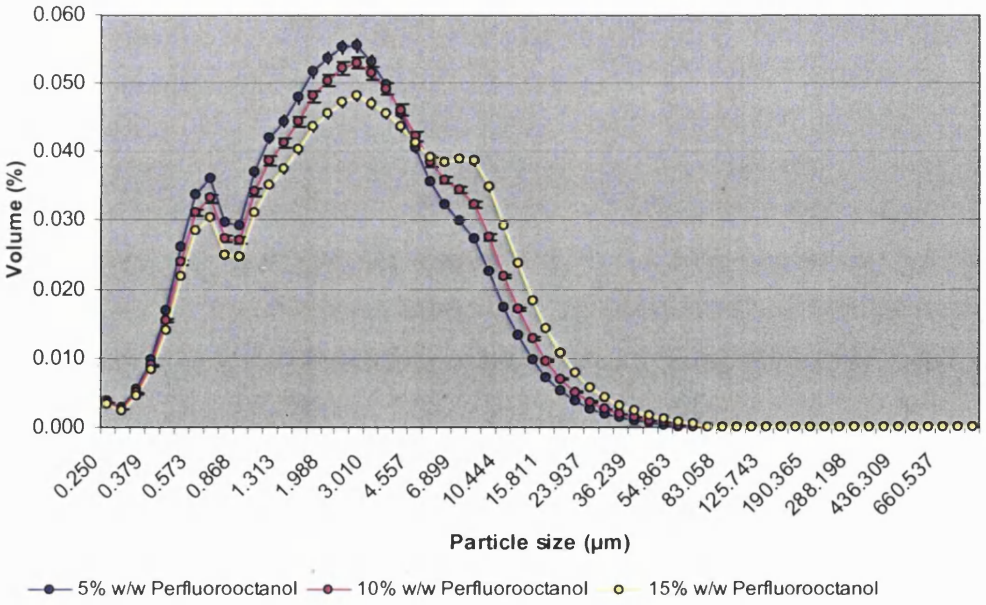


Figure 4.19 *Particle size distribution for 0.2% w/w formoterol fumarate suspension pMDIs containing: (1) 0.1% w/w mPEG-DSPE and 5% w/w PFOH; (2) 0.1% w/w mPEG-DSPE and 10% w/w PFOH; and (3) 0.1% w/w mPEG-DSPE and 15% w/w PFOH (n=3 ±SD).*

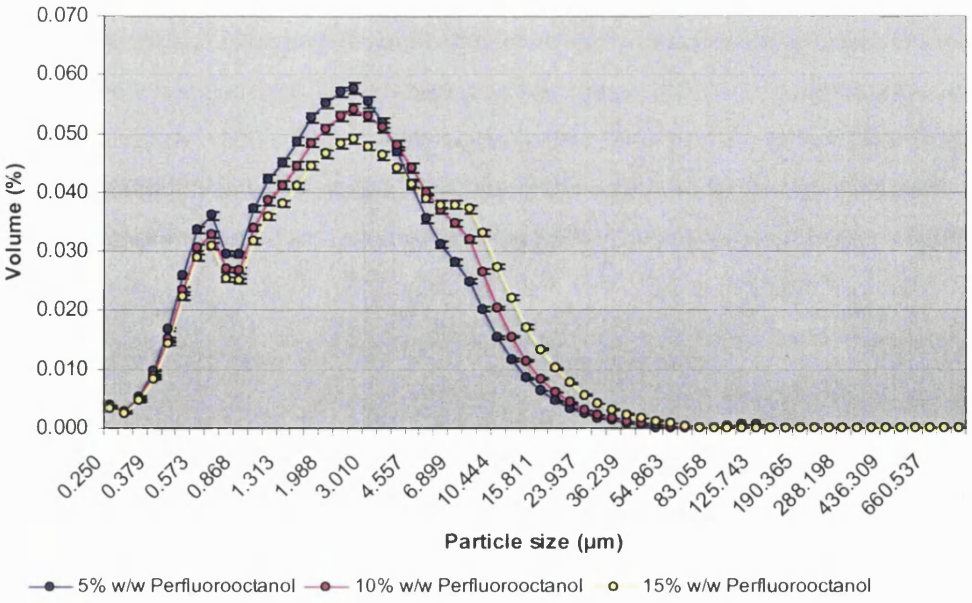


Figure 4.20 *Particle size distribution for 0.2% w/w terbutaline sulphate suspension pMDIs containing: (1) 0.1% w/w mPEG-DSPE and 5% w/w PFOH; (2) 0.1% w/w mPEG-DSPE and 10% w/w PFOH; and (3) 0.1% w/w mPEG-DSPE and 15% w/w PFOH (n=3 ±SD).*

4.4.3 *In vitro* characterisation

The evaluation of the suspensions aerosol spray performance was carried out according to the flow chart given in figure 4.21. First, the effect of aerosol composition, i.e. addition of mPEG-DMPE (composition 1), mPEG-DSPE (composition 2) and DSPE-PEG-DSPE (composition 3), on the spray performance was analysed. Further, suspensions A, B and C were compared regarding their spray performance, i.e. an assessment of the effect of cosolvent concentration in aerosols on aerodynamic size distribution (for compositions refer to table 4.4).

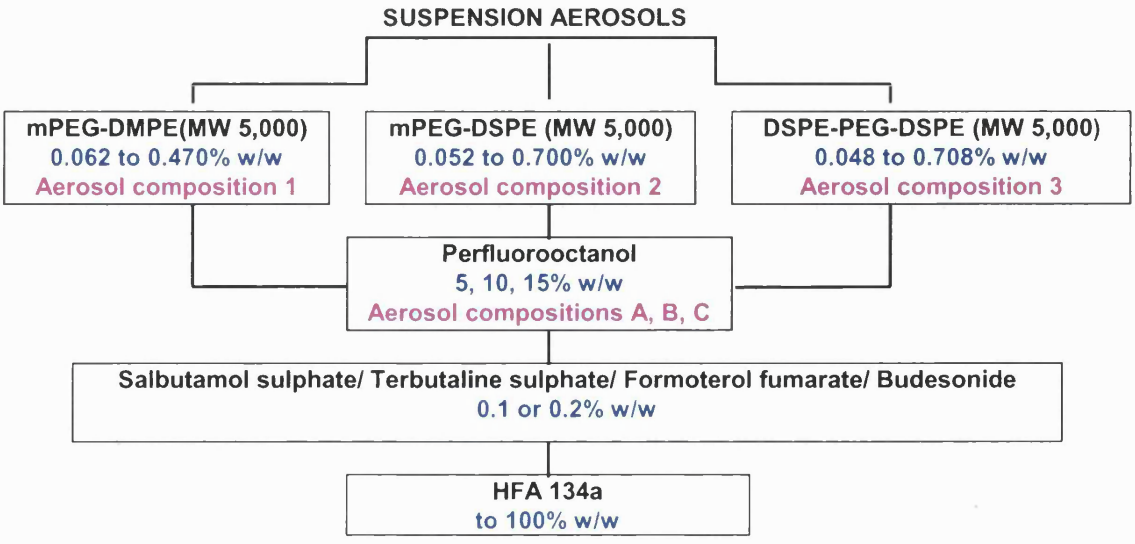


Figure 4.21 *Flow chart for aerodynamic size distribution analysis.*

The *in vitro* drug deposition profile of Becotide®, a micronised beclometasone dipropionate pMDI containing a blend of CFC-11 and CFC-12 was investigated using TI and ACI under the same experimental conditions as for investigation of the fluoroalcohol-based suspension formulations. The FPF for the commercial preparation Becotide® was found to be 55.7 ± 1.1% measured by TI. Becotide® is known to exhibit a high degree of physical stability during its shelf-life of 2 years. The FPF delivered by the Becotide® confirms that it is well formulated as a physically stable suspension. The recovery from the impaction apparatus using this methodology was determined to be 103.4 ± 1.8%. In contrast to the Becotide® formulation, the drug/HFA 134a pMDI controls deposited a very small proportion of their dose on stage 2 of the TI, that is a significant fraction of the drug particles were larger than an MMAD of 6.4 µm after aerosolisation of the dose into the impinger. The low FPF exhibited by the drug suspension (2.2 ± 0.4%, 3.6 ± 1.6% and 4.3 ± 1.4% for budesonide, formoterol fumarate and terbutaline sulphate, respectively) suggests that the drug particles are aggregating within the HFA solvent. This was confirmed by the Spraytec measurements (section 4.4.2.3), which showed that the cloud particle size of the drug particles was significantly higher in control samples ($P < 0.05$) upon suspension within HFA. The combination of PFOH and PEG-phospholipids, with addition of drugs gave a significantly larger stage 2 deposition in the TI compared to drug alone in HFA 134a ($P \leq 0.05$, ANOVA). The stage 2 depositions from the mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE HFA suspensions were not significantly different ($P > 0.05$, ANOVA) with approximately 70-80% of the emitted particles depositing in this part of the apparatus. The high FPF implies that the fluoroalcohol-based suspensions displayed a high degree of physical stability. Therefore the addition of PFOH to the ternary drug/PEG-phospholipid/ PFOH suspensions resulted in both enhanced stage 2 deposition and physical stability of the pMDI formulation compared to the drug/HFA 134a blend (table 4.8). Size measurement using the Spraytec suggested an increase in cloud particle size in the suspension with increasing fluoroalcohol concentration. Whilst the particle size of all drug suspensions at 15% w/w fluoroalcohol did increase significantly ($P < 0.05$, ANOVA) upon suspension within the HFA solvent, the median volume based diameter of the HFA drug PFOH suspensions, Dv50, was still under 5 µm for the drug suspensions at all levels of PFOH investigated inferring a high degree of physical suspension stability (table 4.8).

The effect of the type of pegylated phospholipid (mPEG-DSPE, mPEG-DSPE, DSPE-PEG-DSPE) incorporated in the formulation on the aerosol spray performance with respect to the MMAD and FPF was investigated by comparing compositions 1, 2 and 3 and that of adding PFOH at different levels by comparing compositions A, B and C. Figures 4.22-4.24 and 4.25-4.27 show a comparison of the drug deposition profiles of compositions 1, 2 and 3 and A, B and C, respectively, for budesonide, formoterol fumarate and terbutaline sulphate

aerosol samples. Calculated particle size distribution parameters, i.e. MMAD and GSD as well as FPF and percentage recovery of each aerosol are listed in table 4.9.

Table 4.8 Cloud particle size and FPF of the suspension formulations (n = 3 ± SD).

Formulation [a]	Dv50 ± SD (µm) [b]	TI FPF (%)[c]
Becotide®	-	55.7 ± 1.1
B 1-A	2.63 ± 0.04	84.5 ± 1.3
B 1-B	2.99 ± 0.03	79.3 ± 1.5
B 1-C	3.56 ± 0.04	69.4 ± 2.2
B 2-A	2.66 ± 0.04	86.3 ± 1.6
B 2-B	2.89 ± 0.03	76.4 ± 0.9
B 2-C	3.37 ± 0.04	68.2 ± 1.1
B 3-A	2.64 ± 0.04	83.9 ± 1.5
B 3-B	3.00 ± 0.04	76.2 ± 2.4
B 3-C	3.49 ± 0.04	69.8 ± 2.1
B Control	38.12 ± 0.04	2.2 ± 0.4
FF 1-A	2.32 ± 0.03	86.4 ± 1.5
FF 1-B	2.68 ± 0.04	80.6 ± 1.8
FF 1-C	3.01 ± 0.04	73.1 ± 2.7
FF 2-A	2.45 ± 0.03	84.0 ± 2.3
FF 2-B	2.70 ± 0.04	77.4 ± 2.8
FF 2-C	3.08 ± 0.04	72.4 ± 1.2
FF 3-A	2.54 ± 0.03	84.9 ± 2.0
FF 3-B	2.70 ± 0.04	78.5 ± 2.6
FF 3-C	3.00 ± 0.04	73.0 ± 1.9
FF Control	11.31 ± 0.03	3.6 ± 1.6
TS 1-A	3.74 ± 0.03	85.3 ± 1.6
TS 1-B	3.97 ± 0.03	77.9 ± 1.7
TS 1-C	4.36 ± 0.03	70.5 ± 2.6
TS 2-A	3.75 ± 0.03	88.6 ± 3.2
TS 2-B	3.94 ± 0.03	75.7 ± 2.6
TS 2-C	4.76 ± 0.03	69.0 ± 1.5
TS 3-A	3.77 ± 0.03	82.6 ± 2.6
TS 3-B	4.02 ± 0.04	75.1 ± 2.2
TS 3-C	4.57 ± 0.03	69.3 ± 1.8
TS Control	16.60 ± 0.04	4.3 ± 1.4

[a]1,2,3=mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively; A,B,C= PFOH at 5, 10 and 15% w/w, respectively. B,FF,TS=budesonide, formoterol fumarate and terbutaline sulphate, respectively. [b]Dv50 is the Spraytec 50th percentile value. [c]TI FPF is the % of the drug that has deposited in stage 2 of the twin impinger.

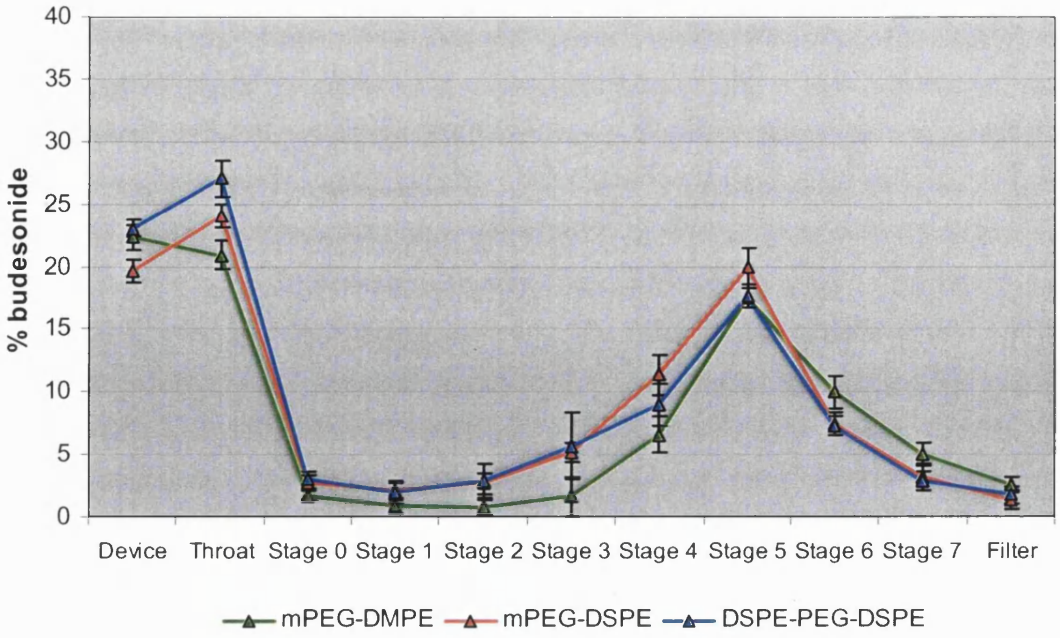


Figure 4.22 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing budesonide (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and 5% w/w PFOH (compositions 1, 2 and 3) (n=3 ±SD).*

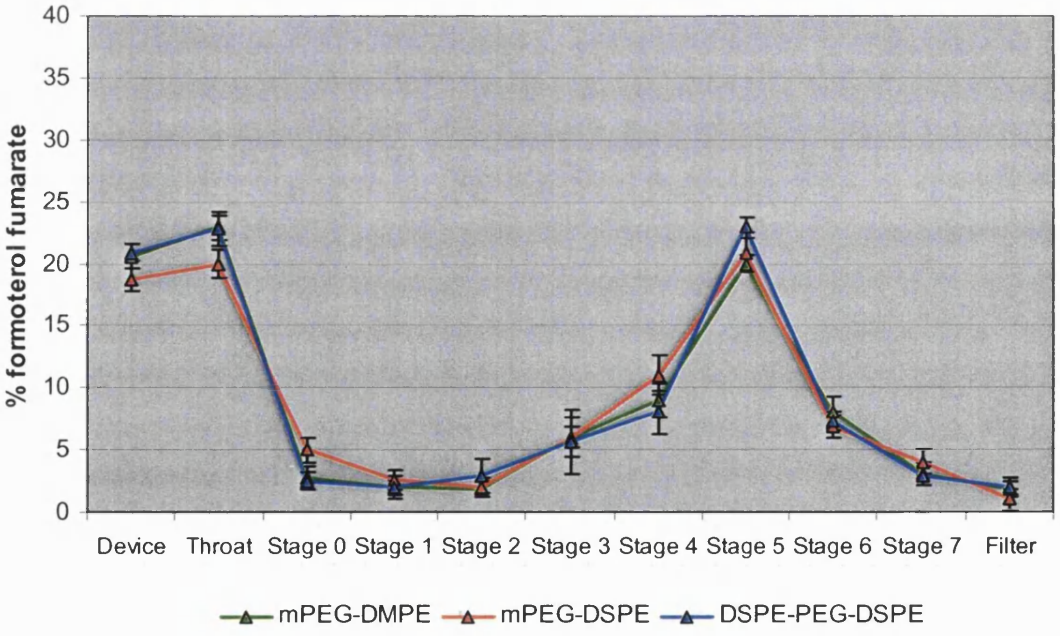


Figure 4.23 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing formoterol fumarate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and 5% w/w PFOH (compositions 1, 2 and 3) (n=3 ±SD).*

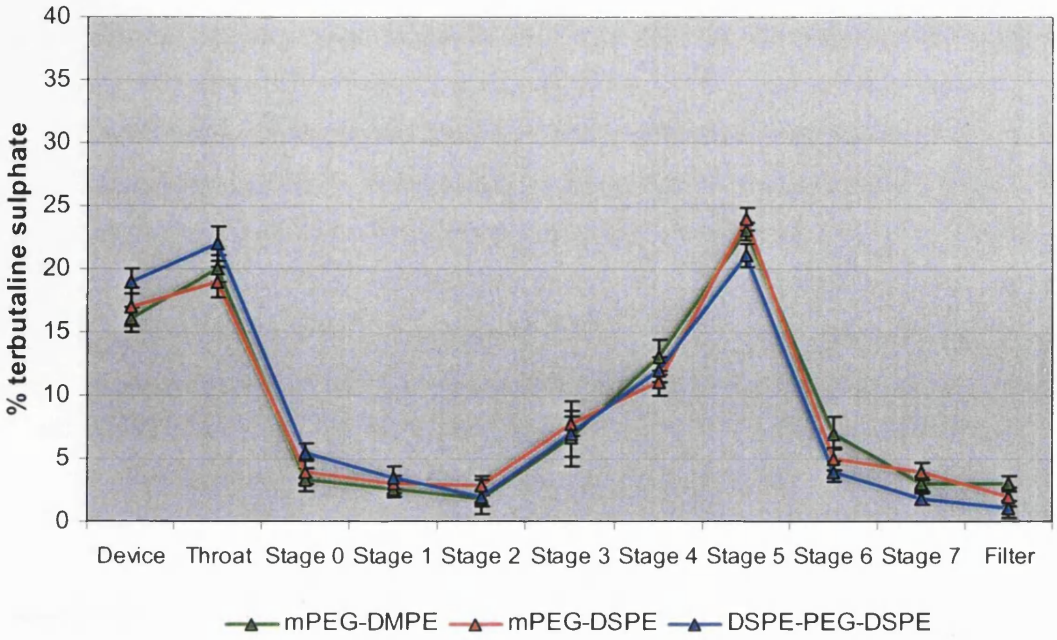


Figure 4.24 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing terbutaline sulphate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and 5% w/w PFOH (compositions 1, 2 and 3) (n=3 ±SD).*

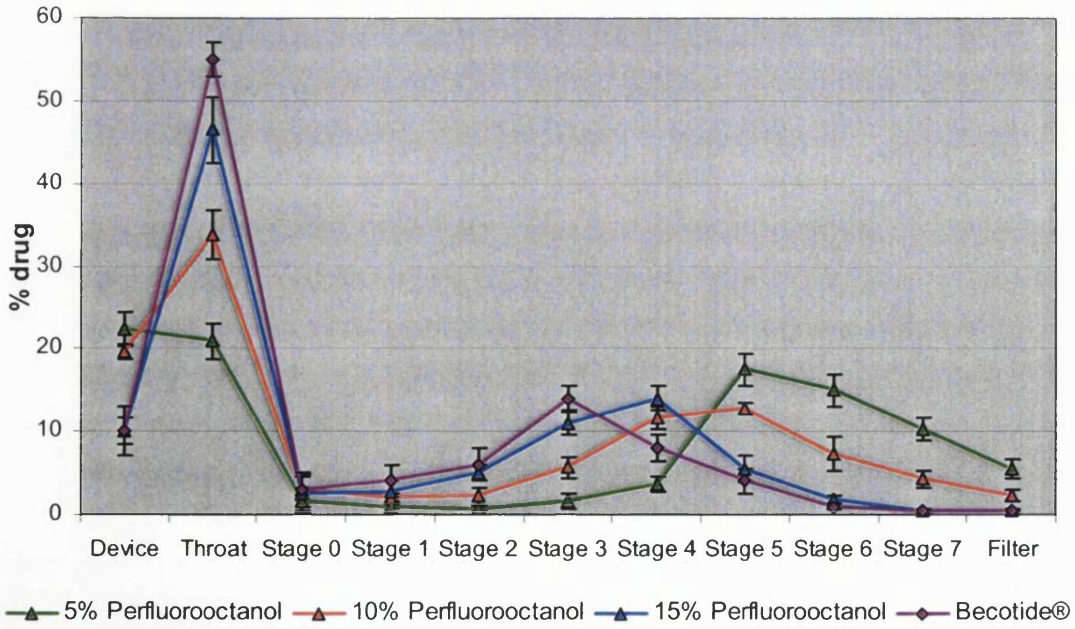


Figure 4.25 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing budesonide at 0.1% w/w, mPEG-DMPE at 0.13% w/w and PFOH at 5, 10 or 15% w/w (compositions A, B and C) (n=3 ±SD).*

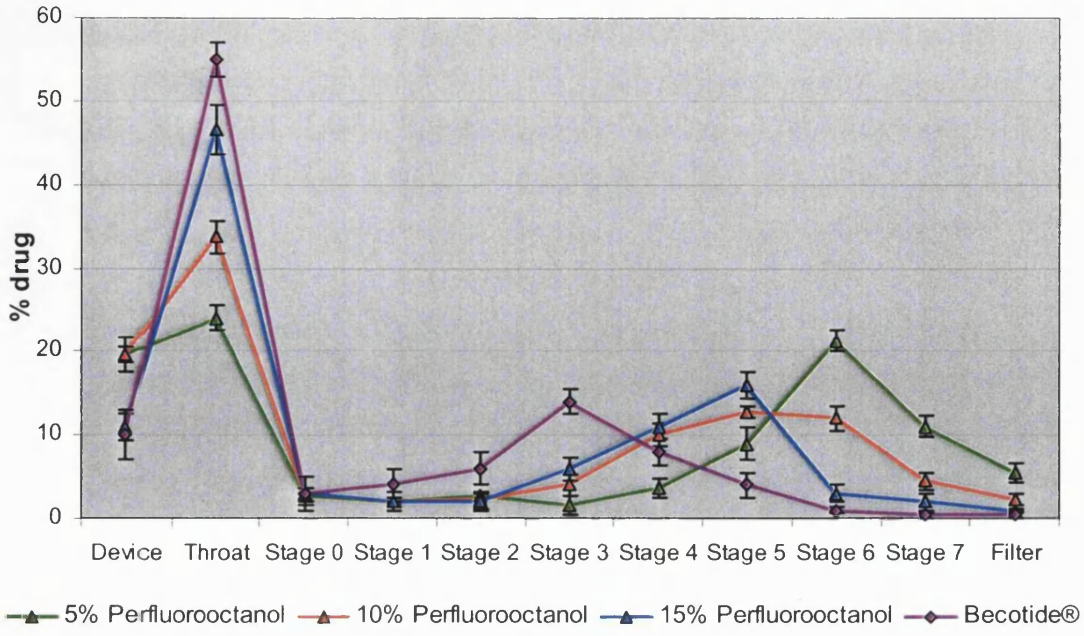


Figure 4.26 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing formoterol fumarate at 0.1% w/w, mPEG-DMPE at 0.13% w/w and PFOH at 5, 10 or 15% w/w (compositions A, B and C) (n=3 ±SD).*

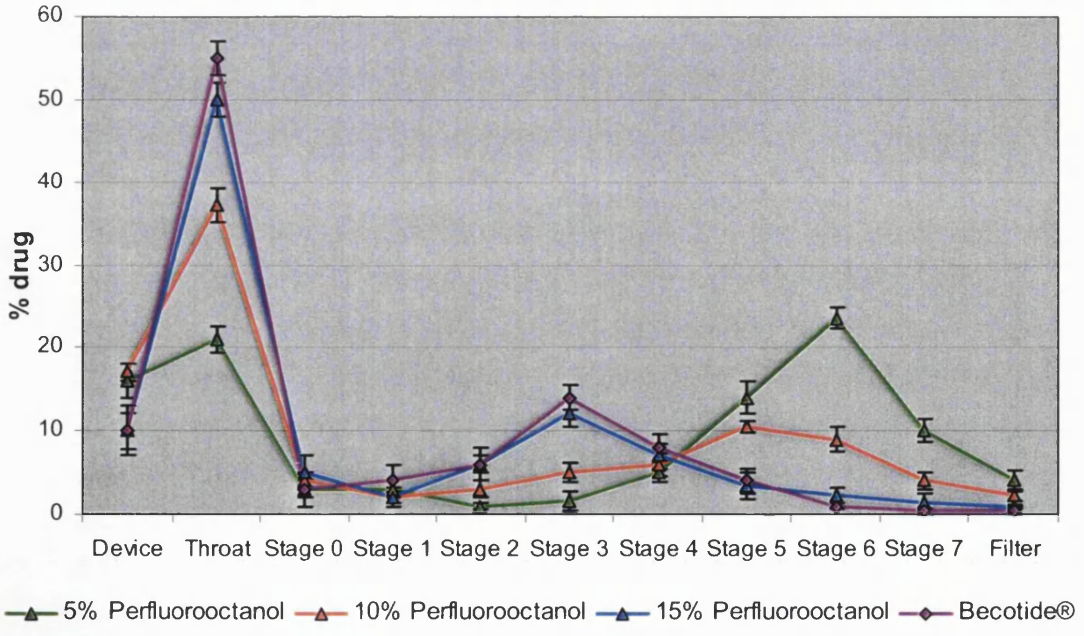


Figure 4.27 *Comparison of ACI drug deposition profiles for an HFA 134a pMDI suspension formulation containing terbutaline sulphate at 0.1% w/w, mPEG-DMPE at 0.13% w/w and PFOH at 5, 10 or 15% w/w (compositions A, B and C) (n=3 ±SD).*

Table 4.9 Spray performance parameters of (a) budesonide, (b) formoterol fumarate, and (c) terbutaline sulphate aerosols tested (n=3; ±SD).

(a)

Formulation	MMAD	GSD	FPF Ex Device	% Recovery
1-A	1.1 ± 0.0	2.1 ± 0.1	75.0 ± 3.1	100.1 ± 2.8
1-B	1.7 ± 0.1	2.4 ± 0.1	62.9 ± 1.6	103.8 ± 4.1
1-C	3.5 ± 0.2	2.2 ± 0.0	54.9 ± 1.8	96.5 ± 6.2
2-A	1.2 ± 0.1	2.3 ± 0.0	73.0 ± 4.4	98.8 ± 3.2
2-B	2.0 ± 0.1	2.0 ± 0.1	63.0 ± 1.8	99.5 ± 4.2
2-C	3.8 ± 0.2	2.5 ± 0.2	51.8 ± 3.2	102.8 ± 2.9
3-A	0.9 ± 0.1	2.7 ± 0.2	68.5 ± 4.8	99.7 ± 2.0
3-B	1.8 ± 0.0	2.8 ± 0.1	56.3 ± 0.8	98.3 ± 4.5
3-C	4.0 ± 0.1	2.1 ± 0.1	49.0 ± 2.7	100.3 ± 2.1

(b)

Formulation	MMAD	GSD	FPF Ex Device	% Recovery
1-A	1.3 ± 0.1	2.5 ± 0.1	70.1 ± 2.1	103.4 ± 3.5
1-B	2.2 ± 0.1	2.0 ± 0.2	55.3 ± 2.9	100.3 ± 2.8
1-C	4.6 ± 0.2	2.1 ± 0.0	49.1 ± 1.7	99.3 ± 5.2
2-A	1.5 ± 0.0	1.8 ± 0.1	69.2 ± 3.9	100.7 ± 3.1
2-B	2.5 ± 0.1	2.2 ± 0.1	53.6 ± 2.5	105.4 ± 4.7
2-C	4.4 ± 0.1	2.1 ± 0.1	53.8 ± 3.3	98.3 ± 2.3
3-A	1.6 ± 0.1	2.6 ± 0.1	69.9 ± 2.7	99.2 ± 1.6
3-B	2.7 ± 0.1	2.5 ± 0.1	54.4 ± 4.9	98.0 ± 5.1
3-C	4.7 ± 0.2	1.9 ± 0.1	49.9 ± 2.0	104.7 ± 3.2

(c)

Formulation	MMAD (μm)	GSD	FPF Ex Device	% Recovery
1-A	1.2 ± 0.1	1.8 ± 0.2	73.4 ± 2.2	105.2 ± 3.5
1-B	2.4 ± 0.1	2.5 ± 0.1	58.9 ± 1.8	100.4 ± 3.1
1-C	3.7 ± 0.0	1.7 ± 0.2	49.7 ± 1.9	99.9 ± 2.7
2-A	1.5 ± 0.1	2.0 ± 0.1	71.2 ± 2.8	97.4 ± 5.3
2-B	2.6 ± 0.2	2.8 ± 0.1	60.6 ± 3.1	102.3 ± 3.7
2-C	3.5 ± 0.1	2.1 ± 0.2	52.3 ± 3.0	100.5 ± 1.9
3-A	1.0 ± 0.1	2.1 ± 0.1	75.9 ± 2.6	97.6 ± 3.6
3-B	2.4 ± 0.1	2.5 ± 0.1	64.7 ± 1.9	99.3 ± 4.8
3-C	3.9 ± 0.2	1.8 ± 0.1	50.4 ± 3.9	100.7 ± 1.1

*1,2,3=mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively; A,B,C= PFOH at 5, 10 and 15% w/w, respectively.

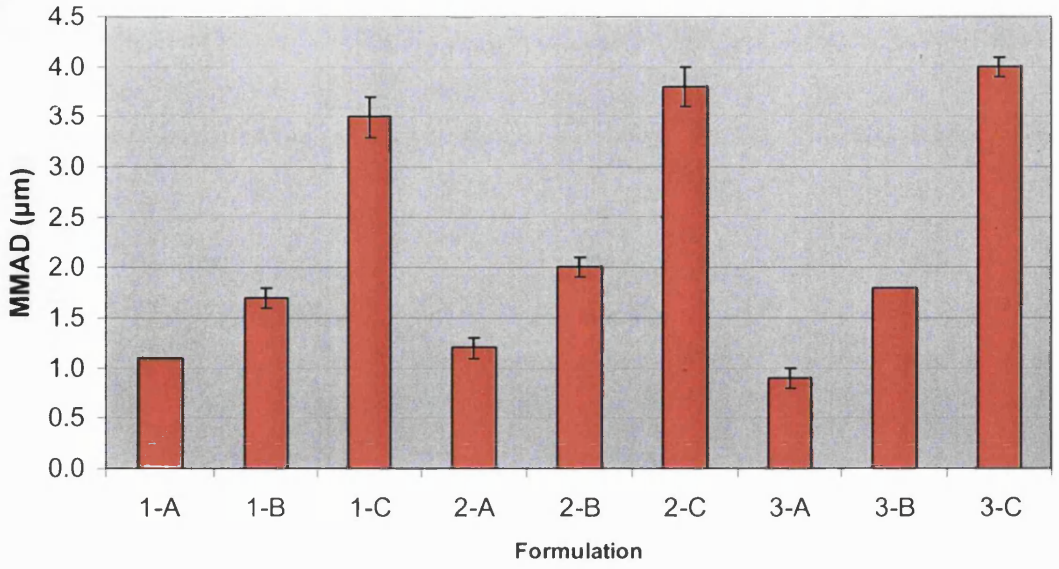


Figure 4.28 *MMAD for HFA 134a pMDI suspension formulations containing budesonide (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

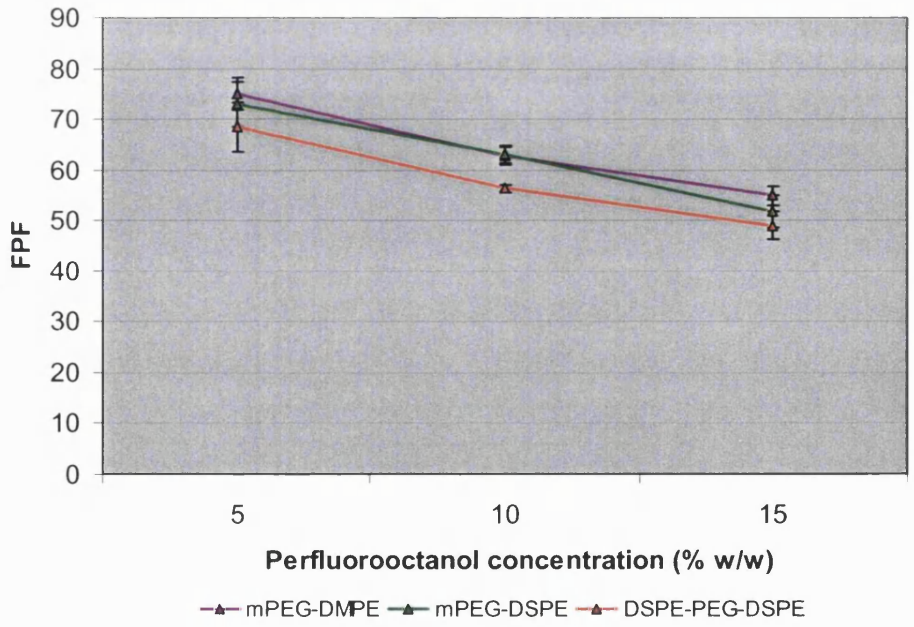


Figure 4.29 *Fine particle fraction ($FPF_{4.7\ \mu m}$) for HFA 134a pMDI suspension formulations containing budesonide (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

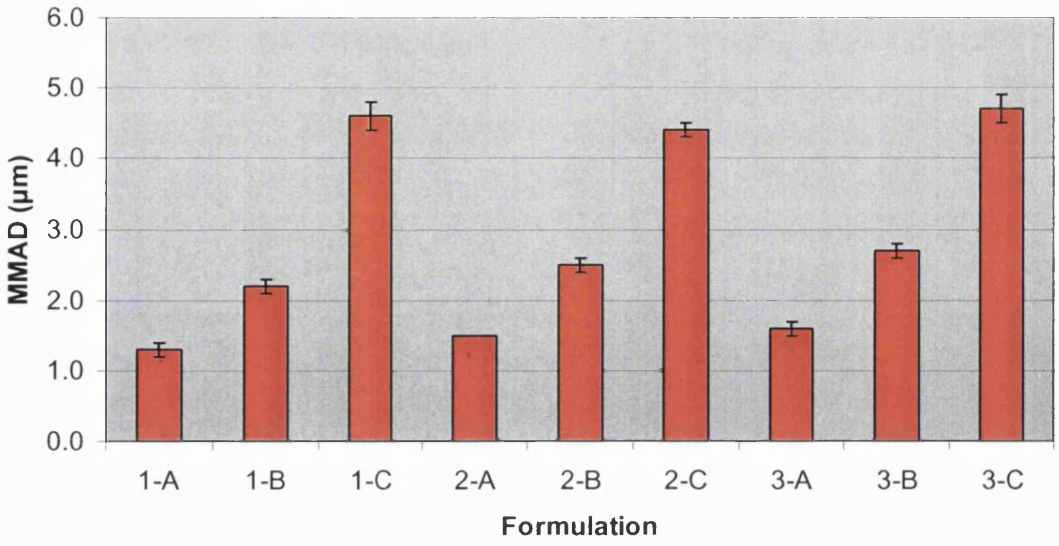


Figure 4.30 *MMAD for HFA 134a pMDI suspension formulations containing formoterol fumarate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

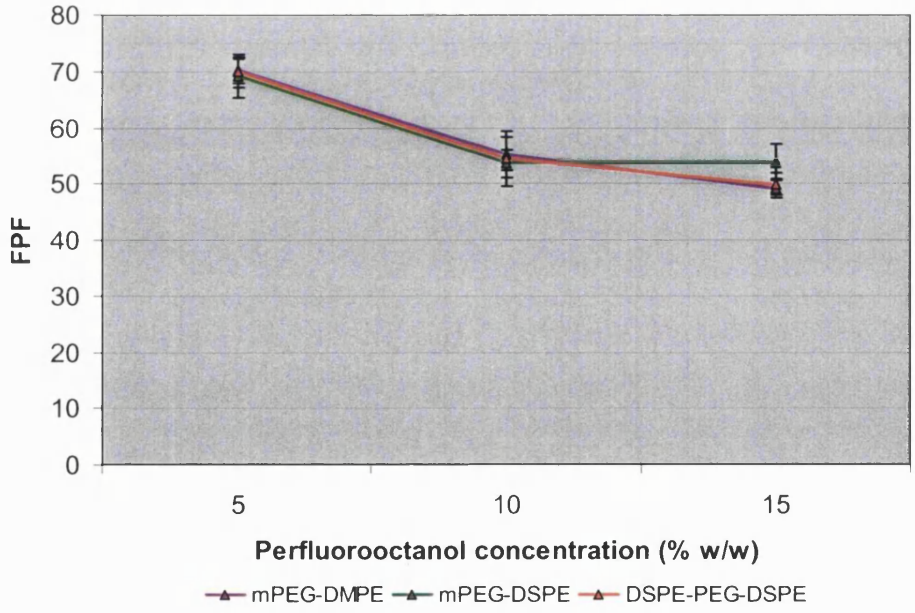


Figure 4.31 *Fine particle fraction ($FPF_{4.7\ \mu m}$) for HFA 134a pMDI suspension formulations containing formoterol fumarate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

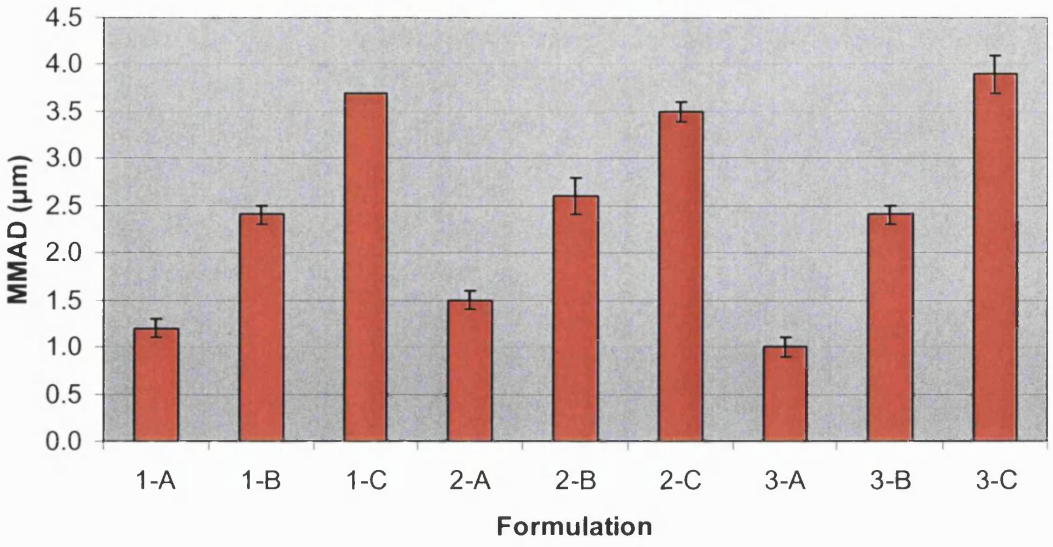


Figure 4.32 *MMAD for HFA 134a pMDI suspension formulations containing terbutaline sulphate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

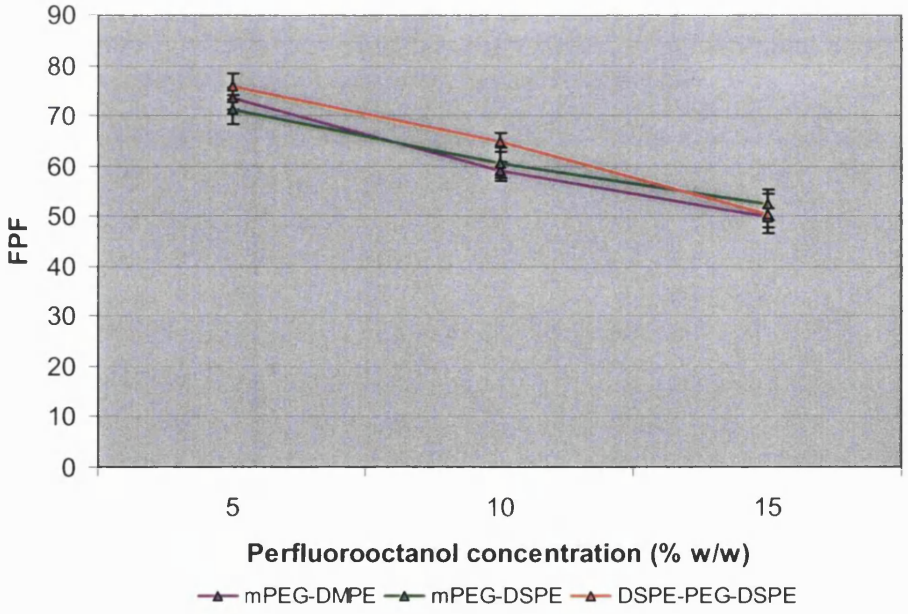


Figure 4.33 *Fine particle fraction ($FPF_{4.7\ \mu m}$) for HFA 134a pMDI suspension formulations containing terbutaline sulphate (0.1% w/w), mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE (0.13% w/w) and PFOH at 5, 10 and 15% w/w (n=3 ±SD).*

The spray performance of PEG-phospholipid and PFOH suspension pMDIs was compared to the commercially available Becotide® pMDI. Figures 4.25-4.27 illustrate the deposition pattern of Becotide®. The MMAD was found to be 3.4 ± 0.23 µm (literature value is 3.5 µm (Leach *et al.*, 1998)). PFOH suspensions containing a PEG-phospholipid showed a far higher FPF (> 70%) compared to Becotide® with an FPF of 55.7 ± 1.1%. Furthermore, a shift of the main drug deposition from the upper bronchi (main deposition on stage 3) to the lower bronchioles and alveoli (main deposition on stage 5) can be achieved with the novel aerosols, i.e. a reduced oropharyngeal deposition compared to Becotide® and an improved bronchial deposition apart from formulations containing 15% w/w PFOH which exhibited a shift in deposition to the left (i.e. increased deposition in upper stages). This was especially apparent for terbutaline sulphate suspension formulations (figure 4.27).

The fine particle fraction ($FPF_{4.7\mu m}$) was determined for all suspension formulations for both the TSI APS system and the ACI. Results obtained with the TSI system were compared with those obtained via the ACI. The cut-off point of stage 2 on the ACI was assumed to be ~4.7 µm, which is the nominal cut-off point as indicated by the manufacturer). The $FPF_{4.7\mu m}$ and particle size results were analysed in order to evaluate comparability of the 2 systems.

Figure 4.34 compares $FPF_{4.7\mu m}$ determined for terbutaline sulphate formulations as measured with the ACI to that from the TSI APS system. $FPF_{4.7\mu m}$ values showed no statistical difference between the TSI system and the ACI ($P > 0.05$). $FPF_{4.7\mu m}$ decreased with increasing concentration of PFOH ($P < 0.05$). Characterisation of $FPF_{4.7\mu m}$ for suspension formulations on the TSI APS system and ACI showed that $FPF_{4.7\mu m}$ is inversely proportional to PFOH concentration (figures 4.29, 4.31, 4.33).

The MMAD and GSD were used to compare the size distribution data from the TSI APS and the ACI. The ACI size distributions for formulations containing 0.1% w/w mPEG-DMPE, mPEG-DSPE or DSPE-PEG-DSPE and 15% PFOH evidenced finer particles than those determined by APS. Correlation between TSI and ACI-measured size distributions can be improved by fitting an extension to the TSI impactor as previous published work has shown that an extension is necessary for pMDIs with higher concentrations of alcohol (Myrdal *et al.*, 2004; Mogalian and Myrdal, 2005).

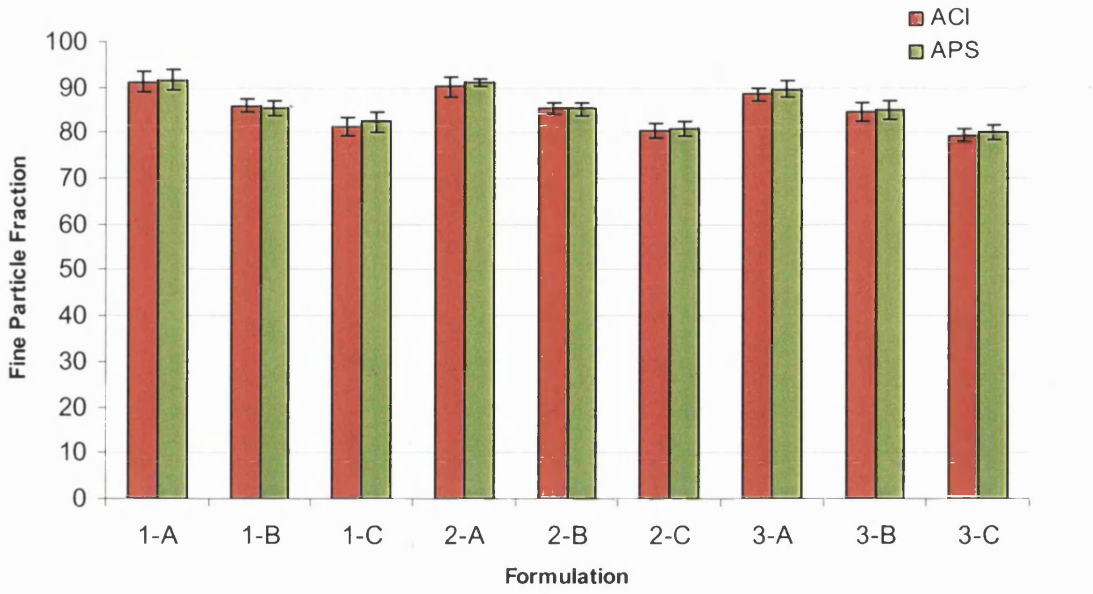


Figure 4.34 *Characterisation of $FPF_{4.7\mu m}$ particle distribution for terbutaline sulphate suspension formulations using the TSI APS system and ACI (n=3 ±SD).*

The concentration of PFOH in the suspension MDI formulations appeared to have an effect on MMAD and $FPF_{4.7\mu m}$. Table 4.10 shows the influence of formulation on the delivery characteristics from the different pMDIs by comparing MMAD values. As illustrated in table 4.10, for all terbutaline sulphate suspension pMDIs, the MMAD values from the ACI correlated well with MMAD values from the TSI APS system (figure 4.35). Overall, the TSI APS system showed similar patterns of particle distribution compared to the ACI for each of the suspension formulations tested and is sensitive enough to detect increases in PFOH concentrations. The MMAD and FPF values from the ACI are illustrated in figures 4.28-4.33.

The formulations with 5% PFOH (1-A, 2-A and 3-A) were more efficient at delivering drug than the formulations with 15% PFOH (1-C, 2-C and 3-C) and had lower MMAD values and higher $FPF_{4.7\mu m}$. This result may be explained by the slightly increased residual particle MMAD, along with the fact that the size of the droplets was possibly larger owing to incomplete evaporation of PFOH as the droplets pass through the impactor stage resulting in lower vapour pressure.

Table 4.10 A comparison of size distribution measurements for terbutaline sulphate suspensions using the TSI APS system and the 8-stage ACI (n=3 ±SD).

Formulation*	ACI		APS	
	MMAD (µm)	GSD	MMAD (µm)	GSD
1-A	1.2 ± 0.1	1.8 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
1-B	2.4 ± 0.1	2.5 ± 0.1	2.1 ± 0.1	2.3 ± 0.0
1-C	3.7 ± 0.0	1.7 ± 0.2	3.3 ± 0.2	2.0 ± 0.1
2-A	1.5 ± 0.1	2.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.2
2-B	2.6 ± 0.2	2.8 ± 0.1	2.4 ± 0.1	1.6 ± 0.3
2-C	3.5 ± 0.1	2.1 ± 0.2	3.0 ± 0.2	1.2 ± 0.3
3-A	1.0 ± 0.1	2.1 ± 0.1	0.9 ± 0.1	2.2 ± 0.1
3-B	2.4 ± 0.1	2.5 ± 0.1	2.0 ± 0.3	1.4 ± 0.1
3-C	3.9 ± 0.2	1.8 ± 0.1	3.5 ± 0.1	1.0 ± 0.0

*1,2,3=mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE, respectively; A,B,C= PFOH at 5, 10 and 15% w/w, respectively.

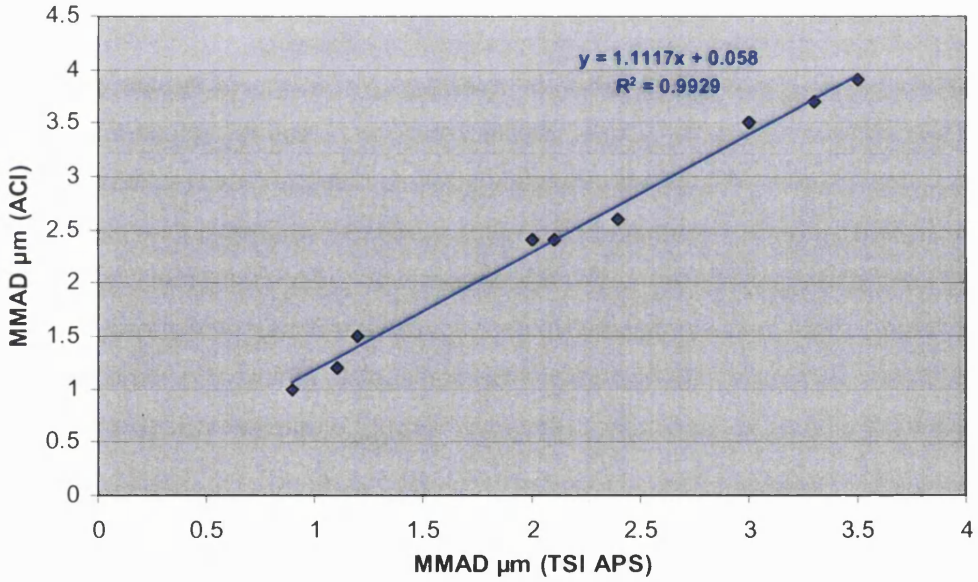


Figure 4.35 *Comparison of MMADs for terbutaline sulphate suspension formulations as determined by the TSI APS system and ACI (n=3 ±SD).*

In this work, the physical stability of suspension pMDIs containing a fluorinated alcohol and PEG-phospholipids within HFA was determined both directly through Turbiscan measurements and indirectly using a TI, an ACI and an APS TSI after aerosolisation. These four methods demonstrated good agreement throughout the study which indicates that they could be reliably used to estimate the physical stability of the suspensions.

Of the few fluorinated alcohols employed to try and enhance the physical stability of the drugs studied within HFA, PFOH was chosen and was used in combination with PEG-

phospholipids. No difference was noted between the three phospholipids investigated mPEG-DMPE, mPEG-DSPE and DSPE-PEG-DSPE ($P < 0.05$). The commercially available grades of mPEG-DMPE MW 5000, mPEG-DSPE MW 5000 and DSPE-PEG-DSPE MW 5000 are known to be similar in terms of physicochemical properties since many of the physicochemical properties are determined by molecular weight. All three PEG-phospholipids used had the same MW of 5000. Importantly the solubility in a range of solvents is dependent on the molecular weight of PEG-phospholipids. In this study, varying the type of PEG-phospholipid was found to have no significant effect on the particle size of the manufactured product as well as no significant influence on the physical stability of the pMDI suspension.

PFOH could be used in combination with PEG-phospholipid to enhance the stability of the budesonide, formoterol fumarate and terbutaline sulphate suspension pMDIs using HFA 134a. Increasing the concentration of PFOH had a significant effect on the stability of the final HFA pMDI formulation whereas in the absence of the fluoroalcohol, drug particles aggregated when suspended in HFA 134a. The inclusion of PFOH was obviously crucial to the physical stabilisation of all the drug formulations within the HFA suspensions since without it none of the formulations showed any evidence of physical stability. However, it was found that increasing PFOH concentration increased MMAD and reduced FPF ($P < 0.05$). Thus the positive effect on suspension stability has to be balanced against the respiratory benefit that results from using the fluorinated molecule/PEG-phospholipid combination.

In order for PFOH to influence the suspension stability of budesonide, formoterol fumarate or terbutaline sulphate drug molecules within the HFA system, it must modify the interactions of the micronised drug with other particles and the vessel in which they are contained (Bagchi, 1973). Typically this could be due to an affinity of the fluoroalcohol for the solid particulates hence adsorption takes place. Adsorption involves the accumulation of molecules at an interface and is favoured by a decrease in free energy of the adsorbing system ($-\Delta G$). When adsorption is occurring directly in the suspending media it may be enhanced for 'poor' solvent systems (with respect to the sorbate) as the free energy change is negative ($-\Delta G$) compared to 'good' solvents where the free energy change is positive ($+\Delta G$) (Bagchi, 1973). If a stabilising compound is rendered insoluble within the dispersion media it can induce flocculation (Napper, 1968). Flocculation (i.e. particle aggregation) occurs at volume fractions of solvent that correspond to the upper solubility limit of the compound. Also, PFOH being miscible within the HFA 134a suspension media (21.7% w/w), it could potentially be acting as a suspension aid and any suspension stabilisation could be a dynamic process (Napper, 1968), that is involving continual adsorption and desorption of the excipients at the solid/liquid interface.

Applying the traditional theories of suspension stabilisation, the enhanced stability of HFA suspensions conferred by the combination of PFOH, PEG-phospholipids and HFA could be due to steric stabilisation. Therefore PFOH, which displayed miscibility in HFA 134a, could possibly be enhancing the stability of the particles within these propellants via classical steric interactions (Cassidy *et al.*, 2000) because this was the only excipient found to be soluble in the HFA propellant and thus possibly extend into the continuous phase of the system.

One trace impurity present in HFA solvents is water (≤10 µg/g) (Solvay Fluor und Derivate, 2006). Yu *et al.*, 2003 showed the effect of water on zeta potential using a series of homologous solvents. The simple addition of 0.5% water to a non-polar system provided a one order of magnitude rise in zeta potential. Researchers also linked a decrease in the magnitude of zeta potential within suspension systems to a decrease in dielectric constant. Kosmulski, (1999) has suggested that the role of trace impurities is often overlooked. PFOH is a fluorinated molecule that represents a source for the counterion charge, which can have an important role in pharmaceutical systems. Water is far less likely to associate with the hydrophobic steroid budesonide than with formoterol fumarate and terbutaline sulphate. Therefore, the presence of ionic species/impurities within this system are likely to be situated at the drug-solvent interface for systems containing hydrophilic drugs, which will influence the degree of surface charge and hence the zeta potential of the particles (Kitahara *et al.*, 1967; Napper, 1976). Further work is required to determine the influence of such trace impurities upon the zeta potential in non-polar suspension systems.

Moreover, PFOH seems to exhibit a unique ability to act as stabiliser within the HFA system for a range of hydrophilic and hydrophobic molecules investigated in this study and this may be because it is made up of alcohol (hydrophilic) and a carbon chain (hydrophobic). Due to this amphiphilic nature, PFOH may form a loop and tail conformation at a hydrophilic/hydrophobic interface. If during the formation of the suspension the molecule adsorbs to the drug budesonide via the hydrophobic moieties, that is the hydrocarbon chain, it may produce a hydrophilic alcohol coating exposed to the exterior of the particle. This external alcohol moiety in addition to trace impurities, for example water may induce the charging of the particle surface. PFOH is miscible in HFA 134a (i.e. inferring an association can occur between the two molecules). If PFOH forms a second layer on the drug particle this may confer an element of steric stabilisation within the HFA propellants thereby reducing the zeta potential of the suspension as particles are 'coated' by the fluorinated alcohol.

The addition of PFOH appeared to increase the physical stability of the suspension within the HFA. Stability was directly dependent on the amount incorporated in the formulation. However, lower concentrations displayed the smallest increase in size upon suspension within the HFA propellants and the best aerosolisation properties which implies that care should be taken when deciding on the optimum amount of PFOH that could be used.

4.5 Conclusion

This work has shown that a combination of PEG-phospholipids and PFOH can stabilise various drug-HFA suspensions. Long standing problems with pMDI suspension formulations include creaming of the suspension, coarse drug suspension, drug flocculation and adhesion to dispensing device. These problems can be overcome for the drugs investigated with a pharmaceutical formulation containing a polar fluorinated molecule in conjunction with suitable PEG-phospholipids. The formulations comprising a drug, propellant HFA 134a, a polar fluorinated molecule and an excipient soluble in the polar fluorinated molecule give rise to improved aerosol drug suspension characteristics, i.e. increase of phase separation times (creaming or sedimentation), production of a finer suspension, reduction of particle adhesion to the can walls and inhibition of particle flocculation. They provide stable dispersion for the pulmonary delivery of the three drugs investigated: budesonide, formoterol fumarate and terbutaline sulphate.

Suspensions containing PEG-phospholipid and PFOH produced a significantly larger ($P \leq 0.05$, ANOVA) stage 2 deposition of the TI compared to drug alone in HFA 134a. Manipulating the physicochemical properties of the excipients could further enhance the physical stability of the HFA suspensions. Suspension pMDIs containing PEG-phospholipids, PFOH and budesonide, formoterol fumarate or terbutaline sulphate produced aerosol clouds in the respirable range. Incomplete evaporation of PFOH may cause a reduction in $FPF_{4.7\mu m}$. All formulations formed a stable suspension system. PFOH cosolvent was effective in stabilising PEG-phospholipid-containing pMDI suspension formulations in the hydrofluoroalkane propellant having dispersed therein drug particulates.

Although the technical challenges of measuring the zeta potential directly within HFA systems are still unresolved, particle charge might have an important role to play in pMDI stabilisation. Thus, there is an obvious need to further develop specialised equipment in order to allow the direct measurement of zeta potential within pressurised non-polar solvents thereby enabling the systematic development of physically stable HFA based suspensions.

CHAPTER 5

GENERAL DISCUSSION

Pulmonary administration of drugs has numerous advantages in the treatment of pulmonary diseases due to direct targeting to the respiratory tract. It enables avoidance of first pass effects, reduces the amount of drugs administered, targets drugs to specific sites and reduces their side effects. The reformulation of inhalation products with HFAs brought about by the phase out of CFCs triggered a renewed interest in the science of pMDIs (Clarke *et al.*, 1993; Smith, 1995; Peart *et al.*, 1998; Kalinkova, 1999; McDonald and Martin, 2000; Price *et al.*, 2000; Smith, 2003). Physicochemical differences between CFC and HFA propellants include solvency, vapour pressures and corresponding differences in aerosolisation properties (Vervaet and Byron, 1999). The poor solvent strength of HFA 134a means that solution formulations are frequently not practical and rules out the use of conventional surfactants to stabilise suspensions (Dalby *et al.*, 1990). On the other hand, partial solubility of drugs in HFA propellants may lead to poor suspension stability through particle growth (Purewal, 1998). In this work, the use of excipients, namely phospholipids and alcohol, in solution and suspension HFAs has been studied in order to investigate novel pMDI formulation strategies.

Liposomes are potentially useful drug delivery systems for pulmonary administration. This work demonstrated the possibility of delivering solubilised phospholipid and drugs via the pulmonary route using an HFA pMDI. Solution HFA 134a based pMDIs were formulated using phospholipid as vesicle forming material, ethanol as a cosolvent and salbutamol base or budesonide as the model drug (Chapter 2). The liquid present as the collection medium in each stage of the TI or the MSLI provided the appropriate environment for liposome formation from deposited phospholipid aerosols. Liposomes were formed by this method as a result of rapid hydration followed by spontaneous vesicle formation when aerosol droplets deposited onto a moist surface. The concept of spontaneous liposome formation from phospholipid/drug-containing HFA-based aerosols and the slow release of drug were thus demonstrated *in vitro* (Chapter 3).

Ethanol was used in the solution pMDIs to alter the solvent properties. However, ethanol is known to have an effect on vapour pressure, liquid density (adding ethanol to a formulation reduces the density of the mixture), and emitted droplet size of pMDIs (Vervaet and Byron, 1999). Ethanol cosolvent was chosen because of its miscibility with the HFA propellant and the positive influence on the solubility of organic molecules due to its higher polarity compared to that of HFAs. However, the extent of drug solubility in the propellant/cosolvent mixture must be considered carefully alongside the vapour pressure of the resultant pMDI. Ethanol decreases vapour pressure and results in an increased aerosol droplet size that may adversely affect lung deposition patterns. HFA 134a and ethanol mixtures show

positive deviation from ideality. Low solubility of drug in HFA 134a may be improved with the addition of ethanol as a cosolvent but solubility is not easily predicted in this system. The cosolvent was investigated in HFA 134a pMDIs in terms of solvency, spray performance, and resulting droplet size. Drug and phospholipid solubilities increased non-linearly with increasing ethanol levels (section 2.4.4), and in general, increasing the proportion of ethanol had the effect of increasing the VMD (volume median diameter) (section 3.4.5). Significant differences in droplet size was detected between formulations containing 5, 10 and 15% w/w ethanol, indicating that addition of ethanol to propellant systems has to be balanced against significant increases in droplet size. The significance of these differences for lung deposition needs to be confirmed by investigating evaporation rates and *in vivo* deposition. The addition of ethanol also increases the moisture uptake capacity of the pMDI formulation. Therefore small amounts of water (0.10 to 1.09% w/w) were added to salbutamol base formulations to investigate the consequences of uptake of small amounts of water. Formulations were found to remain stable. Higher concentrations of water were not investigated (section 2.4.7).

Because the formulation of the solution pMDI systems necessitated the inclusion of a low volatility cosolvent, the aerosol fraction available for inhalation may be diminished. Kirk (1972) and Bell *et al.* (1973) reported that the formulation of isoprenaline as a solution-type pMDI using ethanol as a cosolvent dramatically decreased the therapeutic fraction of the emitted aerosols compared to suspension products. In this study, the inclusion of 15% w/w ethanol had a significant effect on the respirable fraction compared to formulations containing less than 15% w/w ethanol (section 3.4.3.3). The FPF decreased with increasing ethanol concentration (figures 3.25-3.27). This was primarily a function of retarded propellant evaporation due to the presence of ethanol, but this could also be due to the presence of PC in the formulation. Therefore maximum solubility benefit above a certain ethanol concentration is cancelled by lower therapeutic efficiency since the gain in solubility by increasing ethanol concentration is negated by a decrease in respirable deposition. Although there is more drug per unit volume in the aerosol plume, it may not reach the desired location in the lung, as seen *in vitro*. The deposition of the therapeutically active agent in the optimum quantity, at a desired location within the respiratory tract is the principal aim of inhalation therapy. As shown, this can be achieved with the appropriate amount of cosolvent, which increases drug solubility, without compromising the respirable deposition and the aerodynamic particle size (figures 3.28-3.29). Thus, a balance has to be created between the various formulation ingredients of a solution pMDI in order to achieve maximum deposition efficiency. The results obtained from the current investigation can serve as a model for the approach that can be used to optimise pMDI formulations, thus

enabling formulation of therapeutically effective solution pMDIs.

The choice of propellant in the formulation was based on data obtained from using both HFA 134a and HFA 227 propellants. The results favoured the use of HFA 134a and smaller concentrations of ethanol could be used to solubilise materials (section 2.4.3). Moreover, HFA 134a is preferred in terms of spray characteristics when a dose is actuated. This is because HFA 134a and HFA 227 differ significantly in vapour pressure; 570 and 390 kPa at 20°C, respectively. Higher vapour pressure leads to more efficient atomisation and finer sprays. Addition of cosolvent and non-volatile additives lowers the propellant vapour and pack pressures, although those for HFA 134a systems remain higher than for equivalent HFA 227 systems (Brambilla *et al.*, 1999). Pittroff and Jannick (2004) identified 21 inhalation HFA products, 6 of which use propellant HFA 227 and 15 use HFA 134a. Of these, 10 are solution formulations and 11 are suspensions. Most of the solution formulations include ethanol. No big difference in delivery performance is reported between optimised formulations based on either HFA 227 or HFA 134a (Nagel *et al.*, 2002). In addition, there is no difference in therapeutic efficacy of formulations based on the two propellants, or between solutions and suspensions (Zeidler and Corren, 2004). Both propellants have advantages and disadvantages for their use. HFA 227 has the lower vapour pressure, which can lead to larger droplet size distributions and consequently bigger MMADs. Particle diameters can be reduced for HFA 134a due to its higher vapour pressure; however, the higher velocity of the particles can lead to higher oropharyngeal deposition (table 3.10).

Salbutamol sulphate, a hydrophilic drug, did not form a solution system in the three-component system (HFA 134a-ethanol-GMO, SPC or EPC), thus it was not investigated in terms of its controlled delivery potential (section 2.4.4.1). Salbutamol sulphate particles precipitated out of solution and sedimentation occurred due to density difference (densities for salbutamol sulphate and HFA 134a are 1.31 and 1.21 g/ml, respectively). Water is well known to influence the forces of interactions. When small amounts of water (0.26 to 1.98% w/w) were added to the formulation, it was found that it had an effect on the sedimentation rate of the drug formulation (section 2.4.7.1). Therefore, water addition to the non-aqueous pMDI suspensions did not systematically lead to destabilisation. Indeed, as has been previously reported by Malbrel and Somasundaran (1989) who studied alumina suspended in cyclohexane with aerosol OT (dioctyl sodium sulfosuccinate), water first had a stabilising effect on the suspension (by reducing its settling rate), before destabilising the suspension and restabilising it at higher water content. Similarly, Rogueda (2001) found that the zeta potential of a micronised formoterol fumarate suspension in HFA 227 with water content between 11 and 20 ppm increased from 20 to 75 mV as water content increased. However, data to determine if this zeta potential increase led to increased stability were not reported.

The results of this study are contrary to the belief that HFA suspensions are destabilised by water. Work by Griffiths *et al.*, (2004) has shown how water can separate out silica particles suspended in DFP with polymers. This work indicates that the destabilisation mechanism is due to some specific particle-water phase behaviour. An explanation for the stabilising effect of water is that it may express charges on particle surfaces and enhance repulsive forces, or act as a flocculation bridge, a protective layer, or even form separate phases with particles. Overall, there is no unique mode of action for water in HFAs; it is formulation dependent and depending on drug solubility it may not systematically lead to destabilisation. In fact, it is possible to find formulations that use water as a stabiliser, as has been claimed in recent patents (DeStefano and Kelash-Cannavo, US20040184994, 2004: US6565833, 2003; Adjei and Cutie: US2003091512, 2003). However, much remains to be done to understand the nature of interaction forces between particles and the factors influencing them (Roberts, 2004).

In this formulation approach, the aqueous central compartment of liposomes or spaces between successive bilayers may be exploited to entrap drugs whilst hydrophobic drugs may be incorporated into the bilayers. Entrapment of materials in liposomes may protect them from *in vivo* metabolic degradation or clearance, and provide a sustained release or targeted delivery system (Kirby and Gregoriadis, 1999). Liposomes generated from SPC and EPC-based formulations were MLVs and LUVs as shown by TEM (sometimes SUVs were also present) (figures 3.2-3.5). The multilamellarity of MLVs generated by this formulation approach suggests that these vesicles may not be very good candidates for efficient entrapment of hydrophilic drugs, although the high level of lamellarity provides many barriers for drug diffusion and may hence prolong the drug release. The results, however, show an increased entrapment of salbutamol base, a slightly hydrophilic drug, with increasing concentration of phospholipid (figure 3.10). This could be due to an increased entrapment of drug within LUVs rather than MLVs. Increased entrapment of the drug may be due to the fewer bilayers of liposomes generated permitting more drug to be entrapped when liposomes were formed. Another explanation could be due the drug's low hydrophilicity, which would lead to precipitation in the excess water medium of the impinger.

Overall, this study has shown the potential of liposomes as a carrier system for salbutamol and budesonide drug delivery in the respiratory tract. SPC- and EPC-based pMDIs successfully delivered phospholipid to a TI and an MSLI. GMO-based pMDIs failed to form vesicles in the impinger although small 'particles' were observed, and showed erratic aerosol droplet size measurements indicating the unsuitability of this formulation for delivering liposomes using this approach. Compared to a conventional

salbutamol/budesonide solution preparation, delivery of a drug in a liposomal formulation from pMDIs may be advantageous and liposomes formed in this approach were shown to successfully extend drug release (figures 3.33-3.34). Drug particles can be retained within the liposomal carriers for prolonged periods. Vesicles formed within the lungs provide a sustained release of encapsulated material. If fusion of vesicles with the airways surface and/or lipid exchange between vesicle bilayers and surfactant are major mechanisms of drug release, then liposomes with more than one bilayer are likely to provide a more sustained release of entrapped materials than LUVs. Drug entrapment within liposomes formed following the hydration of aerosol droplets emitted from pMDIs containing a solution of the drug and phospholipid may be dependent on the physicochemical properties of the drug molecule and more drugs with varying physicochemical properties need to be investigated in such systems. A drug's physicochemical character may require the delivery of a relatively high lipid dose for example. The presence of a high lipid concentration within the pMDI formulation will further complicate the production of a respirable aerosol directly and indirectly as it requires higher amounts of cosolvent to dissolve. Results with the hydrophobic model compound suggest lipophilic drugs might usefully be employed in such systems. The hydrophilic drug salbutamol sulphate was not investigated because it did not form a solution system in our model formulation. Rendering a slightly hydrophilic drug, such as salbutamol base, more lipophilic through ionic complexation with a lipid of opposite charge incorporated in the liposome bilayer enhanced the applicability of this type of compound. Inclusion of the negatively charged compound DCP into vesicle bilayers increased drug encapsulation. Previous studies with liposomes (MLVs) prepared by conventional methods have illustrated that hydrophobic drugs are incorporated into liposomes to a higher degree than hydrophilic moieties (Juliano and Stamp, 1978). Results from this study, however, illustrated an increase in salbutamol base encapsulation with increasing DCP concentration (figure 3.10). Salbutamol resides in the aqueous channels of liposomes and remains unassociated with the lipid bilayers (Farr *et al.*, 1988). Incorporation of the negatively charged lipid, DCP, into EPC or SPC liposomes resulted in an increased uptake of salbutamol. One possible explanation is that the inclusion of a charged lipid into PC bilayers increased liposome size through electrostatic separation of the bilayers (Bangham *et al.*, 1967; Johnson, 1973) and is a method by which uptake of drugs associated with the entrapped aqueous volume may be improved (Alpar *et al.*, 1981). The improved entrapment of salbutamol could be due to the formation of a lipophilic ion-pair between the positive centre of salbutamol and the negative moiety of DCP. The resulting increased salbutamol entrapment in the presence of DCP reflects the relative hydrophobicity of the complex in SPC/DCP or EPC/DCP liposome systems. For ion-pair formation, a 50:50 molar ratio of salbutamol

and DCP would confer greatest hydrophobicity to salbutamol. Another plausible explanation is that the drug is entrapped in the aqueous core of the LUVs, and DCP is merely increasing the size of these vesicles thereby increasing the aqueous core compartment of the vesicles. Another method for rendering a slightly hydrophilic drug more lipophilic could involve the chemical synthesis of a lipophilic prodrug. The potential toxicity of charged lipids *in vivo* (Adams *et al.*, 1977) could invalidate the former approach, whilst with prodrugs of negligible intrinsic pharmacological activity, drug availability is likely to be a complex function of liposomal efflux as well as prodrug to drug reaction kinetics. Liposomes can be effectively formed in the compartments of the impinger, wherein they may remain for prolonged periods. A prolonged retention in the airways may alter the pharmacokinetics of liposome associated materials: increasing local concentrations, whilst decreasing levels at sites distant from the lung. Although offering a positive result *in vitro,* the usefulness of such systems has yet to be established *in vivo* in disease states that may benefit from such formulations. Stability and/or residence of the vesicles in the lung will greatly influence the effective duration of drug release *in vivo.* Obviously, the ability of this novel approach to prolong the residence times of relevant drugs within the lung will be a complex function of other factors such as pulmonary clearance and the integrity of liposomal structure within respiratory secretions. Nevertheless, such *in vitro* studies will provide a mechanism through which prospective aerosol formulations may be evaluated. Drugs ideal for such formulations are those administered via the inhaled route that would benefit from the increased control of their release profile as a means to usefully match their biological requirements.

Due to the poor solvent properties of HFAs, suspensions are often the only formulation option for respiratory drug delivery. Research in this area has focused on two main themes over the past 5 years: new design of stabilisers and particle engineering such as porous particle formation, surface modification, and excipient design. Non-aqueous suspensions raise particular formulation challenges. Their preparation requires careful consideration of the interactions between drug particulates in liquefied propellant, and also interactions with various components of the pMDI device (Ross and Gabrio, 1999; Smyth, 2003). The ability of pMDI suspension formulations to retain homogeneity is fundamental to achieving consistent performance. It has been shown that suspensions that are not well stabilised lead to poor delivery performance and manufacturing difficulties (Miller and Schultz, 1992). The results in this thesis show that combining PFOH and PEG-phospholipids can successfully stabilise various drug pMDI suspensions to reduce particle interactions so as to improve their delivery characteristics (Chapter 4). The suspension formulations have proved effective in

solving some of the known problems of HFA suspensions such as fast phase separation time (sedimentation and creaming), aggregation, aerosolisation properties and drug losses via device adhesion. Overall, the formulations comprising a drug, propellant HFA 134a, a polar fluorinated molecule and an excipient soluble in the polar fluorinated compound gave rise to improved aerosol drug suspension characteristics, production of a finer suspension, reduction of particle adhesion to the can walls and inhibition of particle flocculation. They provided stable dispersions for the pulmonary delivery of the three drugs investigated: budesonide, formoterol fumarate and terbutaline sulphate and produced a significantly larger ($P \leq 0.05$) FPF compared to drug alone in HFA 134a (table 4.8).

Budesonide, formoterol fumarate and terbutaline sulphate suspensions in HFA 134a alone were unstable, with flocculation and phase separation occurring quickly and simultaneously and contributing to very poor product performance. This lack of stability can originate from a number of sources, amongst which are strong attractive forces between particles, as well as those between particles and the inhaler components. The role of the excipients used in this work is therefore to provide a protective sheath that can reduce or overcome the attractive forces. The efficiency of excipients can be judged on the performance of the end product. Little research has been published to date quantitatively addressing the nature and range of the interactions that exist between the drug particles in suspension and the excipients used (Michael *et al.*, 2000; Young *et al.*, 2003). Theoretical explanations are borrowed from the field of colloid and surface science but are not sufficiently developed to include the specificities of HFA systems such as the properties of fluorine atoms.

It is well documented that the addition of excipients to the formulations has a range of effects on the aerosol performance (Brambilla *et al.*, 1999; Smyth, 2003; Stein and Myrdal 2004). Addition of excipients mainly leads to size increase of the aerosol droplets and reduction of the FPF. It may not be possible to detect this effect below certain concentrations (1-5% w/w). The reason for changes in aerosol properties has been attributed to a vapour pressure drop. Other reasons put forward include the modification of the dynamic surface tension of the HFAs by the kinetics of absorption of the excipients (Stein and Myrdal 2004). Such mechanisms might have caused the reduction in FPF in our systems accompanying increased PFOH levels. Incomplete evaporation of PFOH is another possible explanation. Some publications have made references to the stabilising power of insoluble surfactants (Beausang *et al.*, 2003; Beausang *et al.*, 2004); however, their mode of action is more akin to secondary particulate systems and solid surface modifiers than solubilised stabilisers. This could reflect the effect PEG-phospholipids have in our formulations.

Manipulating the physicochemical properties of the excipients could further enhance the physical stability of the HFA suspensions. However, knowledge of solubilities in HFAs is very scarce, and stabilisers previously used in CFCs are insoluble in HFAs. The range of excipients believed to be helpful can be found in many formulation patents. The stabilisers oleic acid, sorbitan trioleate, cetyl pyridinium chloride, soya lecithin, polyoxyethylene, polyoxyethyleneglycol and their copolymers and many fatty acids have been used. However, most of these are not adequate on their own in HFAs, as their natural solubilities are below any useful concentration range. One approach would be to add a stabiliser with other excipients. For instance, the conventional stabiliser oleic acid has been used in some HFA-based pMDI products where ethanol is also used in the formulation as a cosolvent (Purewal and Greenleaf, 1989). Many other stabilisers have been reported in the patent literature that have been used in HFA-based pMDI formulations, for example polyfluoroalkyloxyethylenes (Steele *et al.*, 1991), perfluorinated carboxylic acids or esters (Schultz and Quessy, 1991a,b), perfluorinated sulfonamido alcohol phosphate esters (Moris *et al.*, 1992), perfluoroalkanoic acid (Johnson, WO1992/000107, 1992), fluorosurfactants, e.g. fluoropolyether (Johnson, US5376359, 1994), diols/diacids (Duan *et al.*, WO9421228, 1994a), hydroxyacid, mercapto acid or amino acid (Duan *et al.*, WO9421228, 1994b), sodium lauryl sulphate, cholesterol (Adjei *et al.*, 1995), polyethoxylated surfactants, e.g. PEGs and poloxamers (Somani and Booles, 1992), polymers with recurring structural units (amide or carboxylic acid ester), e.g. PVPs, PVAs, PVP/VA copolymers (Mistry and Gibson, 1992, 1993). Efficient excipient concentration ranges are structure dependent, but typically are between 0.1 and 1% w/w. However, when more than 1% w/w excipient is used, attention must be paid to the influence of the additive on aerosolisation. HFA suspension formulations can use more widely accepted excipients such as oleic acid, cyclodextrins and pegylated phospholipids, but these need the addition of a cosolvent. Ethanol is most often used (Ganderton *et al.*, 2003; Saso *et al.*, 2004). PEG is also known to be used and fluorinated alcohols and ethers have also been employed successfully (Rogueda, P., AB: WO0203958, 2002). This work has illustrated the usefulness of PFOH in such suspension systems.

Control drug formulations in HFA alone were unstable and exhibited a large degree of aggregation and drug adhesion. pMDIs, like all other colloidal systems, suffer from attractive forces that are always present between colloidal particles and result in a potential for aggregation. These attractive forces generally have their origins in intermolecular forces (Khan *et al.*, 1997). Interactions between colloidal particles are usually described by the DLVO. In summary, particle interactions in HFAs are governed by attractive van der Waals forces. These can be overcome by repulsive

forces such as electrostatic forces or steric forces and lead to stable suspensions. van der Waals forces are quantum mechanical in nature and can arise even between perfectly spherical molecules which posses no permanent dipole (Israelachvili, 1992). Fluctuations in the electron cloud density, which are of very short duration, result in the appearance of short-lived, instantaneous dipoles. These instantaneous dipoles result in the observation of finite attractive forces. As aggregation is not always a desired effect, stability needs to be imparted on these systems. Stability can be achieved if repulsive forces can be introduced, and if these are of sufficient magnitude to overcome the attractive forces. The two most widely used methods of stabilisation involve the use of electrostatic effects (applicable in aqueous systems) or steric effects (applicable to both aqueous and non-aqueous systems). Typically, steric stabilisers are employed in pMDI formulations as the systems are non-aqueous in nature. Various stabilisers are reported to have been used for HFA formulations of pMDIs as discussed previously. However, the application of the DLVO theory for the stabilisation of drug suspensions in non-aqueous pMDI media has not been fully investigated because the relevance of electrostatic forces in non-aqueous liquids, or in liquids with low dielectric constants and low conductivity, is still controversial (Vervaet and Byron 1999; Smyth 2003) due to the absence of an ionic double layer (Pugh *et al.*, 1983; Kitahara, 1974). Non-aqueous formulations are known to be able to carry charges, which can stem from the dissociation of surface groups on particles surfaces, or can result from the adsorption of charged species on the particle surface. Charges on the surface of inhalation drugs have been measured by Sidhu *et al.*, (1993) and Wyatt and Vincent (1992) in CFCs. Measurements were reproducible, and corresponding zeta potentials were much higher than in aqueous systems. The measurement of interparticle forces is particularly difficult with micronised particles as these do not have a defined geometry or surface composition. These interactions can be modulated by modifying the liquid properties, through the addition of excipients for instance.

As well as comparing forces, it is interesting to study interaction energies. If particles have a kinetic energy larger than Brownian energy, suspensions will be driven to aggregate. Another source of instability could be the influence of gravity; particles have a tendency to phase separate; the effect is directly related to size. Reducing the size of the particles in the nanometre range can increase the stability by reducing the gravitational component, as can density matching. The possibility of matching the density of propellant blends with that of micronised triamcinolone acetonide was found to improve pMDI delivery efficiency (Williams and Repka, 1998). Density matching could also be achieved by modifying the particle density. The performance of suspensions can also be improved by using propellant mixtures (HFA 134a with HFA

227) (Williams and Repka, 1998), and the addition of CO_2 (Keller and Herzog, US6461591, 2002) or N_2O (Keller *et al.*, US6475467, 2002), can improve drug delivery.

The problem of drug adhesion observed in control formulations, and which is commonly observed in suspension pMDIs, was successfully overcome by using a polar fluorinated molecule in conjunction with suitable PEG-phospholipids. Within a suspension-based pMDI system, there is a considerable risk for irreversible adhesion of micronised drug particulates to internal canister wall. An increase in the force of adhesion between drug particles and canister walls may result in a decrease in emitted dose (Michael et al. 2001; Young *et al.,* 2003). When considering low dose drugs (such as formoterol fumarate: about 6–12 µg/dose) the possibility of drug loss to the canister wall becomes critical, since the internal surface area of the canister may theoretically approach the projected surface area of the total suspended drug (Rogueda *et al.*, 2003a; Rogueda *et al.*, 2003b). Consequently, a more rational approach into the understanding of these interactions would be beneficial in formulating pMDI suspensions.

The relationship between solubility and chemical structure is poorly understood. Current understanding found no correlation between solubility values and hydrophilic–lipophilic balance parameters (Blondino and Byron, 1998). The best prediction model to date arose from the comparison of octanol/water partition coefficient values with solubility (Dickinson *et al.*, 2000). Thus, solubility in HFAs remains a mystery and hinders further testing and development of stabilisers. Any attempt at understanding structure versus solubility would need to include molecular configuration considerations with respect to the fluorinated atoms. HFAs are polar molecules by virtue of the highly electronegative fluorine atoms (Byron *et al.* 1994). Therefore, orientation of the dipole ($-CF_2-H$) in relation to the solute is important. Most of the surfactants that have a measurable solubility in HFAs tend to be oxygen rich. It has been suggested by Rogueda (2004) that the solubilisation mechanism in HFAs is driven by a non-covalent interaction similar to hydrogen bonding, dubbed halogen bonding, by reference to the work done of Metrangolo *et al.* (2003) on the aggregation of fluorinated and non-fluorinated alkanes. According to this theory, the hydrogen atoms on the HFA act as electron acceptors from the oxygen atoms in the solute. In this context, identifying excipients that might be used as stabilisers is difficult, in addition to the issue about their potential toxicity, or compatibility with the compounds formulated. This also explains ethanol solubilising mechanism in HFA propellants.

The results presented in this thesis are believed to be representative for this kind of

model suspension system, where drug particulates have been proven to have a certain degree of stability in the presence of added stabilisers. It was found that adding PFOH and PEG-phospholipids to drug particulate suspensions in a low polarity media like HFA led to a decrease in inter-particulate cohesion. In addition, the use of such excipients had a positive effect in decreasing particle-particle interactions and drug adhesion. PFOH might be playing the role of any alcohol in the stabilised formulations. It could be forming a layer at the interface, or helping to solubilise some of the drug, or at least acting as a viscosity enhancer. It could be that the phospholipids do provide stability, i.e. prevent agglomeration, and that any extra PFOH helps to modify the viscosity and slow down the phase separation process. With microparticles, phase separation will never be prevented as gravity is always there, it can be slowed down but rarely stopped. We might however be able to prevent agglomeration, that's the likely effect of the phospholipids in these formulations. Yet again these are observations, not quantitative measurements (the polymer adsorbed layer was never measured for instance). When PEG-phospholipids or PFOH were used alone, there was no stability enhancing effect observed. However, that could be drug dependent, for instance salbutamol suspensions are stable even without excipients. Further experiments (in particular phase diagrams and spectroscopy) would be useful to quantitatively study the effect of PFOH and PEG-phospholipids in the pMDI suspensions.

HFA suspensions are almost universally unstable due to the attraction between drug particles, and between drug particles and device surfaces. Because of the difficulties with effective electrostatic stabilisation, there is only one way at present to improve the stability of the suspension, which is through the addition of stabilisers. There is a need for more work to better understand the relationship between chemical structure and solubility, as well as particulate properties and stability. Specifically for HFA pMDIs, the choice between a solution and a suspension is one that rests with the chemistry of the drug and its compatibility with the propellants. Furthermore, formulations are only one part of the drug delivery space; devices and patients also play very important roles.

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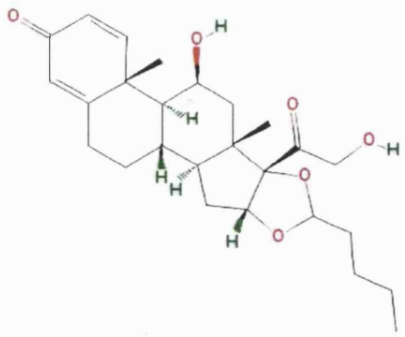
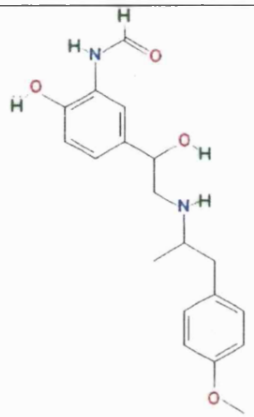
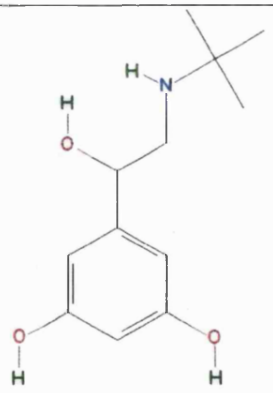
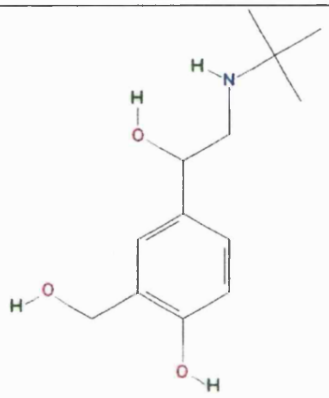
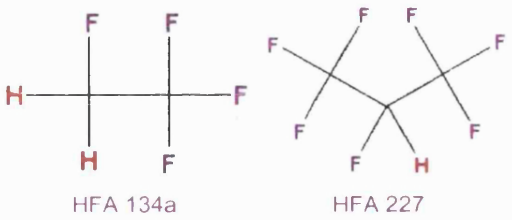
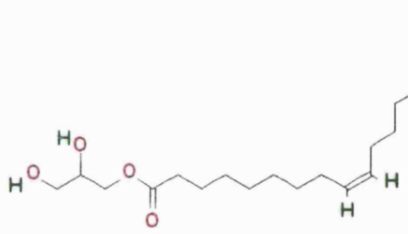
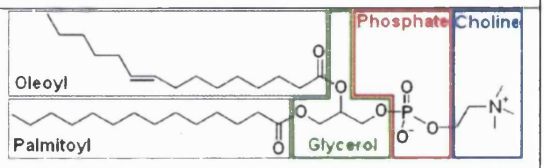
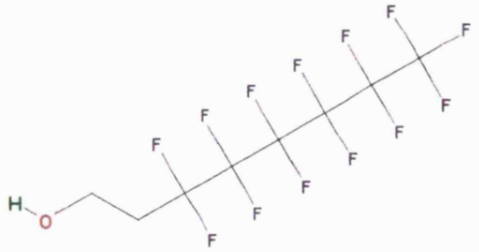
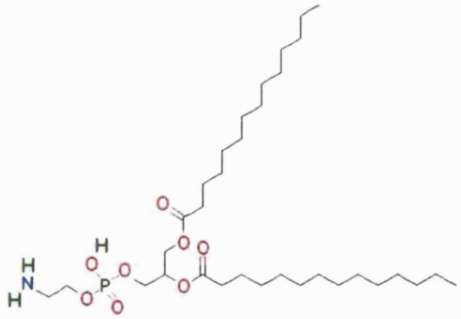
APPENDIX I CHEMICAL STRUCTURES*

COMPOUND PROPERTIES	CHEMICAL STRUCTURE
Compound: Budesonide **Molecular Weight:** 444.56 g/mol **Molecular Formula:** $C_{26}H_{36}O_6$ **LogP:** 2.5 **Hydrogen Bond Donor Count:** 2 **Hydrogen Bond Acceptor Count:** 6 **Rotatable Bond Count:** 5 **Tautomer Count:** 9 **Topological Polar Surface Area:** 93.1	
Compound: Formoterol fumarate **Molecular Weight:** 344.405 g/mol **Molecular Formula:** $C_{19}H_{24}N_2O_4$ **LogP:** 2.2 **Hydrogen Bond Donor Count:** 4 **Hydrogen Bond Acceptor Count:** 5 **Rotatable Bond Count:** 8 **Tautomer Count:** 6 **Topological Polar Surface Area:** 90.8	
Compound: Terbutaline **Molecular Weight:** 225.284 g/mol **Molecular Formula:** $C_{12}H_{19}NO_3$ **LogP:** 1.4 **Hydrogen Bond Donor Count:** 4 **Hydrogen Bond Acceptor Count:** 4 **Rotatable Bond Count:** 4 **Tautomer Count:** 5 **Topological Polar Surface Area:** 72.7	
Compound: Salbutamol **Molecular Weight:** 239.311 g/mol **Molecular Formula:** $C_{13}H_{21}NO_3$ **LogP:** 1.4 **Hydrogen Bond Donor Count:** 4 **Hydrogen Bond Acceptor Count:** 4 **Rotatable Bond Count:** 5 **Tautomer Count:** 3 **Topological Polar Surface Area:** 72.7	

* http://www.chemblink.com; http://www.chemindustry.com

Compound: Hydrofluoroalkane 134a **Molecular Weight:** 102 g/mol **Molecular Formula:** $C_2H_2F_4$ **Compound:** Hydrofluoroalkane 227 **Molecular Weight:** 170 g/mol **Molecular Formula:** C_3HF_7	 HFA 134a HFA 227
Compound: Glycerol monooleate **Molecular Weight:** 356.54 g/mol **Molecular Formula:** $C_{21}H_{40}O_4$ **LogP:** 5.4 **Hydrogen Bond Donor Count:** 2 **Hydrogen Bond Acceptor Count:** 4 **Rotatable Bond Count:** 19 **Topological Polar Surface Area:** 66.8	
Compound: Phosphatidylcholine **Molecular Weight:** 759.578 g/mol **Molecular Formula:** $C_{42}H_{82}NO_8P$	
Compound: 1H,1H,2H,2H-Perfluorooctan-1-ol **Molecular formula:** $C_8H_5F_{13}O$ **Molecular weight:** 364.104 g/mol **Hydrogen Bond Donor Count:** 1 **Hydrogen Bond Acceptor Count:** 14 **Rotatable Bond Count:** 6 **Topological Polar Surface Area:** 20.2	
Compound: 1,2-Dimyristoylphosphatidylethanolamine (DMPE) **Molecular Weight:** 635.853 g/mol **Molecular Formula:** $C_{33}H_{66}NO_8P$ **Hydrogen Bond Donor Count:** 2 **Hydrogen Bond Acceptor Count:** 9 **Rotatable Bond Count:** 35 **Topological Polar Surface Area:** 134	
Compound: 1,2-Distearoylphosphatidylethanolamine (DSPE) **Molecular Weight:** 748.065 g/mol **Molecular Formula:** $C_{41}H_{82}NO_8P$ **Hydrogen Bond Donor Count:** 2 **Hydrogen Bond Acceptor Count:** 9 **Rotatable Bond Count:** 43 **Topological Polar Surface Area:** 134	

APPENDIX II P-VALUES

Table 1 P-values calculated from ANOVA performed for MMAD and FPF means of salbutamol base solution aerosol packs containing ethanol at 5, 10, 15 and 20% w/w.

		ANOVA: P-values	
Formulation (GMO or PC% w/w, salbutamol base% w/w)		**MMAD**	**FPF**
GMO			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.002
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
SPC			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.002
EPC			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.002
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.001
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.9, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.002
1.0, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
1.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
1.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
1.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.001
1.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
1.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
1.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.002
1.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.002
1.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001

Table 2 P-values calculated from ANOVA performed for MMAD and FPF means of budesonide solution aerosol packs containing ethanol at 5, 10, 15 and 20% w/w.

		ANOVA: P-values	
Formulation (GMO or PC% w/w, budesonide% w/w)		**MMAD**	**FPF**
GMO			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.002
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.002
0.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.002
0.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	< 0.001
0.9, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.003	0.001
1.0, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
1.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
SPC			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.001
0.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.001
EPC			
0.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
0.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.001
0.5, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
0.6, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.001
0.7, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001
0.8, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.002
0.9, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	< 0.001
1.0, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
1.1, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.002	0.001
1.2, 0.1	Ethanol (5%, 10%, 15%, 20%)	< 0.001	0.001
1.3, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	0.001
1.4, 0.1	Ethanol (5%, 10%, 15%, 20%)	0.001	< 0.001

Table 3 P-values calculated from Fisher's LSD test for salbutamol base formulations.

		LSD (test): P-values	
Formulation (GMO% w/w, SPC% w/w, EPC% w/w)		**MMAD**	**FPF**
0.1, 0.1, 0.1	Ethanol 5% (GMO, SPC, EPC)	0.296	0.222
0.2, 0.2, 0.2	Ethanol 5% (GMO, SPC, EPC)	0.136	0.318
0.3, 0.3, 0.3	Ethanol 5% (GMO, SPC, EPC)	0.291	0.189
0.4, 0.4, 0.4	Ethanol 5% (GMO, SPC, EPC)	0.239	0.120
0.5, 0.5, 0.5	Ethanol 5% (GMO, SPC, EPC)	0.258	0.325
0.1, 0.1, 0.1	Ethanol 10% (GMO, SPC, EPC)	0.412	0.121
0.2, 0.2, 0.2	Ethanol 10% (GMO, SPC, EPC)	0.329	0.238
0.3, 0.3, 0.3	Ethanol 10% (GMO, SPC, EPC)	0.248	0.313
0.4, 0.4, 0.4	Ethanol 10% (GMO, SPC, EPC)	0.325	0.215
0.5, 0.5, 0.5	Ethanol 10% (GMO, SPC, EPC)	0.652	0.288
0.1, 0.1, 0.1	Ethanol 15% (GMO, SPC, EPC)	0.369	0.297
0.2, 0.2, 0.2	Ethanol 15% (GMO, SPC, EPC)	0.254	0.241
0.3, 0.3, 0.3	Ethanol 15% (GMO, SPC, EPC)	0.146	0.369
0.4, 0.4, 0.4	Ethanol 15% (GMO, SPC, EPC)	0.541	0.111
0.5, 0.5, 0.5	Ethanol 15% (GMO, SPC, EPC)	0.187	0.196

Table 4 P-values calculated from Fisher's LSD test for budesonide formulations.

		LSD (test): P-values	
Formulation (GMO% w/w, SPC% w/w, EPC% w/w)		**MMAD**	**FPF**
0.1, 0.1, 0.1	Ethanol 5% (GMO, SPC, EPC)	0.145	0.136
0.2, 0.2, 0.2	Ethanol 5% (GMO, SPC, EPC)	0.187	0.124
0.3, 0.3, 0.3	Ethanol 5% (GMO, SPC, EPC)	0.291	0.199
0.4, 0.4, 0.4	Ethanol 5% (GMO, SPC, EPC)	0.369	0.219
0.5, 0.5, 0.5	Ethanol 5% (GMO, SPC, EPC)	0.258	0.382
0.6, 0.6, 0.6	Ethanol 5% (GMO, SPC, EPC)	0.269	0.214
0.7, 0.7, 0.7	Ethanol 5% (GMO, SPC, EPC)	0.321	0.114
0.8, 0.8, 0.8	Ethanol 5% (GMO, SPC, EPC)	0.228	0.189
0.1, 0.1, 0.1	Ethanol 10% (GMO, SPC, EPC)	0.297	0.125
0.2, 0.2, 0.2	Ethanol 10% (GMO, SPC, EPC)	0.201	0.225
0.3, 0.3, 0.3	Ethanol 10% (GMO, SPC, EPC)	0.312	0.298
0.4, 0.4, 0.4	Ethanol 10% (GMO, SPC, EPC)	0.336	0.268
0.5, 0.5, 0.5	Ethanol 10% (GMO, SPC, EPC)	0.369	0.352
0.6, 0.6, 0.6	Ethanol 10% (GMO, SPC, EPC)	0.485	0.129
0.7, 0.7, 0.7	Ethanol 10% (GMO, SPC, EPC)	0.129	0.265
0.8, 0.8, 0.8	Ethanol 10% (GMO, SPC, EPC)	0.364	0.226
0.1, 0.1, 0.1	Ethanol 15% (GMO, SPC, EPC)	0.401	0.321
0.2, 0.2, 0.2	Ethanol 15% (GMO, SPC, EPC)	0.259	0.121
0.3, 0.3, 0.3	Ethanol 15% (GMO, SPC, EPC)	0.139	0.118
0.4, 0.4, 0.4	Ethanol 15% (GMO, SPC, EPC)	0.221	0.239
0.5, 0.5, 0.5	Ethanol 5% (GMO, SPC, EPC)	0.349	0.119
0.6, 0.6, 0.6	Ethanol 15% (GMO, SPC, EPC)	0.325	0.201
0.7, 0.7, 0.7	Ethanol 15% (GMO, SPC, EPC)	0.212	0.289
0.8, 0.8, 0.8	Ethanol 15% (GMO, SPC, EPC)	0.112	0.271

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Alouache, A. I., Kellaway, I. W. and Taylor, K. M. G., (2006). Pressurised Metered Dose Inhaler Solution Formulations To Generate Controlled Release Colloids. In: Dalby, R.N., Byron, P.R., Peart, J., Suman, J.D. and Farr, S.J., (Eds), *Respiratory Drug Delivery X.* Interpharm Press, Buffalo Grove, IL.

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