DEVELOPMENT AND EVALUATION OF TERBUTALINE SULPHATE LOADED-BIODEGRADABLE MICROSPHERES FOR PULMONARY DELIVERY

by

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To my father Abdallah, my late mother Hyatt, my aunty saeda, my brothers, my sisters and my husband

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LIST OF ABBREVIATION

µm	**Micron**
MDIs	**Metered dose inhalers**
CFC	**Chlorofluorocarbons**
pMDI	**Propellant-driven metered dose inhalers**
HFAs	**Hydrofluroalkanes**
DPIs	**Dry powder inhalers**
PLA	**Poly(lactic acid)**
PLGA	**Poly(lactide-co-glycolide)**
w/o	**Water-in-oil**
o/w	**Oil-in-water**
w/o/w	**Water-in-oil-in water**
HLB	**Hydrophile-lipophile balance**
TBS	**Terbutaline sulphate**
pK_a	**Acid dissociation constant**
GFR	**Glomerular filtration rate**
Mwt	**Molecular weight**
DCM	**Dichloromethane**
rpm	**Rotation per minute**
w/v	**Weight over volume**
w/w	**Weight over weight**
Sod.oleate	**Sodium oleate**
PBS	**Phosphate buffer solution**
UV	**Ultraviolet**
S.D.	**Standard deviation**
SEM	**Scanning electron microscopy**
FTIR	**Fourier transformed infra-red**
DSC	**Differential scanning calorimetry**
ANOVA	**Analysis of variance**
PVA	**Poly vinyl alcohol**
Tg	**Glass transition temperature**

NGI	**Next generation cascade impactor**
MMAD	**Mass median aerodynamic diameter**
GSD	**Geometric standard deviation**
FPF	**Fine particle fraction**

PEMBANGUNAN DAN PENILAIAN MIKROSFERA TERBIODEGRADASI BERMUATAN TERBUTALIN SULFAT UNTUK PENGHANTARAN PULMONARI

ABSTRAK

Mikrosfera terbutalin sulfat (TBS) pelepasan tertahan dibangun menggunakan polimer PLA R 203H dan PLGA RG 504H. Mikrosfera disediakan menggunakan kaedah emulsi berganda pemeruapan pelarut dan amaun TBS terperangkap dalam mikrosfera ditentukan dengan spectrometer UV. Pengaruh surfaktan (PVA dan natrium oleat) dan gelatin di dalam fasa luar dan pH di dalam fasa internal ke atas ciri-ciri fizikal mikrosfera dikaji. PVA (0.5 and 5%), natrium oleat (0.1 and 0.5%) dan pH (4, 5.8 and 7.4) didapati mempengaruhi ciri-ciri fizikal (saiz, hasil, muatan drug dan kecekapan pemerangkapan) mikrosfera. Sebaliknya, gelatin (25, 50 and 100 mg) tidak ada kesan terhadap ciri-ciri fizikal mikrosfera PLA dan PLGA. Pada keadaan optimum (PVA 0.5%, natrium oleat 0.1% and pH 7.4) muatan drug, kecekapan pemerangkapan, hasil dan purata saiz partikel adalah 0.85%, 34.99%, 87.11% dan 6.55 μm masing-masing untuk PLA dan 0.76 %, 31.17 %, 84.52% dan 8.64 μm masing-masing untuk PLGA. Profil terma DSC memperlihatkan Tg mikrosfera PLA dan PLGA berganjak ke nilai rendah. Tambahan pula, spectrum FTIR juga memperlihatkan anjakan ciri-ciri puncak TBS di dalam mikrosfera. Ini menunjukkan interaksi molekul telah berlaku di antara TBS dengan polimer di dalam mikrosfera. Imbasan mikroskop electron memperlihatkan struktur permukaan mikrosfera PLA lebih berliang daripada PLGA. Tambahan pula, pelepasan drug dari mikrosfera PLA lebih cepat dari pada PLGA. Penambahan gelatin ke dalam formulasi didapati memanjangkan pelepasan drug dari mikrosfera. Pelepasan drug pada fasa

cepat peringkat awal (6 jam) dari PLA dan PLGA tanpa gelatin masin-masing adalah 76.31% and 55.4% dan selebihnya dibebaskan dalam masa 24 jam and 72 jam. Sebaliknya, pelepasan drug pada fasa cepat peringkat awal dari PLA dan PLGA dengan gelatin masing-masing adalah 35.4% and 22.4% dan selebihnya dibebaskan dalam masa 144 jam dan > 144 jam. Pelepasan drug dari mikrosfera PLA dan PLGA tanpa gelatin mengikut kinetik tertib pertama. Walaubagaimanapun, pelepasan drug dari PLA dan PLGA dengan gelatin masing-masing mengikut kinetik Higuchi dan bi-eksponential tertib pertama. Purata saiz aerosol (MMAD) PLA terhidrat semula (2.53 μm) dan PLGA terhidrat semula (3.50 μm) yang dihasilkan menggunakan nebulizer lebih kecil daripada MMAD PLA (11.10 μm) dan PLGA (11.47 μm) yang dihasilkan oleh Rotahaler. Sebagai tambahan, FPF dari PLA (49.54%) and PLGA (37.50%) yang dihasilkan oleh nebulizer lebih tinggi daripada FPF dari PLA (11. 89%) and PLGA (10.57%) yang dihasilkan oleh Rotahaler. Sebagai kesimpulan, mikrosfera bermuatan TBS adalah formulasi yang terbaik untuk TBS pelepasan tertahan penghantaran pulmonari menggunakan nebulizer.

DEVELOPMENT AND EVALUATION OF TERBUTALINE SULPHATE LOADED-BIODEGRADABLE MICROSPHERES FOR PULMONARY DELIVERY.

ABSTRACT

Sustained-release terbutaline sulphate (TBS) microspheres were developed using PLA R 203H and PLGA RG 504H polymers. The microspheres were prepared using the double emulsion solvent evaporation method and the amount of TBS entrapped in the microspheres was determined by UV spectrometry. The influence of surfactants (PVA and sodium oleate) and gelatin in the external phase and the pH in internal phase on the physical characteristics of the microspheres were investigated. PVA (0.5 and 5%), sodium oleate (0.1 and 0.5%) and pH (4, 5.8 and 7.4) were found to influence the physical characteristics (size, yield, drug loading and entrapment efficiency) of the microspheres. Conversely, gelatin (25, 50 and 100 mg) had no effect on the physical characteristics of both PLA and PLGA microspheres. At optimum level (PVA 0.5%, sodium oleate 0.1% and pH 7.4) the drug loading, entrapment efficiency, yield and mean particles size of PLA were 0.85%, 34.99 %, 87.11% and 6.55 µm while that of PLGA were 0.76%, 31.17%, 84.52% and 8.64 µm respectively. The DSC thermal profiles revealed that the Tg of PLA and PLGA microspheres shifted to a lower value when TBS incorporated into microspheres. Moreover, the FTIR spectra also showed a shift in the characteristic peak of TBS in microspheres. This indicates that molecular

interaction had occurred between TBS and polymers within the microspheres. The scanning electron microscope revealed that the surface structure of PLA was more porous than that of PLGA microspheres. Furthermore, the release of drug from PLA microspheres was faster than from PLGA microspheres. An addition of gelatin to the formulation was found to prolong the release of drug from the microspheres. Drug release at the initial rapid phase (6 h) from PLA and PLGA without gelatin was 76.31% and 55.4% respectively while the remaining amount was released within 24 h and 72 h respectively. In contrast, drug release at the initial rapid phase from PLA and PLGA with gelatin was 35.4% and 22.4% respectively while the remaining amount was released within 144 h and >144 h respectively. The drug release from both PLA and PLGA microspheres without gelatin fitted first order release kinetics model. However, drug release from PLA and PLGA with gelatin followed the Higuchi and bi-exponential first order release kinetics models respectively. The mean aerosols size (MMAD) of rehydrated PLA (2.53 μm) and rehydrated PLGA (3.50 μm) generated using nebulizer were smaller than the MMAD of PLA (11.10 μm) and PLGA (11.47 μm) produced by a Rotahaler. In addition, the fine particle fraction (FPF) of PLA (49.54%) and PLGA (37.50%) aerosolized by a nebulizer were higher than the FPF of PLA (11.89%) and PLGA (10.57%) produced by a Rotahaler. In conclusion, TBS- loaded PLA microspheres is a promising candidate for pulmonary delivery of sustained-release TBS using a nebulizer.

CHAPTER 1: GENERAL INTRODUCTION

1.1 RESPIRATORY SYSTEM

The human respiratory system is a complicated organ system of very close structure-function relationships. The system consists of two regions: the conducting airway and the respiratory region. The airway is further divided into many folds: the nasal cavity and the associated sinuses; the nasopharynx, oropharynx, trachea, bronchi and bronchioles. The respiratory region consists of respiratory bronchioles, alveolar ducts and alveolar sacs (Travis *et al*., 1999).

$Beta_2$-adrenergic receptors exist throughout the airways such as on the epithelium, smooth muscle, alveoli and specialized cell types including on the Clara and mucus-secreting cells (Nijkamp, 1993). The existence of epithelial β_2 -receptors is of particular importance to respiratory function, as it mediates various functions in man and animals including smooth muscle relaxation, clearance of alveolar fluid, influence of ion fluxes, as well as modulating the release of bronchodilating mediators (Abraham *et al*., 2003).

The respiratory system has very efficient defence and clearance mechanisms for foreign particles and infectious agents inhaled on inspiration. The nose traps almost all particles with an aerodynamic diameter of more than 5 μm (Fig 1.1). Aerodynamic diameter refers to the way particles behave in air rather than to their actual size. The mucociliary blanket of the airway epithelium disposes of particles with an aerodynamic diameter 3-5 μm. The ciliary beat drives the mucous blanket toward the trachea, and particles that land on the mucociliary

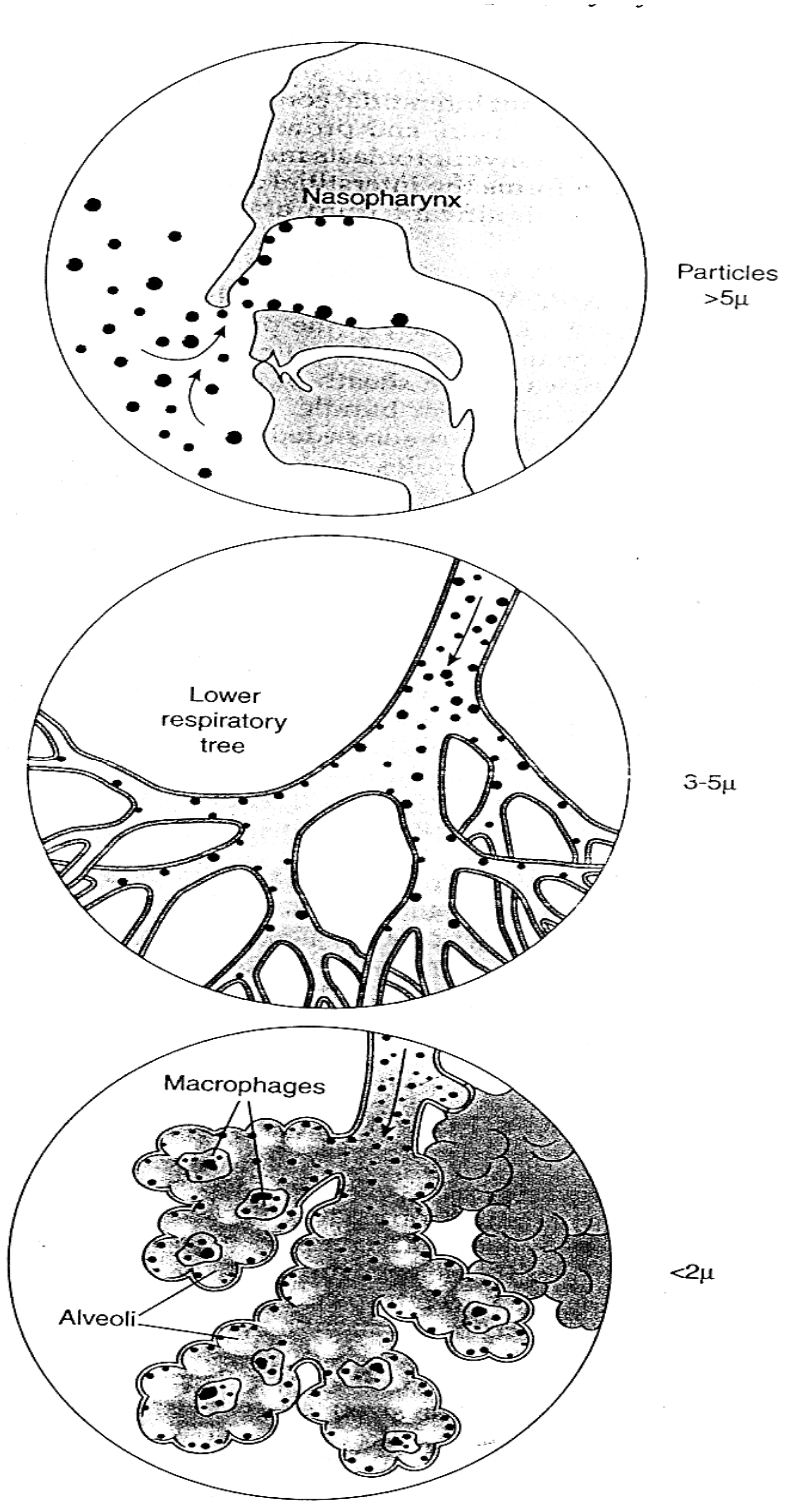

Figure 1.1 Deposition of particles in the respiratory tract (adapted from Travis *et al*., 1999).

blanket of the airway epithelium are thus removed from the lungs and swallowed or coughed up. Alveolar macrophages protect the alveolar space by phagocytizing viable and non-viable particles which have aerodynamic diameter less than 2 μm. Very small particles that behave like gas are not phagocytosed but exhaled (Travis *et al*., 1999). Nevertheless, Everard (2003) reported that by generating airborne particles in 1-5 μm range they were able to exploit a relative weakness in the pulmonary defence mechanisms and deposit foreign materials relatively effectively in the lung.

1.2 ASTHMA

Asthma is a heterogeneous common chronic condition characterized by endobronchial inflammation with consequent bronchial hyperesponsiveness (Currie *et al*., 2005). This leads to variable airflow obstruction and typical symptoms such as cough, breathlessness, chest tightness, wheezing and reduced exercise tolerance. The precise aetiology of asthma remains uncertain, but genetic and environmental factors such as viruses, allergen exposure, early use of antibiotics, and the numbers of siblings have all been implicated in its inception and development (Sandford *et al*., 2000).

1.2.1 Drugs used for treatment of asthma

Beta$_2$-adrenoceptor agonists and glucocoticoids are at present the most effective drugs for the treatment of airway obstruction and inflammation, with theophylline, leukotriene receptor antagonists and anticholinergics functioning as second- or third-line therapy. For decades, there has been no newly developed drug available to supplement or even replace beta$_2$-adrenoceptor

agonists or glucocorticoids (Rabe and Schmidt, 2001). However, a combination therapy consisting of inhaled corticosteroids and long acting beta$_2$-adrenoceptor agonists (single-inhaler combination product) e.g. budesonide/formoterol and salmeterol/fluticasone can be considered as a new addition to the pharmacological management of asthma (Balanag *et al*., 2006). Recently, efforts have been made to develop a dry powder inhalation system consisting of vasoactive intestinal peptide analogue (IK312532-DPI) to treat pulmonary diseases such as asthma (Ohmori *et al*., 2006).

1.2.1(a) Bronchodilators

Beta$_2$-adrenoceptor agonists such as salbutamol and terbutaline have been the standard therapies for the symptomatic treatment of asthma (Mohammed *et al*., 2000). At present, good clinical and experimental experience with short (e.g. fenoterol, salbutamol and terbutaline) and long acting (e.g. salmeterol and formoterol) beta$_2$-adrenoceptor agonists seems to suggest that it is rather unlikely that novel bronchodilators which are better tolerated and more effective will be developed. Beta$_2$-adrenoceptor agonists are believed to cause airway smooth muscles to relax by increasing intracellular levels of cyclic adenosine monophosphate and opening potassium channels. Attempts have been made to imitate these effects with other substances such as nonselective phosphodiesterase inhibitors and potassium channel openers. However, these drugs were shown to be far less effective as bronchodilators compared to beta$_2$-adrenoceptor agonists, and their application at higher doses in order to cause smooth muscle relaxation was limited with marked side-effects (Rabe and Schmidt, 2001).

1.2.1(b) Anti-inflammatory drugs

Airway tissue inflammation is considered to be the main mechanism in the development and maintenance of asthma. So, limiting exposure to inflammatory

triggers and reducing the inflammatory process using anti-inflammatory drugs are the main thrusts in the management of asthma. The first-line anti-inflammatory drugs of inhaled corticosteroids (e.g. budesonide, beclometasone dipropionate, flunisolide and fluticasone), may be adequate to fully control symptoms in mild cases (Sears and Lotvall, 2005). However, for many patients, additional drug therapy, typically long acting $beta_2$-adrenoceptor agonists that relax the smooth muscle in the airway, is needed for long term treatment of moderate to severe asthma (Barnes, 2006; Sin and Paul Man, 2006).

1.2.1(c) Cromones and other drugs

Cromones including disodium cromoglycate and nedocromil have been used in the treatment of asthma for many years. Clinical trials in children and adults with asthma have shown that inhaled corticosteroids (such as fluticasone propionate) and cromones (such as nedocromil) alleviate asthma symptoms, lung dysfunction and decrease nonspecific bronchial hyperreponsiveness (Vatrella *et al*., 2002). In addition, other drugs such as methylxanthines, leukotriene receptor antagonists, anti-cholinergics and antihistamines have also been used in the treatment of asthma.

1.2.2 TREATMENT OF ASTHMA VIA INHALATION AEROSOLS

Aerosols are an effective method to deliver therapeutic agents to the respiratory tract (Sham *et al*., 2004). Nebulizers, metered dose inhalers, or dry powder inhalers are commonly used for this purpose (Cohn *et al.,* 2003; Dalby and Suman, 2003).

1.2.2(a) Nebulizers

A nebulizer is a device designed for the purpose of producing an aerosol (Fig 1.2). The device works by converting liquid asthma medication into aerosol droplets. The droplets are then inhaled into the lower respiratory tract through a mask worn over the nose and mouth of a patient. Nebulizers can be classified into two categories namely, jet nebulizers and ultrasonic nebulizers. Several advantages of nebulizers have been reported:

- Some patients like infants, young children and elderly patients cannot master the coordinated effort needed to correctly use the metered dose inhaler, or dry powder inhalers (O´Driscoll, 1997).
- Some patients feel more comfortable using nebulizers, enjoying the way the mist feels in their lungs (Win and Hussain, 2005).
- The inhaled droplets produced by nebulizers may alter the mucus viscosity in the airways and a nebulized drug or saline solution may help patients with bronchiectasis to expectorate (Sutton *et al*., 1988).

Although the use of nebulizers is encouraging, nebulizers exhibit certain disadvantages in that they are cumbersome to use and costly.

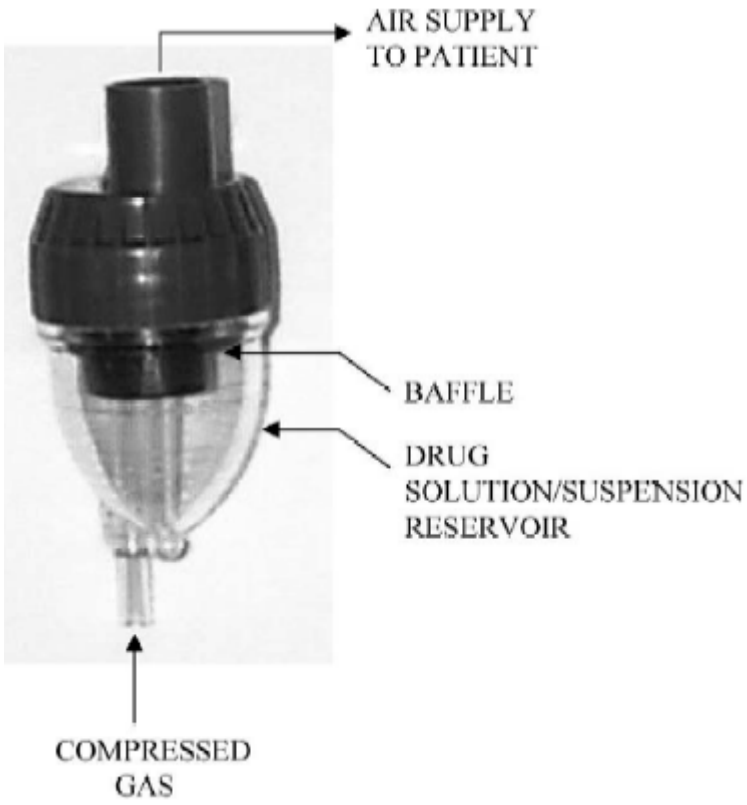

Figure 1.2 Example of an Air jet nebulizer (adapted from Dalby and Suman, 2003).

1.2.2(b) Metered dose inhalers (MDIs)

Metered dose inhalers are a well-known dosage form for treatment of respiratory diseases (Fig 1.3). Aerosolized beta agonists and anti-allergic compounds were first formulated as pharmaceutical aerosols in 1956 using chlorofluorocarbons (CFCs). The MDI formulation comprises an active ingredient and one or more propellants. In addition, it may also contain formulation additives, such as surfactants and co-solvents. The propellant system is the main ingredient in MDI formulations and serves as a solvent and dispersion medium for drug substance and other excipients. It also serves as an energy source for generating an aerosol cloud on actuation while the dose is emitted from the metering valve (Williams *et al*., 1998).

CFC propellants possess several desirable characteristics that have made them an excellent choice for use as metered dose inhaler propellants. They are chemically stable and as a result, are not metabolized but are instead rapidly re-emitted into the atmosphere when the patient exhales. CFC propellants offer the additional advantage of extremely low toxicity and are not flammable at atmospheric pressure and temperatures (Kempsford *et al*., 2005). Moreover, they are inexpensive to produce and have been widely available since the 1970s. However, scientific research has unearthed substantial evidence that CFCs and other chlorine-containing chemicals contribute to the depletion of the stratospheric ozone layers (Molina and Rowland, 1974). CFCs production in the United States came to a virtual stop on January 1, 1996, when the use of CFC in air-conditioning, refrigeration and

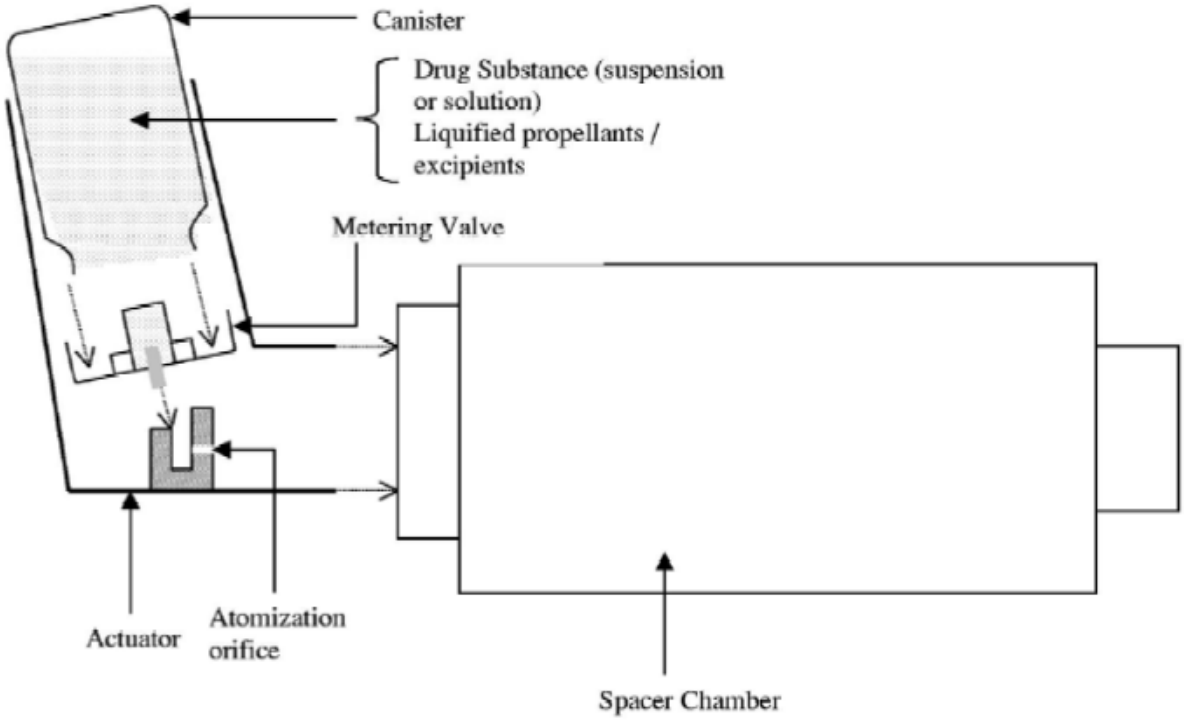

Figure 1.3 Basic components of a pMDI (adapted from Smyth, 2003).

production of foam was phased out. However, production of pharmaceutical-grade CFCs for use in MDIs, has continued. This is because MDIs are considered essential for the health of patients with asthma and chronic obstructive pulmonary disease. Efforts have been made to consider an alternative to ozone-depleting CFC using other classes of environmental friendly propellant such as hydrofluroalkanes (HFAs). For instance, HFAs propellants (e.g. HFA-133a and HFA-227) have been recommended to be used in the delivery of inhaled medication. This is because in most cases, HFAs meet safety standards and are found to be as effective as their predecessor, the CFC propellant. As a result, many MDIs containing CFC were replaced by HFAs for example salmeterol (Chopra *et al*., 2005).

1.2.2(c) Dry powder inhalers (DPIs)

The requirement to replace ozone-depleting CFCs propellants, has led to the pharmaceutical industry re-evaluating the potential of dry powder inhalers. However, the delivery efficiency of DPI currently is not high, as in some cases only 7-30% of the inhaled dose of the drug are deposited in the lung depending upon the devices or brands used (i.e. Spinhaler®, Diskhaler®, Rotahaler® (Fig 1.4), Turbuhaler® and Novolizer®) (O´connor, 2004). The site of deposition and deposition patterns of the inhaled aerosol from DPIs are influenced by two major interdependent factors: (a) the patient (anatomical and physiological aspects of the respiratory tract as well as mode of inhalation) and (b) the physical properties of the aerosol cloud (Timsina *et al*., 1994). However, dry powder inhaler posses several advantages over other delivery methods. They are propellant-free, portable, easy to operate and low-

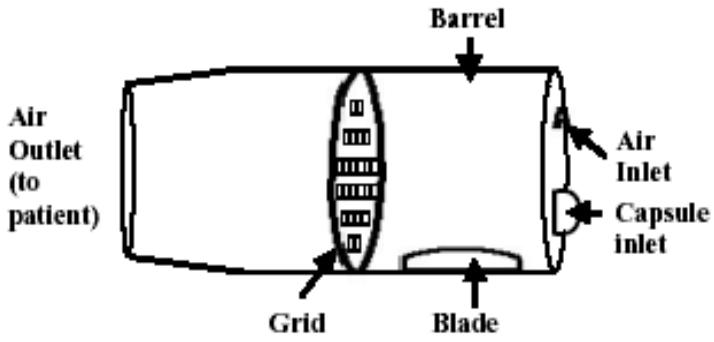

Figure 1.4 Schematic diagram of the Rotahaler device (adapted from Chew *et al*., 2002).

cost devices. Moreover, the stability of the formulation is improved as a result of the dry state (Bosquillon *et al*., 2001).

1.3 ADVANTAGES OF PULMONARY DRUG DELIVERY

The pulmonary route of drug administration delivers adequate therapeutic levels of potent bronchodilators in respiratory tract and provides a better clinical response whilst reducing their distribution to other organs (Lai *et al*., 1993). This route provides an excellent example of targeted drug therapy. Indeed, aerosol delivery has long been viewed as a promising technique in the treatment of lung cancer (Koshkina *et al*., 2003).

The lung has a large surface area (up to 100 m^2), thin absorptive barrier, low enzymatic metabolic activity, and good blood supply. These characteristics make the lung an attractive target for the non-invasive administration of aerosolized systemically-active peptide and protein drugs (Yamamoto *et al*., 2005).

The pulmonary delivery route as well as nasal, rectal, and oral routes, have attracted much attention, in attempts to improve the quality of patients lives, because no repeated injections are required (Kawashima *et al*., 1999). Contrary to the oral route of drug administration, pulmonary inhalation is not subject to first pass metabolism. Therefore, expensive biotechnology drugs like toxic chemotherapeutics are ideal drug candidates for local pulmonary administration (Sharma *et al*., 2001).

The main advantage of the treatment of the respiratory tract diseases via inhalation aerosols therapy is that a relatively low drug concentration reaches systemic circulation. Consequently, the intensity and incidence of the side effects of inhaled drugs is, in many instances, markedly reduced compared to administration via the oral route (Tsapis *et al*., 2003).

Several drugs such as bronchodilators, anti inflammatory agents, mucolytics, anti viral agents, antibiotics agents, and pentamidine are all routinely given as aerosolized formulations (BNF 43, 2002). In addition, a number of drugs for example insulin, cyclosporin, interferon, antitrypsin, protease inhibitors, deoxyribonucleases, recombinant adenoviruses and others have been reported to have high potential for delivery via the respiratory route (Waldrep *et al*., 1998; Karathanasis *et al*., 2005).

1.4 SUSTAINED RELEASE MICROSPHERES FOR PULMONARY DRUG DELIVERY

The advantages of sustained release drug delivery to the respiratory tract are numerous. They include extended duration of action, reduction in drug use, improved management of therapy, improved compliance and reduction in side effects (Zeng *et al*., 1995). Moreover, lower dosage regimens may provide considerable cost savings especially those that involve expensive therapeutic agents (Saks and Gardner, 1997). A number of methods have been investigated as potential pulmonary sustained release systems for short acting drugs. These include the incorporation of drugs in liposomes and other biodegradable microspheres (Zeng *et al*., 1995).

The efficacy of a liposomal sustained release delivery system to the respiratory tract has been proven by Juliano and McCullough (1980). They showed that the chemotherapeutic agent, arabinoside entrapped within liposomes had a longer half-life of release in the lungs than did a free drug (8 versus 1 h, respectively).

Furthermore, retention of liposomes within the lung provided more specific pharmacological activity and minimized systemic exposure (reduced gastrointestinal and myelotoxic side-effects). In another study, Taylor *et al*. (1989) showed that liposomal disodium cromoglycate administered to healthy human volunteers were still detectable at 25 h, whereas an equivalent dose of drug inhaled as a solution was not detected within the same period. This investigation clearly shows the applicability of liposome-mediated pulmonary sustained release in humans. Nebulisation of liposomes, however, can cause its structural disruption with the resultant release of the encapsulated drug. Even at low temperatures, liposomes are unstable during storage thus limiting their practicality as commercial formulations (Taylor *et al*., 1993). However, dry liposome powders containing corticosteroids have been developed for inhalation and they have been observed to have improved stability (Darwis and Kellaway, 2001).

1.4.1 Biodegradable microspheres as drug delivery systems

Microspheres are defined as homogeneous, monolithic particles measuring about 0.1-1000 μm and are widely used as drug carriers for controlled release. Microspheres have significant importance in biomedical applications

as the administration of drugs in the form of microspheres usually improves treatment through the localization of active substance at the site of action thus enabling prolonged drug release. Furthermore, sensitive drugs such as peptides and proteins may be protected against chemical and enzymatic degradation when entrapped in microspheres (Crotts and Park, 1997).

Biodegradable microspheres produced from natural and synthetic polymers have been extensively investigated as drug transporters via a number of different routes. A number of these particles have many desirable characteristics for ensuring both targeted and sustained drug release. Another characteristic is that biodegradable microspheres can be prepared over a wide range of particle sizes, which is a decisive factor in the *in vivo* deposition of particulate carriers. Accordingly, biodegradable microspheres can be used to deliver drugs to various organs, such as the liver, the kidney, the reticuloendothelial system and the lungs.

1.4.2 Biodegradable polymers for microspheres formulation

A wide variety of natural and synthetic biodegradable polymers have been investigated for use in drug targeting or prolonged drug release. Natural polymers remain attractive primarily because they are natural products of living organisms readily available, relatively inexpensive and capable of a multitude of chemical modifications. The majority of investigations into the use of natural polymers as matrices in drug delivery systems have centered on proteins (e.g. collagen, gelatin, and albumin) and polysaccharides (e.g. starch, dextran, inulin, cellulose and hyaluronic acid) (table 1.1) (Hincal and Calis,

2000). Collagen has unique structural properties, therefore, it has been fabricated into wide variety of forms including crosslinked films, meshes, fibres and sponges. However, certain properties of collagen have adversely influenced its use as a drug delivery vehicle. These properties include poor dimensional stability due to swelling *in vivo*, poor *in vivo* mechanical strength and low elasticity, possible occurrence of an antigenic response, tissue irritation due to residual aldehyde crosslinking agents, and variability in drug release kinetics (Sinha *et al*., 2003). Apart from this, non-collagenous proteins like albumin, gelatin, casein, fibrinogen in the form of microspheres and nanoparticles continue to be exploited as drug delivery systems. The development of collagen has been some what overshadowed by advances made in both synthetic absorbable polymers (e.g. poly lactide and poly glycolide) and non-absorbable polymers such as silicone rubber and hydrogels. The most widely used and studied class of biodegradable polymers has been polyesters, including poly(lactic acid) (PLA) which was investigated as a drug delivery material as early as 1971, poly(glycolic acid) (PGA), first marketed in 1970 as a biodegradable suture, and poly(lactide-co-glycolide) (PLGA). By varying the monomer ratios in polymer processing conditions, the resulting polymer can exhibit drug release capabilities for months or even years (Matschke *et al*., 2002). PGA is the most hydrophilic member of the poly (α-ester) series and is insoluble in organic solvents. In contrast, PLA is amorphous and more hydrophobic than PGA, owing to the extra methyl group in its structure (Fig 1.5) and is thus a good candidate for drug matrix release. It is available in the form of D(-), L(+), and racemic (DL) (Conti *et al*., 1992). Ramachandani and Robinson (1998) reported that PLGA had been

Table 1.1. EXAMPLES OF BIODEGRADABLE POLYMERS USED IN DRUG DELIVERY SYSTEMS

Natural polymers			Synthetic polymers		
(i) Animal		(ii) Plant	(i) Animal		(ii) Plant
Proteins	Polysaccharides	Polysaccharides	Proteins	Polysaccharides	Polysaccharides
Albumin	Chitin	Starch	Poly(lactic/glycolic acid)	Poly(ε-caprolactone)	Polyalkylcyanoacrylate
Collagen	Chitosan	Dextrin		Polyanhydrides	
Gelatin	Hyaluronic acid	Dextran			
Fibrinogen	Poly(β-hdroxybutyric acid)	Alginic acid			
Casein		Poly(ortho esters)			
Fibrin					
Poly(lactic acid)					

extensively used in biomaterial applications such as tracheal replacement, ligament reconstruction, surgical dressings, and dental repairs as well as functioning as transporters in drug delivery systems. Various classes of drugs such as anticancer agents (Hussain *et al*., 2002), antibiotics (Atkins *et al*., 1998; Gavini *et al*., 2004), antimalarials (Schlicher *et al*., 1997), and local anesthetics Le Corre *et al*., 1997) have been incorporated into poly(lactic acid) or poly(lactide-co-glycolide).

(a) (b)

Figure1.5. Chemical structure of (a) poly(lactic acid) and (b) poly(glycolic acid) (adapted from Hincal and Calis, 2000).

1.4.3 Microspheres preparation

Several processes are available for the preparation of drug-loaded, biodegradable microparticulates. The selection of the technique depends on the nature of the polymer, the drug and the intended use. In preparing for the controlled release microspheres, the choice of the optimal method to use is of utmost importance to ensure efficient entrapment of the active substance in

microspheres (Hincal and Calis, 2000). Some pharmaceutically acceptable microencapsulation techniques using hydrophobic biodegradable polymers such as poly(lactide-co glycolide) and poly(lactic acid) as matrix materials for microspheres preparation include emulsion solvent evaporation and solvent extraction process, phase separation (coacervation) and spray drying (Jain, 2000).

1.4.3.1 Emulsion solvent evaporation methods

The emulsion solvent evaporation method is the most common process for microspheres preparation as it is simple, reproducible and economical (Goto *et al*., 1984). The method consist of single emulsion solvent evaporation and double emulsion solvent evaporation procedures.

1.4.3.1(a) Single emulsion solvent evaporation procedure

Single or simple emulsions are classified according to the nature of their continuous or dispersed phase, i.e. as either water-in-oil (w/o) or oil-in-water (o/w) emulsions. An emulsifier is present in each system (w/o or o/w) to stabilize the emulsion. The procedure consists of dissolving the polymer in a volatile organic solvent such as methylene chloride. The drug to be encapsulated into the microspheres is then either dissolved or suspended in the same solution. The mixture is then emulsified in an aqueous phase containing an emulsifier that does not solubilize the polymer. Various types of ionic surfactants e.g. sodium oleate, and non ionic surfactants e.g. Span 80, Tween 80, and polyvinyl alcohol are used as emulsifiers. Lipophilic (oil-soluble, low HLB) surfactants are used to stabilized w/o emulsions whereas

hydrophilic (water-soluble, high HLB) surfactants are used to stabilize o/w emulsions. As the w/o or o/w emulsion is formed, the solvent is allowed to evaporate from the microparticles formed, leaving behind solid microparticles containing the drug. Ultimately, the microparticles are isolated by centrifugation or filtration and are subsequently lyophilized (Jalil and Nixon, 1989; Jalil and Nixon, 1990; Atkins *et al*., 1998).

1.4.3.1(b) Double emulsion solvent evaporation procedure

Double emulsions have promising applications in the food, cosmetic, and pharmaceutical industries, as well as in other fields like agriculture and microsphere production (van der Graaf *et al*., 2005). There are two main types of double emulsion: water-in-oil-in-water (W/O/W) emulsions, and oil-in-water-in-oil (O/W/O) emulsion. W/O/W emulsion is more common than O/W/O emulsion. The technique comprises four steps: (1) primary emulsification: an aqueous solution of the active agent (internal water phase, W1) is emulsified into an organic solution containing the biodegradable polymer and lipophilic surfactant (oil phase, O); (2) re-emulsification: the primary emulsion (W1 / O) is further emulsified into a second aqueous phase containing a stabilizer (external water phase, W2) to form a W1/ O/ W2 double emulsion; (3) solidification: the organic solvent is removed by evaporation or extraction and solid microparticles are formed; (4) separation and purification: the microparticles are collected by centrifugation or filtration and are subsequently lyophilized (Meng *et al*., 2003).

1.4.3.2 Phase separation (coacervation) method

The coacervation process is mainly used to encapsulate water-soluble drugs like peptides, proteins, and vaccines. Proteins encapsulated by this technique include diphtheria toxoid (Johansen *et al*., 1999). In the coacervation process, the drug is dispersed in solid form into a solution containing a solvent and polymer. Silicon oil or light liquid paraffin is added to this dispersion phase at a defined rate, reducing the solubility of the polymer in its solvent. The polymer-rich liquid phase (coacervate) encapsulates the dispersed drug particles and embryonic microspheres are subjected to hardening and washing step using heptane or hexane.

1.4.3.3 Spray drying method

In the spray drying method, the biodegradable polyesters are dissolved in a volatile organic solvent, such as dichloromethane or acetone. The drug in solid form is dispersed in the polymer solution by high speed homogenization, and then atomized in a stream of heated air to form droplets. The solvent evaporates instantaneously yielding microspheres ranging from 1 to 100 μm in size depending on atomizing conditions. The microspheres are collected from the airstream by a cyclone separator. Residual solvent is then removed by vacuum drying. The process can be operated under aseptic condition, and in closed loop configurations. Besides this, spray drying in a nitrogen atmosphere is also technically feasible. The important advantages of this technique over other encapsulating techniques are its proven reproducibility, well-defined control of particle size, controlled drug release properties of resulting microspheres and tolerance to small changes in polymer

specifications. The disadvantages include high capital investment. Besides this drawback, encapsulation of protein using this method requires lyophilisation of protein before dispersion and homogenization in organic polymer solution. These processing conditions are likely to induce aggregation and denaturation of sensitive proteins and antigens. Protein encapsulated by this technique includes recombinant human erythropoietin (Bittner *et al*., 1998).

1.5 PULMONARY DEPOSITION OF AEROSOL

The aerosolized particles or droplets will deposit in the airways by gravitational sedimentation, inertial impaction and diffusion (Biddiscombe *et al*., 2003). Inertial impaction in the lung occurs when particles of sufficient momentum are unable to follow the curved streamlines of air within the airways during inhalation due to centrifugal forces in curved tubes. In contrast, sedimentation of particles within the airways often occurs due to the action of gravity, while deposition in the airways via diffusion is pronounced with ultrafine aerosols. In general, larger drug particles (particles > 1 μm in diameter) are deposited by the first two mechanisms in the airways while smaller particles (particles < 0.1 μm in diameter) get into the peripheral region of the lungs by diffusion (Martonen *et al*., 2002). In order to achieve optimal drug delivery to the lungs aerosol particle size and mode of inhalation are the two most important factors to be considered (Biddiscombe *et al*., 2003). Four types of methodology are used for assessing lung deposition of inhaled particles: *in vitro* particle-sizing methods, direct assay of drug concentrations

in the lung tissue, pharmacokinetic studies and lung imaging methodology (Snell and Ganderton, 1999; Mobley and Hochhaus, 2001).

1.5.1 *In vitro* particle-sizing methods

In vitro measurements of the aerodynamic diameter of a particle using particle-sizing apparatus (e.g. cascade impactors) is vital in determining its respiratory deposition (Wiggins, 1991). The most frequently used *in vitro* particle-sizing methods are based on the cascade impaction technique. The technique enables the collection and fractionisation of an aerosol cloud through a simulated throat in a manner which imitates the *in vivo* situation (Timsina *et al*., 1994). Cascade impactor utilizes the relationship between velocity and mass wherein larger particles impact on the upper stages of the chamber with finer particles reaching the lower stages of the chamber (Timsina *et al*., 1994). Another advantage of the method is that it is the simplest and cheapest method to assess lung deposition of inhaled medication.

1.5.2 Direct assay of drug in lung tissue

The lung deposition of inhaled particles can be determined directly in animals by using aerosols labelled with a dye or through radioactivity, and examining the lungs directly or by autoradiography after dissection. However, such animal studies are expensive, time consuming and limiting due to the lack of suitable animal models for examining lung deposition of inhaled medication (McConville *et al*., 2000).

1.5.3 Pharmacokinetic studies

Pharmacokinetic study is the most commonly used method to assess lung deposition *in vivo*. It is easy to perform, but limited due to problems in trying to relate the amount of drug in plasma or urine to total lung deposition. A sizeable proportion of the drug present in plasma may be due to gastrointestinal absorption of the swallowed fraction resulting from orally deposited dose and possible drug metabolism (Lindström *et al*., 2004). Börgstrom and Nilsson (1990) quantified the percentage of inhaled doses deposited in the lung utilizing Charcoal-block method. The method involved the swallowing of charcoal slurry to block gastrointestinal uptake of the orally deposited drug. Lindström *et al*. (2004) utilized the pharmacokinetic method to measure salbutamol concentration in plasma collected over the first 20 min post-inhalation or the amount excreted in urine during the first 30 min post-inhalation. However, Snell and Ganderton (1999) reported that urine or plasma pharmacokinetic at best would only reflect the total lung drug deposition, without providing information about regional patterns of deposition.

1.5.4 Lung imaging

The total lung deposition and the regional deposition pattern of inhaled medication can be quantified using radionuclide imaging methods (Newman *et al*., 2000). The test formulation must be radiolabelled with a gamma-emitting isotope such as ^{99m}Tc and after inhalation the thorax is scanned using external imaging (Snell and Ganderton, 1999). The imaging techniques used include planner (2-D) gamma scintigraphy, single photon emission computed