FORMULATION AND EVALUATION OF RIFAMPICIN-LOADED POLYMERIC PARTICLES FOR PULMONARY DELIVERY

By

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This thesis is dedicated to …

My late father, my mother, my late brother, my wife and my sons

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LIST OF ABBREVIATION

Tuberculosis (TB)

World Health Organization (WHO)

Directly observed therapy, short-course (DOTS)

Isoniazid (H)

Rifampicin (R)

Pyrazinamide (Z)

Streptomycin (S)

Ethambutol (E)

Antitubercular drugs (ATD)

Metered dose inhalers (MDIs)

Dry powder inhalers (DPIs)

Chlorofluorocarbon (CFC)

Hydrofluoroalkanes (HFAs)

Poly (Lactic acid) (PLA)

Poly (glycolic acid) (PGA)

Poly (lactic-co-glycolic acid) (PLGA)

methoxypolyethyleneglycol distearoyl-phosphatidylethanolamine (mPEG-DSPE)

Food and drug administration (FDA)

Oil in water (O/W)

Water in oil (W/O)

Water in oil in water (W/O/W)

Oil in oil (O/O)

Polyvinyl alcohol (PVA)

Drug Loading (DL)

Entrapment efficiency (EE)

Scanning electron microscopy (SEM)

Transmission electron microscopy (TEM)

Photon correlation spectroscopy (PCS)

Volume mean diameter D[4, 3]

Mass median diameter D(v, 0.5)

The size of particle for which 10% of the sample is below this size D(v, 0.1) (the Size of particle for which 90% of the sample is below this size) D(v, 0.9)

Differential scanning calorimetry (DSC)

Fourier transformed infrared (FTIR)

Glass transition temperatures (Tg)

Exothermic crystallization (Tc)

Mass median aerodynamic diameter (MMAD)

Geometric standard deviation (GSD)

Emitted dose (ED)

Fine particle fraction (FPF)

Effective cut-off diameter (ECD)

Oleic acid-albumin-dextrose-catalase (OADC)

Dimethyl sulphoxide (DMSO).

Pure culture of the sensitive strain (H37Rv)

Pure culture of the resistant strain (JB74)

Minimum inhibiting concentration (MIC)

LIST OF PUBLICATIONS

1 Abdullah. J.M.A., Darwis. Y., and Tan, Y.T.F., (2003). Formulation and characterization of rifampicin-loaded Poly (ethylene oxide)-Block distearoyl phosphoidylethanolamine (mPEG-DSPE) polymeric nanoparticles. *14 international symposium of microencapsulation*, 4-6 September 2003, Singapore, Malaysia.

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4 J.M.A. Abdulla., H.H. Haris., P. Ibrahim., Y.T. F. Tan and Y. Darwis., (2004). Susceptibility of *mycobacterium tuberculosis* to rifampicin loaded Methoxy poly- (ethylene oxide)- block-distearoylphosphatidyl ethanolamine. *National TB symposium.* 5-6 Octobar 2004, Penang, Malaysia.

FORMULASI DAN PENILAIAN PARTIKEL POLIMERIK BERMUATAN RIFAMPISIN UNTUK PENGHANTARAN PULMONARI

ABSTRAK

Partikel polimerik dibangunkan menggunakan polimer bioterdegradasikan PLGA dan mPEG-DSPE. Pengaruh berbagai parameter formulasi ke atas ciri-ciri fisikal partikel polimerik dinilai. Parameter formulasi yang dinilai untuk PLGA ialah jenis polimer (RG 502, RG 503H dan RG 504), kepekatan PVA (2.5 dan 5 % w/v) dan perkadaran drug dengan polimer (0.2:1, 0.5:1 and 1:1). Parameter formulasi yang dinilai untuk mPEG-DSPE ialah jenis polimer (mPEG$_{2000}$-DSPE dan mPEG$_{5000}$-DSPE), perkadaran drug dengan polimer (1:5, 1:10 and 1.5:10) dan keliangan turas (0.22 dan 0.45 µm). Formulasi disediakan menggunakan kaedah pemeruapan pelarut dan amaun rifampicin terperangkap di dalam partikel polimer ditentukan menggunakan UV spektrofotometer. Purata saiz partikel mPEG-DSPE (241.5 nm) lebih kecil berbanding saiz partikel PLGA (3.7 µm). Hasil mikropartikel PLGA (90.71 %) tidak dijejas oleh semua factor. Di antara PLGA yang diselidiki, PLGA 503H mempunyai kecekapan pemerangkapan tertinggi iaitu 79.59 % pada kepekatan 5 % dan perkadaran drug dengan polimer 0.2:1. Kecekapan pemerangkapan tertinggi mPEG-DSPE ialah 100% pada perkadaran drug dengan polimer 1:5 dan keliangan turas 0.45 µm. Jenis polimer dan keliangan turas tidak ada kesan ke atas kecekapan pemerangkapan, hasil dan muatan drug. Walaubagaimanapun, perkadaran drug dengan polimer berkadar negatif dengan kecekapan pemerangkapan nanopartikel. Analisis termal menggunakan DSC memperlihatkan Tg nanopartikal tersesar ke nilai rendah. Walaubagaimanapun, spectra FTIR tidak memperlihatkan cirri-ciri puncak drug dan polimer tersesar dan ini bermakna tiada interaksi kimia antara drug dan polimer dalam polimerik partikel.

Pelepasan drug dari PLGA mikropartikel sangat perlahan berbanding mPEG-DSPE nanopartikel. Pelepasan berkadar negative dengan jenis PLGA dan berkadar positif dengan perkadaran drug dengan polimer. Kesan cetusan pelepasan diperlihatkan semasa pekadaran drug dengan polimer mencapai 1:1. Di antara PLGA-PLGA, pelepasan drug dari PLGA 503H mikropartikel berlaku paling cepat (14.11 % dalam masa 12 jam). Pelepasan dari PLGA sesuai dengan kinetik tertib sifar manakala PLGA 502 dan 503H masing-masing mengikut kinetik bieksponential. Sebaliknya, pelepasan dari mPEG-DSPE nanopartikles mengikut kenetik tertib pertama dan pelepasan drug paling cepat (58%) berlaku dalam masa 12 jam. Jenis mPEG-DSPE yang digunakan tiada kesan ke atas profile pelepasan drug dari nanopartikel. Walaubagaimanapun, peningkatan perkadaran drug dengan polimer dan penignkatan keliangan turas akan memanjangkan masa pembebasan drug dari nanopartikel.

MMAD mPEG-DSPE yang dihasilkan oleh nebulizer (2.6 μm) dan Rotahaler® (5.8 μm) yang dicirikan menggunakan NGI adalah lebih kecil dari pada aerosol MMAD PLGA 503H yang dihasilkan oleh nebulizer (6.9 μm) dan Rotahaler® (10.6 μm). Sebagai tambahan, FPF mPEG-DSPE (≈ 40 %) lebih tinggi dari pada FPF PLGA 503H (≈15 %). Seterusnya, kaedah perkadaran agar 1% digunakan untuk menguji keterentanan rifampisin terhadap mikobaterium. MIC mPEG-DSPE untuk strain sensitif drug (H37Rv) (10 μg/ml) dan strain rintang drug (JB74) (25 μg/ml) adalah rendah dari pada rifampisin mentah (masing-masing 35 dan 200 μg/ml). Oleh itu, boleh diambil kesimpulan bahwa mPEG-DSPE nanopartikel adalah pembawa yang sesuai untuk penghantaran rifampisin ke pulmonary.

FORMULATION AND EVALUATION OF RIFAMPICIN-LOADED POLYMERIC PARTICLES FOR PULMONARY DELIVERY

ABSTRACT

Polymeric particles were developed using PLGA and mPEG-DSPE biodegradable polymers. The influence of various formulation parameters on physical characteristics of polymeric particles was investigated. The formulation parameters investigated for PLGA were polymer type (RG 502, RG 503H and RG 504), PVA concentration (2.5 and 5 % w/v) and drug to polymer ratio (0.2:1, 0.5:1 and 1:1). The formulation parameters investigated for mPEG-DSPE were polymer type ($mPEG_{2000}$-DSPE and $mPEG_{5000}$-DSPE), drug to polymer ratio (1:5, 1:10 and 1.5:10) and filter porosity (0.22 and 0.45 µm). The formulations were prepared using a solvent evaporation method and the amount of rifampicin encapsulated in polymeric particles was quantified using a UV spectrophotometry. The mean particle size of mPEG-DSPE (241.5 nm) was smaller than PLGA (3.7 µm). The PLGA microparticles yield (90.71 %) was not affected by all factors. Among the PLGA studied, PLGA 503H had the highest entrapment efficiency with 79.59 % at a PVA concentration of 5 %w/v and drug polymer ratio of 0.2:1. The highest entrapment efficiency of mPEG-DSPE nanoparticles was 100 % at a drug to polymer ratio of 1:5 and filter porosity 0.45 µm. Polymer type and filter porosity had no effect on entrapment efficiency, yield and drug loading. However, drug to polymer ratio was negatively correlated with the entrapment efficiency of nanoparticles. Thermal analysis using DSC showed the Tg of nanoparticles shifted to a lower value. However, the FTIR spectra showed no shift in the characteristic peaks of drug and polymer which indicated no chemical interaction between drug and polymer in polymeric particles.

Drug release from PLGA microparticles was much slower than mPEG-DSPE nanoparticles. The release was negatively correlated with PLGA type and positively correlated with drug to polymer ratio. The burst effect was seen when drug to polymer ratio reached 1:1. Drug release from PLGA 503H microparticles was the fastest (14.11 % in 12 hours) among PLGAs. The release from PLGA 504 fitted zero order kinetics whereas PLGA 502 and 503H followed biexponential first order kinetics. Conversely, the release from mPEG-DSPE followed the first order release kinetics and the fastest drug released form nanoparticles (58%) occurred in 12 hours. The mPEG-DSPE type used had no effect on the drug release profile from nanoparticles. However, increasing drug to polymer ratio and filter porosity would prolong the release of drug from nanoparticles.

The MMAD of mPEG-DSPE generated by nebulizer (2.6 μm) and Rotahaler® (5.8 μm) characterized by NGI was smaller than the MMAD of PLGA 503H aerosols produced by nebulizer (6.9 μm) and Rotahaler® (10.6 μm). In addition, the FPF of mPEG-DSPE (≈ 40 %) was higher than the FPF of PLGA 503H (≈15 %). Furthermore, 1% agar proportional method was used to test the susceptibility of rifampicin against mycobacteriums. The MIC values of mPEG-DSPE for drug sensitive strain (H37Rv) (10 μg/ml) and drug resistant strain (JB74) (25 μg/ml) were lower than raw rifampicin (35 and 200 μg/ml respectively). Therefore, it can be concluded that the mPEG-DSPE polymer is a suitable carrier for pulmonary delivery of rifampicin.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Tuberculosis

Tuberculosis (TB) is a chronic communicable disease caused by the bacterium (*Mycobacterium tuberculosis*) and usually occurs in the lungs (the initial site of infection), but it also can occur in other organs. The complex nature of this pathogen and its ability to evade the immune system has prevented the development of an effective vaccine. TB is a highly contagious, persistent disease characterized by the formation of hard greyish nodules, or tubercles (Pandey *et al.,* 2003).

The World Health Organization (WHO) on 23 April 1993 declared tuberculosis as global public health emergency (Brennan, 1997; Makino *et al*, 2004). The disease infects over 1.8 billion people worldwide and it is responsible for 1.5 million deaths annually (Pandey *et al*., 2003). Frieden *et al*. (2003) also affirmed *Mycobacterium tuberculosis* as being a leading cause of infectious mortality after HIV AIDS worldwide. Frieden *et al*. (2003) noted that there were an estimated of 8–9 million new cases of tuberculosis in 2000, 3–4 million cases were sputum-smear positive. Most cases (5–6 million) were in people aged 15–49 years. Duncan and Barry (2004) said that according to a recent report compiled by the World Health Organization (WHO), the total number of new cases of tuberculosis (TB) worldwide in 2002 had risen to approximately 9 million. This is despite the success of widespread of the ‘DOTS’ (directly observed therapy, short-course) strategy, now covering 180 countries and accessible by over 70% of the world's population.

Despite the availability of effective therapeutic regimens for the treatment of TB, treatment failure and emergence of drug resistant are still problematic. This treatment failure is related in part to patient non-compliance (due to frequent administration of anti-TB drugs). Patient-compliance can be improved by the use of sustained release antitubercular drugs formulations, which reduce the dosing frequency of the drugs. Such system can be designed to target specific regions of the lung, and therefore allow controlled drug delivery to lung, or to the systemic circulation via the lung (Fu *et al*., 2002; Prabakaran *et al*., 2004).

1.2 Drug Therapy in Pulmonary Tuberculosis

The goals of drug therapy are to ensure cure without relapse, to prevent death, to stop transmission and to prevent the emergence of multi-drug resistance tuberculosis (Frieden *et al*, 2003). Directly Observed Treatment, Short-course (DOTS) therapy, which lasts for 6 or 8 months, given under direct observation, is one of the most important components of WHO strategy against tuberculosis.

Tuberculosis is treated in two phases. The initial phase for 2 months involves concurrent use of at least 3 drugs to reduce the bacterial population rapidly and prevent drug resistant bacteria emerging. The second continuation phase for 4-6 months involves fewer drugs and is used to eliminate any remaining bacteria and prevent recurrence. Direct observation of therapy is considered essential to ensure compliance during treatment of tuberculosis. Five drugs are considered essential first line for treatment of tuberculosis (Academy of Medicine of Malaysia 2nd edition. 2002). These are isoniazid (H), rifampicin (R), pyrazinamide (Z), streptomycin (S) (which are bactericidal) and ethambutol (E) (which is bacteriostatic) are used in various combinations as part of WHO recommended treatment regimens. Isoniazid, rifampicin and pyrazinamide are components of all antituberculosis drug regimens currently recommended by WHO. In supervised regimens change of drug regimen should be considered only if the patients fail to respond after 5 months of DOTS.

Patients who cannot comply reliably with the treatment regimen drug administration needs to be fully supervised (directly observed therapy, DOTS) The patients are given daily doses of SHRZ or EHRZ or HRZ under supervision i.e directly observed by health personnel or trained person for the first 2 months followed by HR or SHR or HR, 2 –3 times a week for a further of 4 months (Academy of Medicine of Malaysia 2nd edition. 2002). Frieden et al., (2003) reported that the DOTS method could ensure high rates of treatment completion, reduce development of acquired drug resistance, and prevent relapse.

Second line drugs in TB therapy are reserved for use only if the bacteria are resistant to the first line agents or if the patient experiences toxic side effects to them. The 2nd line drugs are much less active and have a much higher toxicity. Examples of second line drugs are ofloxacin/Ciprofloxacin, ethionamide, aminosalicylate, cycloserine, amikacin/Kanamycin and capreomycin (Pandey *et al*., 2003).

1.3 Respiratory System and Lung Anatomy

The respiratory system consists of the conducting airway and respiratory regions (Figure 1.1). The conducting airway essentially consists of nasal cavity, nasopharynx, bronchi and bronchioles. Airways distal to the bronchioles constitute the respiratory region, which include the respiratory bronchioles, the alveolar ducts and the alveolar sacs. The latter structures (the alveoli), which are the important parts in this study, are composed almost exclusively of a nonciliated epithelial membrane. The alveolar walls contain a dense network of capillaries and connective tissue fibers (Suarez and Hickey, 2000).

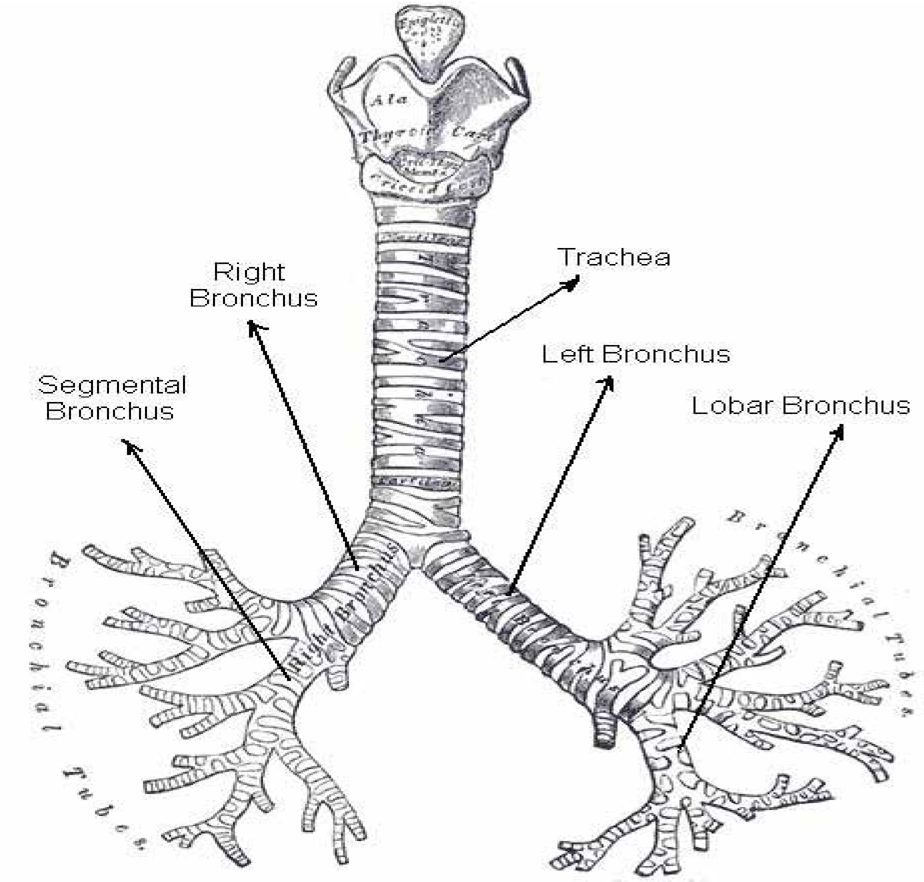
Ala
Right Bronchus
Trachea
Left Bronchus
Segmental Bronchus
Lobar Bronchus
Bronchial Tubes
Bronchial Tubes

Figure1.1: Front view of cartilages of larynx, trachea, and bronchial tree (Gray, 2001)

The lungs have in fact been demonstrated an efficient port of entry to the bloodstream due to: (i) the tremendous surface area of the alveoli (100 m^2), immediately accessible to drug; (ii) a relatively low metabolic activity locally, as well as a lack of first-pass hepatic metabolism; and (iii) the elevated blood flow (5 l/min) which rapidly distributes molecules throughout the body (Fehrenbach, 2001).

The lungs have two separate circulations. The bronchial circulation, which involves small systemic arteries from the aorta supplies oxygen for the relatively high metabolic needs for lungs. The pulmonary circulation, which serves respiratory function, begins in the pulmonary artery; bring venous blood from the right atrium. The pulmonary arteries subdivide extensively and finally terminate in a dense capillary network around the alveoli. Venous blood returns to the left atrium via veins, which coalesce and eventually form the pulmonary venous system. The venous blood from the bronchial circulation returns to the system circulation via the azygous and pulmonary veins (Gray, 2001).

1.4 Pulmonary Drug Delivery Systems

Growing attention has been given to the potential of a pulmonary route as an non-invasive administration for systemic delivery of therapeutic agents due to the fact that the lungs could provide a large absorptive surface area (up to 100 m^2) with extremely thin (0.1 μm – 0.2 μm) absorptive mucosal membrane and good blood supply. Controlled release polymeric systems are approaches that help for improving the duration and effectiveness of inhaled drugs (Fu *et al*., 2002).

Targeting delivery of drugs to the diseased lesions is one of the most important aspects of drug delivery systems. The systems should have novel properties such as increase efficiency of drug delivery, improve release profiles and drug targeting to the diseased site. Among the different dosage forms reported, nanoparticles and microparticles sized polymeric systems occupy unique position in drug delivery technology (Majeti and Kumar, 2000).

The advantages of sustained drug delivery to the respiratory tract are numerous, and include extended duration of action, reduction in drug use, improved management of therapy, improved compliance, reduction in side effects and together with potential cost savings that exist for sustained release therapy (Cook *et al*., 2005).

Malo *et al*. (1989) showed that four times daily treatment of asthma with a corticosteroid resulted in less nocturnal cough attacks and relapses when compared to a twice daily schedule, with no change in the side effect profile. However, excessive dosing frequency is a well-documented cause of non-compliance in patients. In another study Mann *et al*. (1992) reported that, inhaler under-usage was greater with four times daily versus twice daily treatment (57.1% versus 20.2%). Even in twice daily dosing, just 40% of patients complied with the given protocol, despite extensive education at the study onset. An inhaled sustained release formulation, administered once daily, would therefore provide benefit to non-compliant patient groups owing to the convenience of reduced dosing frequency (Cook *et al*., 2005).

Deol and Khuller. (1997) encapsulated antitubercular drugs (ATD) in liposomes. Sustained release of such drugs in the lung would be particularly beneficial since they could be delivered to and retained at the targeted receptors for a prolonged period of time and thus minimize the biodistribution throughout the systemic circulation (Zeng *et al.*, 1995). This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and can contribute in minimizing the risk of emergence of drug-resistance and potential toxicity (Makino *et al*., 2004).

1.5 Advantage of Pulmonary Delivery

The pulmonary delivery route has attracted much attention, as well as nasal, rectal, injections and oral routes, to improve the quality of life of patients, because no dose repeated are required. Further, this route is desirable for delivering drugs because of the following advantages over other routes.

(1) The surface area of a lung is extremely large (approximately 100 m^2) and the mucosal permeation of drug substances is comparatively easy, because the vascular system is well developed and the wall of the alveolus is extremely thin (Yamamoto *et al.*, 2005).

(2) The activity of drug-metabolizing enzymes with intracellular or extracellular is relatively low, it avoids hepatic first-pass metabolism (Suarez and Hickey, 2000).

(3) A very rapid onset of action with very small dose. An oral dose of bronchodilator may take 2–3 h to be fully effective while an inhaled dose usually takes a minimum of 15–30 min (Zeng *et al*., 1995).

(4) Reduces exposure of drug to the systemic circulation and potentially minimizes adverse effects and lower dosage regimens may provide considerable cost saving especially with expensive therapeutic agents (Joshi and Misra, 2001).

1.6 Pulmonary Delivery Devices

Local delivery of medication to the lung is highly desirable, especially in patients with specific pulmonary diseases like cystic fibrosis, asthma, chronic pulmonary infections, or lung cancer. Aerosols are an effective method to deliver therapeutic agents to the respiratory tract. Metered dose inhalers (MDIs), dry powder inhalers (DPIs) or nebulizers are commonly used for this purpose (Finlay, 2001).

There are numerous commercially available devices, and their design is an important factor governing aerosol size and fluid output. Although pressurised metered dose inhalers are the most commonly used inhalation drug delivery system, other delivery systems, such as dry powder inhalers and nebulizers, are widely used as propellant-free alternatives to MDIs (McCallion *et al*., 1996a). Gupta and Hickey, (1991) reported that nebulizer inhalers compared to MDIs or DPIs, generate smaller particles, which are better penetration to the distal region of the lungs and, thus, are more suitable for systemic delivery.

1.6.1 Metered Dose Inhalers (MDIs)

The metered dose inhalers (MDIs) were the first apparatus, which is both reliable and practical (Timsina *et al*., 1994). The fundamental components of

MDIs are an actuator, a metering valve, and a pressurized container that holds the micronized drug suspension or solution, propellant, and surfactant. The high vapor pressure propellant supplies the energy for dispersion in these delivery systems (Suarez and Hickey, 2000). Chlorofluorocarbon (CFC) as a propellant for MDIs has been widely used for pulmonary drug delivery devices (Yamamoto *et al*., 1999).

Chlorofluorocarbons based metered-dose therapeutic aerosols are in the process of being reformulated with more environmentally friendly propellants, such as hydrofluoroalkanes (HFAs). CFCs were reported to destroy ozone layer in the stratosphere and allow excessive ultraviolet radiation to reach the earth's atmosphere (Tashkin, 1999). HFAs were investigated as possible substitutes for CFCs because they shared similar desirable characteristics but non-ozone depleting. Despite the similarities with the CFCs, many additional difficulties were observed. HFAs were demonstrated to have toxic effects, modified the solubilities of drug and incompatibility with MDI components such as valves and container walls (Crowder *et al*., 2001).

The main disadvantages of MDI, especially in young children and elderly who have difficulty to administer the drug alone since it require patient’s hand and breathe coordination. Another disadvantage is release the aerosol at high velocity. This ballistic effect causes deposition of approximately 65% of the medication in the upper respiratory tract (mouth, oropharynx and larynx). It became also known that only a small fraction (10-20%) of the emitted dose reaches the lower airways. The remainder deposits in the extrathoracic and upper airways, are swallowed and subsequently absorbed in the gastrointestinal tract. The low temperature of the CFCs or HFAs discharged from a pMDI frequently also causes children to abruptly stop inhaling. All the disadvantages lead to a suboptimal delivery of drugs to the airways and thereby reduced therapeutic efficacy (Biddiscombe *et al*., 1993).

1.6.2 Dry Powder Inhalers

Dry powder inhalers (DPIs) can be divided into two classes: passive and active.

1. Passive devices depend on the inhalation ability of patient’s to provide the energy needed for dispersion.
2. Active powder-dispersion devices, similar to propellant-driven metered-dose inhalers, which use an external energy source to help the patient to accomplish some part of the aerosol dispersion (Crowder, 2004).

DPIs are the most recent developed devices in respiratory therapy. The majority of these devices are breath-activated inhalers that rely on the patient's inspiratory flow to deaggregate and deliver the drug for inhalation, thereby eliminating the requirement of inhalation coordination inherent in pMDI use. However, with DPIs there is the need to generate at least moderate inspiratory flow in order to accomplish effective drug delivery. The drug in a DPI is in the form of a finely milled powder in large aggregates, either alone or in combination with some carrier substance (Byron *et al*., 1990).

Most of the particles are initially too large to be carried into the lower airways, but the turbulent air stream created in the inhaler during inhalation causes the aggregates to break up into primary particles sufficiently small to be carried into the lower airways. Therefore, the deposition pattern of the particles depends on the inspiratory flow generated by the patient. A very low inspiratory flow is likely to move the dose from the inhaler into the patient’s mouth, with very low deposition in the pulmonary air-ways. Shear, turbulence, and mechanical intervention may be used to aid in the dispersion of aerosols from dry powders (Suarez and Hickey, 2000).

Dry powder generation is often hindered by aggregation of the small particles (Brown, 1987), which is in turn exacerbated by the hygroscopic nature of the drug and its electrostatic charge. The reduction of powder hygroscopic and electrostatic charge may enhance the future prospects of aerosol powder formulation (Ferron, 1977).

1.6.3 Nebulizers

Nebulizers use ultrasound or compressed gas to produce aerosol droplets in the respirable size range from liquids, usually aqueous solutions of drugs. They are widely used therapeutically to deliver corticosteroids, antiallergics, anticholinergics, antibiotics, mucolytics and other agents to the respiratory tract (British National Formulary, 1994). Further, the nebulizers are adaptable to very fine suspensions as well as aqueous solution (Yamamoto *et al*., 1999).

Nebulizers have the advantage over MDIs and DPIs that the drug may be inhaled during normal breathing through a mouth-piece or facemask. Thus, they can be employed to deliver aerosolized drug to patients, such as children, the elderly and patients with arthritis, who experience difficulties using other devices. Nebulizers can also deliver relatively large volumes of drug solutions and suspensions. They are frequently used for drugs that can not be conveniently formulated into an MDI or DPI or where the therapeutic dose is too large for delivery with the alternative systems (McCallion *et al*., 1996a).

1.7 Preparation Techniques for Pulmonary Drug Delivery System

Different drug carriers/delivery systems have been used for controlled drug delivery. In the last two decades, synthetic biodegradable polymers have been increasingly used as carrier to deliver drugs, because they are free from most of the problems associated with the natural polymers. Poly (amides), poly (amino acids), poly (alkyl-α-cyano acrylates), poly (esters), poly (orthoesters), poly (urethanes), poly (acrylamides) and ligands of carbonyl-methoxypolyethyleneglycol (mPEG) and distearoylphosphatidylethanolamine (DSPE) have been used to prepare various drug-loaded devices to improve therapy. Amongst them, the thermoplastic aliphatic poly (esters) such as PLA, PGA, especially PLGA and niosomes (Non-ionic surfactant vesicles), as well as mPEG and DSPE based polymeric micelles have generated so much interest due to their excellent biocompatibility and biodegradability. However, recent approaches to improve patient compliance have involved instituting intermittent drug delivery regimens with the use of polymers by cleaving conventional antitubercular drugs to various types of carrier systems (Dutt and Khuller, 2001; Zhang *et al*., 2003).

1.7.1 Microspheres

Microspheres are defined as homogenous monolithic spherical colloidal particles made of single or multiple type of polymers, typically with a particle size in the range of 1-200 µm, ideally <125 µm (Jain, 2000). Microspheres in strict sense are monolithic. However, the terms microcapsules and microspheres are often used synonymously. In addition, some related terms are used as well, for example, “micro beads” and “beads”. The term sphere and spherical particles are also used for a large size and rigid morphology (Majeti and Kumar, 2000). Microspheres have been used widely as drug carriers for controlled drug release (Hincal and Calis, 1999). Polymers, which have been extensively investigated for drug carriers, are (lactic acid) (PLA), poly (glycolic acid) (PGA) poly (lactic-co-glycolic acid) (PLGA). These polymers have excellent biocompatibility, mechanical strength, ease of fabrication, prolonged in vivo degradation kinetics, and changeable biodegradability properties (Pandey *et al*., 2003 and Zheng *et al*., 2004). The polymers have been fabricated into a variety of devices, such as microspheres, micelles, liposomes, nanospheres, film, implants, and pellets. Furthermore, their application in humans has been approved by food and drug administration (FDA). However, the disadvantages of this types of polymeric system particularly that of PLGA are low entrapment efficiency, burst release, instability of entrapped hydrophilic protein, and its incomplete release (Zheng *et al*., 2004).

1.7.2 Microparticle Preparation

The preparations of lung based drug delivery system have involved several processes. Hincal and Calis’s. (1999) reported that a wide range of microencapsulation techniques. The selection of the technique depends on the nature of the polymer, the drug, the intended use and the duration of therapy (O'Donnell and McGinity, 1997). In preparing controlled release microspheres for efficient entrapment of the active substance, the choice of the method is importance. The microencapsulation methods for hydrophobic biodegradable polymers such as poly (lactide-co-glycolide) and poly (lactic acid) as matrix materials are:

a) Emulsion-Solvent Evaporation and Solvent Extraction.
b) Phase Separation (Coacervation).
c) Interfacial Polymerisation
d) Spray Drying.

1.7.2 (a) Solvent Evaporation and Extraction Process

The solvent evaporation method is widely used to produce microspheres. There are two systems from which to choose, oil in water (O/W) or water in oil (W/O) and (W/O/W). The choice of a particular method is usually determined by the solubility characteristics of the drug.

i. Single Emulsion Process

The method is ideal for water-insoluble drugs in which polymer are first dissolved in volatile organic solvent. The drug is then added to the polymer solution to produce a solution or dispersion of the drug particles. This polymer–solvent–drug solution/dispersion is then emulsified (with appropriate stirring and temperature conditions) in a larger volume of water in presence of an emulsifier to yield an o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process to harden the oil droplets. The solid microspheres obtained are then washed and collected by filtration, sieving, or centrifugation. The microspheres are then dried under appropriate conditions or lyophilised to give the final free flowing microsphere product (Bodmeier and McGinity, 1988; Torres *et al*., 1996; Jain, 2000).

It should be noted that the solvent evaporation process in a way is similar to the extraction method, in the sense that the solvent must first diffuse out into the external aqueous dispersion medium before it could be removed from the system by evaporation (Arshady, 1991; Wu, 1995).

In order to increase the encapsulation of the water-soluble drugs, an oil-in-oil (O/O) emulsification method was developed (Arshady, 1991 and Ramírez *et al*., 1999). A water-miscible organic solvent is employed to solubilise the drug in which polymers are also soluble. This solution is then dispersed into oil such as light mineral oil in presence of an oil soluble surfactant like Span to yield the (O/O) emulsion. Microspheres are finally obtained by evaporation or extraction of the organic solvent from the dispersed oil droplets and the oil is washed off by solvents like n-hexane. This process is also sometimes referred as water-in-oil (W/O) emulsification method (Jalil and Nixon, 1990a).

ii. Double / Multiple Emulsion Process

The process is best suited to encapsulate water-soluble drugs like peptides, proteins, and vaccines, unlike the o/w method which is ideal for water-insoluble drugs. The method is that a buffered or plain aqueous solution of the drug (sometimes containing a viscosity building and/or stabilizing protein like gelatin) is added to an organic phase consisting of polymer solution in organic solvent with vigorous stirring to form the first w/o emulsion. This emulsion is added gently with stirring into large volume water containing an emulsifier like PVA to form the w/o/w emulsion. The emulsion is then subjected to solvent removal by either evaporation or extraction process. The solid microspheres obtained are then washed and collected by filtration, sieving, or centrifugation. The microspheres are then dried under appropriate conditions or lyophilized to give the final free flowing microsphere product (Jain, 2000).

1.7.2 (b) Phase Separation (Coacervation)

Coacervation is a process in which a homogeneous solution of macromolecules undergoes liquid-liquid phase separation, giving rise to a polymer rich dense phase. Coacervation has been classified into simple and complex processes depending on the number of participating macromolecules. In simple polyelectrolyte coacervation, addition of salt or alcohol normally promotes coacervation. In complex coacervation, two oppositely charged macromolecules (or a polyelectrolyte and an oppositely charged colloid) could undergo coacervation through associative interactions (Mohanty *et al*., 2004).

The process consists of decreasing the solubility of the encapsulating polymer by addition of a third component to the polymer solution in an organic solution (Jalil and Nixon, 1990a). At a particular point, the process yields two liquid phases (phase separation): the polymer containing coacervate phase and the supernatant phase depleted in polymer. The drug which is dispersed/dissolved in the polymer solution is coated by the coacervate. Thus, the coacervation process includes the following three steps: (i) phase separation of the coating polymer solution, (ii) adsorption of the coacervate around the drug particles, and (iii) solidification of the microspheres (Jain, 2000).

The main disadvantages of this method are tendency to produce agglomerated particles, problem in mass production, requires large quantities of organic solvent, and difficult to remove residual solvents from the final microsphere product (Takada *et al*., 1995).

1.7.2 (c) Interfacial Polymerization

The method involves the condensation of two monomers at the interface of the organic and aqueous phases. Polyamide capsules are a good example of this system (Conti *et al.,* 1992). The surface polymerization of the monomer surfactants is the advanced method of this technique for preparation of nanocapsules (Shapiro and Pykhteeva, 1998).

1.7.2 (d) Spray Drying

The spray drying technique appears to be attractive for the preparation of microparticles (Baras *et al*., 2000). It can be used for the microencapsulation of antigens. The technique consists of spraying an emulsion of polymer and drug through the nozzle of a spray dryer apparatus; the solvent evaporates very quickly, leaving solid microparticles (Pavanetto *et al*., 1992). The spray drying process involves the following four sequential stages: atomization of the product into a spray nozzle, spray air contact, drying of the sprayed droplets and collection of the solid product obtained. Due to the rapid evaporation of the solvent, the temperature of the droplets can be kept below the drying air temperature, and for this reason spray-drying can be applied to heat-sensitive materials (Broadhead *et al*., 1992).

The main advantages of the spray drying technique are applicable to both heat resistant and heat sensitive drugs, as well as water-soluble and water-insoluble drugs (Jain, 2000 and Mu *et al*., 2005). However, the method is associated with some drawback that included a significant loss of the product during spray-drying, due to adhesion of the microparticles to the inside wall of the spray-drier apparatus, and agglomeration of the microparticles (Takada *et al*., 1995). Another limitation of spray drying is its unsuitability for substances sensitive to mechanical shear of atomization (Maa and Prestrelski, 2000) and amorphous materials which are hygroscopic, more cohesive and difficult to flow and disperse (Hak and Nora, 2003).

1.7.3 Poly (Lactic-Co-Glycolic Acid) (PLGA)

Poly(lactide-co-glycolide) PLGA is a highly biocompatible and biodegradable synthetic polymer, which is hydrolytically degraded into non-toxic oligomer and finally to lactic acid and glycolic acid (Ito and Makino, 2004). In general, poly lactic-co-glycolic acid (PLGA), poly lactic acid (PLA) and poly glycolic acid (PGA) are block copolymers of lactic and/or glycolic acid (Figure 1.2), with the monomers linked by ester bands. The final hydrolytic products are monomers glycolic and lactic acid. Both monomers enter the tricarboxylic acid cycle and can be eliminated from the body as carbon dioxide and water (Jain, 2000).

Chemically, lactic acid, which is a composite of PLGA, contains one more side methyl group and is more hydrophobic than glycolic acid. Therefore, the higher content of lactide, the more hydrophobic is the polymer, the lower water uptake and the slower the degradation rate. In addition, lactic acid in the polymer can either be in its optically active form (L) or as a racemate (D, L), which affects the crystallinity of the polymer. Besides hydrophobicity and crystallinity, MW and poyldispersity are also important molecular properties affecting polymer performance. Several other important bulk properties, like glass transition temperature, melting point, and solubility in organic solvents, water uptake rate and biodegradation rate are closely related to the molecular properties of PLGA polymers (Jain, 2000).

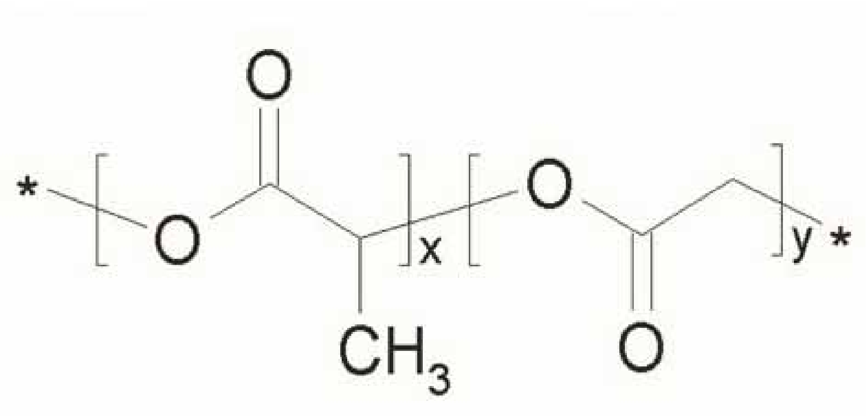

Figure 1.2: Chemical structure of poly lactic-co-glycolic acid (PLGA)

1.7.4 PLGA Microparticles for Lung Delivery

Most previous studies of polymeric pulmonary drug delivery have utilized PLGA since it is readily available and has a long history of safety in humans (Fu *et al*., 2002).

Masinde and Hickey. (1993) prepare poly (lactic acid) (PLA) microspheres with particle sizes between 1 and 11 µm by a solvent evaporation technique. The microspheres were suspended in a non-surfactant solution and subsequently atomized using a jet nebulizer. The particles generated were suitable for drug delivery to the lower airways, having a median diameter of 2 µm and geometric standard deviation of 2.4 µm. Zeng *et al*. (1995) studied tetrandrine antisilicotic alkaloid entrapped in albumin microspheres for delivery to the alveolar region. They observed tetrandrine metabolized in alveolar and incorporate into alveolar macrophages.

Lai *et al*. (1993) reported prolonged protection against bronchoconstriction challenge in rats at least 12 h post-administration with PLGA/isoproterenol microspheres. Edwards *et al*. (1997) studied sustained release of insulin in rats with large porous particles fabricated from PLGA, and showed reduced macrophage uptake and immune response to the larger particles relative to non-porous controls. El-Baseir and Kellaway. (1998) studied the *in vitro* sustained release of beclomethasone diproprionate and nedocromil sodium entrapped in PLA microparticles for 8 and 6 days respectively. However, pulmonary administration of PLA microspheres to rabbits was associated with inflammation at sites adjacent to microparticle deposition, raised neutrophil count and incidence of haemorrhage (Armstrong *et al*., 1996).

PLGA has many limitations as a carrier for drugs in the lungs. First, small amount of PLGA microspheres degrade over the period of weeks to months, but typically deliver drugs are released for a shorter period of time. Such a pattern would lead to an unwanted build-up of polymer in the lungs upon repeat administration (Cook *et al*., 2005). Second, bulk degradation of PLGA microspheres creates an acidic core, which can damage pH sensitive drugs such as peptides and proteins. Surface eroding polymers, such as polyanhydrides, lessen the effect of acidic build-up by increased diffusion rates of soluble fragments away from the particle. Third, PLGA microspheres have hydrophobic surfaces, which result in sub-optimal particle flight into the deep lung (due to particle agglomeration by van der Waals forces) (Fu *et al*., 2002). Additionally, hydrophobic surfaces lead to rapid opsonization (protein adsorption), resulting in a rapid clearance by alveolar phagocytic cells (Cook *et al*., 2005).

1.7.5 Polymeric Nanoparticles

Nanoparticles are colloidal particles ranging in size from 10 to 1000 nm, and they are extensively employed for targeted drug delivery systems. Nanoparticles have several advantages over conventional drug carriers; small particle size, ease of administration, drug targeting to the specific body site, solubilization of hydrophobic drug, avoid the reticuloendothelical system (RES), and reduced side effects of anticancer drugs (Lee *et al*., 2003).

Various drug delivery and drug targeting systems are currently developed or under development. Among drug carriers are soluble polymers, insoluble or biodegradable natural and synthetic polymers, microcapsules, nanocapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles. Each of those carrier types offers its own advantages and has its own shortcomings, so the choice of a certain carrier for each given case can be made only taking into account the whole bunch of relevant considerations (Torchilin, 2001).

Among the various drug delivery systems considered for pulmonary application, biodegradable polymeric nanoparticles demonstrate several potential advantages. In comparison to liposomal formulations, polymeric nanoparticles may exhibit a greater stability in the face of extreme forces generated during the nebulization process, thus eliminating the possibility of drug leakage. A further advantage of nanoparticle formulations is the fact that particles with a diameter of <1 μm are more easily incorporated in the `respirable percentage' of aerosolized droplets (droplets exhibiting a mass median aerodynamic diameter (MMAD) of 1–5μ μm) (Lea *et al*., 2003).

Drug targeting systems like liposomes (Codde *et al*., 1993) or prodrugs (O'Hare *et al*., 1989) have been limited with some disadvantages such as instability of carriers in the body fluid, rapid elimination by undesirable organs, difficulties in modifying macromolecular carriers, possibility of drug inactivation during chemical attachment, liberation rate of drug from the macromolecular-drug conjugates and biodegradation. Drug carrier systems of core-shell type nanoparticles reported by Peracchia *et al*. (1997), or polymeric micelles reported by Yokoyama *et al*. (1990), were attempted to solve the problems mentioned above. Nanoparticles based on core-shell structure or polymeric micelles have many advantages such as long circulation in the body, better drug solubility, drug stability and high drug encapsulation. However, polymeric micelles or core-shell type nanoparticles are found to have limited application for specific drug targeting due to the drug may be freely diffused throughout the body (Jeong *et al*., 2005).

Recently, block copolymers or polymeric conjugates were synthesized to make core-shell type nanoparticles and polymeric micelle. Polymeric micelles represent a separate class of micelles and are formed from polymers consisting of both hydrophilic and hydrophobic monomer units and they are more stable compared to micelles (Torchilin, 2001: Torchilin, 2002). Polymeric micelles have a hydrophobic core and a hydrophilic outer shell, in which hydrophobic segments form the inner-core of the structure, acts as a drug incorporation site, especially for hydrophobic drugs (Jeong *et al*., 1998). At present, polymeric micelles seem to be one of the most advantageous carriers for the delivery of water-insoluble drugs (Deol and Khuller, 1997; Jones and Leroux, 1999).

Use of lipid moieties as hydrophobic blocks capping PEG chains can provide additional advantages for particle stability when compared with conventional amphiphilic polymeric micelles due to the existence of two fatty acid acyls which might contribute considerably to an increase in the hydrophobic interactions between the polymeric chains in the micelle's core (Torchilin, 2002).

Diacyllipid–PEG conjugates micelles have been introduced into the area of controlled drug delivery as polymeric surface modifiers for liposomes (Klibanov *et al*., 1990). Interestingly, diacyllipid–PEG molecule itself represents a characteristic amphiphilic polymer with a bulky hydrophilic (PEG) portion and short but extremely hydrophobic diacyllipid part. The diacyllipid–PEG conjugates were found to form micelles of different sizes in an aqueous environment (Lasic *et al*., 1991). PEG–PE micelles can efficiently incorporate sparingly soluble drugs (Weissig *et al*., 1998a). It seems that the use of PEG-diacyllipid conjugates, which represent micelle-forming amphiphilic polymers with larger hydrophilic blocks and more lipophilic hydrophobic blocks, might result in colloidal particles, which are more stable under physiologic conditions (Torchilin, 1999).

1.7.6 PEG-PE Nanoparticles Preparation

There are two principal methods for the preparation of polymeric micelles, the direct dissolution method and the dialysis method. In each particular case, the choice of the method is usually determined by the extent of the solubility of a micelle-forming in an aqueous medium. If the polymer is marginally soluble in water, the direct dissolution method is employed, whereas if the polymer is poorly soluble in water, the dialysis method is usually employed (Allen *et al*., 1999).

In direct dissolution method, a polymer is dissolved in an aqueous medium at normal or elevated temperature and at a concentration well above its CMC value. Usually, in direct dissolution method the copolymer produce micelles spontaneously in aqueous solution, but in some cases the copolymer and water are mixed at elevated temperatures to ensure micellization (Allen *et al*., 1999; Torchilin, 2001). This method is frequently applied for micelle preparation from block co-polymers possessing a certain degree of solubility in water (Torchilin, 2001).