CHAPTER I

INTRODUCTION

Tuberculosis (TB) is an infectious lung disease, which is one of the leading causes of death worldwide. This disease has become a clinically significant opportunistic disease among the population with high incidence of acquired immunodeficiency syndrome (AIDS). Rifampicin is one of the first line drugs in the therapy of TB. Current medical treatment of TB is accomplished by methods of drug delivery, being mostly oral and/or parenteral. Oral bioavailability of rifampicin, a common anti-TB drug, is found to be fairly high (90-95 %) (Kenny and Srates 1981). However, most single or combination anti-TB drugs are known for their maximum tolerated doses beyond which otherwise result in undesirable toxic effects. Several investigators have proposed the administration of antitubercular drugs in the form of vesicular systems as injectable preparations (Deol et al. 1997), as also microparticulate systems (Quenelle et al. 1999 and Barrow et al. 1998). The objectives of these investigators include reduction of drug dose, dose frequency and toxicity and improvement in patient compliance.

The microparticle drug delivery systems targeting to the alveolar macrophages contribute to improved chemotherapy of TB (Quenelle et al. 1999 and Barrow et al. 1998). Recently, the administration of biodegradable microspheres through the bronchio-pulmonary route for better therapy of TB was reported (O'Hara et al. 1999 and Suarez et al. 2001). The inhalable biodegradable microparticle as a drug delivery system is capable of controlled release that targets the deep lung, and specifically the alveolar macrophages. The bacteria reaching inner lung tissues are phagocytized by alveolar macrophages. Alveolar macrophage (AM) targeted drug delivery can have an important benefit, specifically for intracellular bacterial infections, such as tuberculosis that survive and grow within AMs (Rolee et al. 2001). Finally, an efficient targeted drug delivery system, with the capability for extended release, should also increase patient compliance with long-term therapies by allowing less frequent dosing, and reduced side-effects typically associated with the long-term systemic antibiotic therapy (Danielle et al. 2003). In pulmonary drug

delivery, drug-loaded microparticles can be administered via a dry powder inhaler (DPI), metered dose inhaler (MDI) or nebuliser. The small microparticles having a typical mass median aerodynamic diameter of 1-5 µm are required in order to efficiently penetrate to pulmonary alveoli (Hinds 1982).

Targeting antituberculosis drug delivery to the lung has received increased attention. A number of conventional preparation techniques have been employed to produce the microparticles for inhalation, e.g., spray drying, milling and emulsion-solvent evaporation method. These techniques, such as spray drying, operate at temperatures that can thermally denature heat sensitive compounds, such as proteins. Milling produces powders with broad size distributions. In additional, milling of drugs is inappropriate or ineffective for unstable or wax-like substances particularly if they are unstable at higher temperature. The solvent/emulsion evaporation techniques often require further processing stages to remove solvents. Furthermore, product drying can take several days until acceptable levels of residual solvent in the product can be achieved.

In recent years, a number of processing techniques using supercritical fluids (SCFs), particularly supercritical carbon dioxide (SC), have received increased attention as a method of preparing excipients for pharmaceutical applications. Micronization techniques using SCFs can be categorized into three groups, namely, rapid expansion of supercritical solution (RESS), gas anti-solvent recrystallization (GAS) and supercritical anti-solvent process (SAS) (Yeo et al. 1993 and Subramaniam et al. 1997). In the RESS method, microparticles are formed as a result of a rapid expansion of SC solution (Philips and Stella 1993). This method is suitable for substances with sufficiently high solubility in the SC solvent. It is known as a novel method to prepare solvent-free drug-containing polymeric particles for controlled release delivery (Debenedetti. et al. 1993 and Tom et al. 1993). In the GAS method, SCF is used as a non-solvent which causes precipitation of microparticles from solution. The method is of interest in the formulation of fine peptide/protein powders as well as polymeric particles (Yeo et al., 1993).

An alternative method capable of producing microparticles in a single step at a moderate temperature is SAS. The drug is dissolved in an organic solvent and the solution sprayed into supercritical fluid. The organic solvent is miscible with the SC gas thus causing the drug particles to precipitate. Carbon dioxide (CO_2) is one of the most commonly used supercritical fluids because of its relatively low critical parameters (T_c = 31.1°C, P_c = 73.8 bar). In addition, CO_2 is nontoxic, nonflammable and inexpensive. Microparticles prepared by this method contain negligible amount or none of the residual solvent (Ruchatz et al. 1997). The particles can be produced to meet certain morphological requirements such as size, shape, porosity, by varying necessary process parameters of the system.

The supercritical fluids techniques are used as methods for preparation microparticles of biodegradable polymer or drug, such as poly(L-lactide) and poly(β-hydroxybutyric acid) microspheres (Bleich et al. 1993) and tetracycline (Reverchon et al. 1999). Furthermore, there are several reports of the production of microparticles of drug loaded biodegradable polymers, such as lovastatin loaded DL-PLA (Tom et al. 1993), gentamycin loaded L-PLA (Meyer et al. 1998) and hydrocortisone loaded DL-PLGA microparticles (Ghaderi et al. 2000). This technique can produce drug microparticles suitable for pulmonary delivery, such as budesonide, flunisolide, fluticasone propionate, prednisolone and triamcinolone acetonide microparticles (Steckel et al. 1997) and terbutaline microparticles (Reverchon and Porta 2003). However, no investigator performs rifampicin loaded biodegradable polymers microparticles for pulmonary drug delivery by this technique. So, it is interesting to study the feasibility of using dense CO_2 with the technique known as the supercritical anti-solvent process (SAS) to prepare rifampicin-containing biodegradable microparticles having a diameter in the range of 1-5 μm.

Biodegradable microparticles can be used as drug carriers via pulmonary route. The deposition of aerosolized microparticles onto the lung peripheries can offer a prolonged delivery of active compounds (El-Baseir and Kellaway 1998) and a targeted drug delivery system (Quenelle et al. 1999 and Barrow et al. 1998). In this study, biodegradable polymers such as 50:50 poly (DL-Lactide-co-glycolide) (PLGA), poly(DL-Lactide) (DL-PLA) and poly(L-lactide) (L-PLA) were used as

carrier materials. Formulation variables (polymer type, polymer content, solvent type, drug-polymer concentration) and process variables (operating pressure, temperature, solution feed rate) affecting the drug-polymer particle formation were investigated. The drug-containing microparticles were characterized with respect to their morphology, particle size and size distribution, drug content, encapsulation efficiency, *in vitro* drug release properties. In addition, *in vitro* deposition study was also investigated using the Anderson cascade impactor.

The purposes of the study were as follows:

1. To prepare rifampicin loaded biodegradable microparticles for pulmonary drug delivery by supercritical fluid technique.

2. To study the effect of polymer type, drug/polymer ratio and solvent type on the morphology, particle size, particle size distribution and drug content of rifampicin-loaded polymer microparticles.

3. To study the influence of operating pressure, temperature, solution feed rate, drug-polymer concentration and molecular weight of polymer on the rifampicin-polymer microparticles properties.

4. To study the *in vitro* drug release of rifampicin-loaded microparticles and *in vitro* deposition of microparticles using the Anderson cascade impactor and investigate the stability of processed rifampicin prepared by supercritical antisolvent process.

5. To determine the bactericidal efficacy of the rifampicin-loaded polymer microparticles against *Mycobacterium Tuberculosis*.