CHAPTER I

INTRODUCTION

Recent advances in biotechnology have resulted in the availability of a large number of macromolecules such as peptides and proteins as therapeutic agents with local and systemic effects. Up until now, most therapeutic proteins are administered by injection because of their low stability and bioavailability after oral administration and poor absorption at other mucosal sites (Smith et al., 1992; Couvreur and Puisieux, 1993). Pulmonary delivery has received much attention as a non-invasive administration route for proteins and peptides because of a large alveolar surface area (100 m^2) suitable for drug absorption, extensive vasculature, a thin alveolar epithelium membrane (0.1-0.2 μm) and low enzymatic activity (Patton and Platz, 1992; Okamoto et al., 2002; Smola, Vandamme, and Sokolowski, 2008). However, difficulties associated with proteins/peptides aerosols included protein denaturation during manufacture, aerosolization and storage, poor powder flowability resulting in inefficient dry powder delivery, rapid clearance from the lungs due to mucociliary clearance, enzymatic degradation and phagocytosis by alveolar macrophages (Kobayashi, Kondo, and Juni, 1996; Shoyele and Slowey, 2006).

Liposomes are promising vehicles for pulmonary drug delivery because they are prepared with phospholipids endogeneous to the lung as surfactants and can significantly alter the pharmacokinetics and pharmacodynamics of entrapped drugs (Zeng, Martin, and Marriott, 1995). Liposomes are applied for drug delivery, gene therapy and immunization (Schreier, Gonazales-Rothi, and Stecenko, 1993; Sharma and Sharma, 1997). Some proteins and enzymes, such as insulin (Huang and Wang, 2006) and superoxide dismutase (Lo, Tsai, and Kuo, 2004), have been encapsulated in liposomes to improve their pulmonary delivery and activity preservation. Most of them were administered intratracheally into the respiratory tract in liquid-based liposomes (Schreier et al., 1993). Nebulizers have been used extensively for the delivery of liposomes (Lange et al., 2001; Zaru et al., 2007). However, stability and

leakage are problems associated with aqueous dispersions with nebulizers (Niven, Carvajal, and Schreier, 1992). Among formulations employed for pulmonary drug delivery, dry powders have been considered as a promising dosage form because of the stability of drugs and formulations (Prime et al., 1997). Liposomal dry powders have many advantages for pulmonary administration with respect to controlled delivery, increased potency, prevention of local irritation, reduced toxicity, uniformed local drug deposition, free of propellant, patient compliance, high dose carrying capacity and improved drug stability (Kellaway and Farr, 1990; Cryan, 2005; Chougule, Padhi, and Misra, 2006). In addition, liposomes have immunological adjuvant activity by increased antigen uptake and localization to lymph nodes (Allison and Gregoriadis, 1974; Frezard, 1999; Perrie et al., 2008).

Liposomal dry powders have been prepared by several techniques such as lyophilization (Joshi and Misra, 2001a, b; Lu and Hickey, 2005), micronization (Desai et al., 2002) and spray drying (Lo et al., 2004). Mobley (1998) and Desai et al. (2002) examined the effects of lyophilization and jet milling on the efficacy of a liposomal formulation and found significant leakage due to stresses induced in the separate drying and milling process. Some researchers have proposed spontaneous production of liposomes upon dispersion of micronized phospholipids based powders in an aqueous environment (Desai et al., 2002; Desai, Hancock, and Finlay, 2003). However, this technique is associated with significant loss of product in the milling process and observed suboptimal dispersion due to the auto-adhesive properties of powders.

Spray drying technique has been considered as a modern one-step process for the production of small particles (<5 μm) for pulmonary administration (Shoyele and Cawthorne, 2006). Moreover, spray drying is a very simple and industrially applicable method for the bulk preparation of lipid mixture for secondary liposome production (Kikuchi, Yamauchi, and Hirota, 1991; Alves and Santana, 2004). The spray drying technique was used in the past to dry preformed liposomes in order to stabilize their contents during storage (Goldbach, Brochart, and Stamm, 1993a, b). Recent studies have used the spray drying technique in the preparation of liposomal

powders as an efficient drug carrier to protect the integrity, stability, functionality and to control release of peptides or proteins (Lo et al., 2004; Grenha et al., 2008) and other drugs (Chougule, Padhi, and Misra, 2006, 2008).

The feasibility of preparing liposomal powders that spontaneously form liposomes in an aqueous environment, thereby creating reservoirs for the encapsulation of drugs, has been investigated (Payne and Salmon, 1985; Nanba, Toshiya, and Sakakibara, 1989; Kikuchi et al., 1991; Skalko-Basnet, Pavelic, and Becirevic-Lacan, 2000; Desai et al., 2003; Alves and Santana, 2004; Weers, Tarara, and Tzannis, 2005). Phospholipids are known to orient into a liposomal configuration through a spontaneous, entropic process in a water-rich environment. Such conditions exist in the airways of the respiratory tract. Therefore, it is feasible to hypothesize that spontaneous liposome formation would occur following pulmonary deposition of liposomal dry powders. Moreover, incorporation of drugs within such matrix should result in the creation of a reservoir of liposomally encapsulated drug that is subsequently released at a controlled rate. However, most studies involved the use of organic solvents such as chloroform and ethanol to dissolve lipid, additives and drugs for formulating the dried lipid particles by spray drying technique.

In this research, the spray drying technique was studied to dry preformed liposomes simultaneously with protein solution into powders which spontaneously formed liposomes and entrapped the protein into liposomal structure after reconstitution with aqueous buffer solution. Hydrogenated soybean phosphatidylcholine (HPC) was used as the structural lipid for liposome formation. Lysozyme was chosen as a model protein in this study because it is commercially available and well characterized (Rosenberger, 1996). In addition, lysozyme is also of therapeutic value in the prevention and treatment of respiratory disorders either alone or in combination with other therapeutic agents (Cantor and Shteyngart, 2004, 2008). To reduce the tendency of phospholipid to form highly cohesive powders, additional additives were introduced in the formulations. Carbohydrates such as sucrose, lactose, trehalose and mannitol were evaluated as an additive for formulation of the liposomal powders by spray drying technique. Glycine was also investigated

for preparation of the spray-dried HPC liposomal powders as a co-additive and an anti-adherent. In addition, a systematic assessment of the effect of the HPC/additive ratio, the glycine amount and the HPC/Chol ratio on the properties of the spray-dried liposomal powders was also carried out.

A literature search revealed that no studies have been reported on statistically optimizing the quantitative aspects of the effects and relationships among various spray drying conditions for preparing liposomal powders. The optimization study requires a large number of experiments to describe the effect of spray drying condition on the physical properties of the liposomal powders. Screening and optimizing of the spray drying condition could be simplified by the use of a statistical design that requires only a small number of experiments, thereby eliminating the need for time-consuming experimentation (Montgomery, 2005). Thus in this present study, a 2^3 full factorial design was applied to assess the effects of three spray drying factor variables: inlet temperature (°C), pump speed (%) and total solid content (%w/w) on spray-drying process yield, moisture content, particle size of the spray-dried lysozyme-loaded liposomal powders and entrapment efficiency of lysozyme in the reconstituted liposomes. Entrapment efficiency of lysozyme into liposomes was determined after the liposomal powders were re-hydrated at physiologically relevant conditions. First order models were employed and their adequacy was investigated by adding central points to the design dataset to evaluate a possible response curvature. The response surface methodology was performed by central composite design to optimize the spray-drying conditions. This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. Contour plots of central composite design describing the variable effects on the response were built to define the proper conditions for yield, moisture content, particle size and entrapment improvement. In addition, model prediction efficiency was assessed by check point analysis in the optimum condition which gave high yield, low moisture content, low particle size and high entrapment as indicated from the contour plots. Predicting how the spray drying parameters would affect characteristics of liposomal powders will be useful in future development of spray-dried liposomal powders for pulmonary delivery.

The purposes of this study were as follows:

1. To develop the preparation of liposomal powders by spray drying technique using different additives

2. To investigate the effect of the formulation parameters including HPC/additive weight ratio and glycine amount on the properties of the spray-dried liposomal powders without lysozyme and the reconstituted liposomes

3. To evaluate the influence of HPC/Chol molar ratio on the properties of the spray-dried liposomal powders with lysozyme and the reconstituted liposomes

4. To prepare lysozyme-loaded liposomes by dehydration-rehydration technique and to investigate the effect of lysozyme-loading into liposomes prior to spray drying on the physicochemical properties of spray-dried lysozyme-loaded liposomal powders

5. To optimize the spray drying process factors, namely inlet temperature (°C), pump speed (%), and total solid content (%w/w) for preparing the lysozyme-loaded liposomal powders using factorial design and central composite design