Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 305 (2005) 180–185
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Note

Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery

Lyle G. Sweeney [a], Zhaolin Wang [a], Raimar Loebenberg [b], Jonathan P. Wong [c], Carlos F. Lange [a], Warren H. Finlay [a,*]

[a] *Department of Mechanical Engineering, University of Alberta, Aerosol Research Laboratory of Alberta, Edmonton, AB, Canada T6G 2G8*
[b] *Faculty of Pharmacy, University of Alberta, Edmonton, AB, Canada T6G 2G8*
[c] *Defense R&D Canada-Suffield, Box 4000, Medicine Hat, AB, Canada T1A 8K6*

Received 10 September 2004; received in revised form 12 August 2005; accepted 6 September 2005

Abstract

Spray-freeze drying was utilized to manufacture a liposomal powder formulation containing ciprofloxacin as a model active component. The powder forms liposomally encapsulated ciprofloxacin when wetted. Aerosol properties of this formulation were assessed using a new passive inhaler, in which the powder was entrained at a flow rate of 60 l/min. A mass median aerodynamic diameter (MMAD) of 2.8 μm was achieved for this formulation. Using the experimental dispersion testing data, ciprofloxacin concentration in the airway surface liquid (ASL) was calculated using a Lagrangian deposition model. The reconstitution of the powder in various aqueous media gave drug encapsulation efficiencies as follows: 50% in water, 93.5% in isotonic saline, 80% in bovine mucin, 75% in porcine mucus and 73% in five-fold-diluted ex vivo human cystic fibrosis patient sputum.

Keywords: Aerosol; Ciprofloxacin; Liposomes; Powder; Spray-freeze drying

Inhalation drug delivery is an effective pathway to treat many topical and some systemic illnesses (e.g. Finlay, 2001). Indeed, the interest in inhalation treatment is increasing yearly; an example being the peptides and proteins produced through biotechnology for pulmonary delivery (Bosquillon et al., 2004; Steckel et al., 2003; Adjei and Gupta, 1998). One particular type of drug delivery system that has been explored with a number of antimicrobial and anticancer drugs is liposomal encapsulation of the active component (e.g. Wong et al., 2003; Oh et al., 1995). In successful circumstances, this could alter the pharmacokinetics to an extent that drug retention time is increased and drug toxicity is reduced, thereby prolonging the half life of the drug in the body.

Typically, liposomal formulations have been delivered by nebulization. Concerns arise from drug stability and leakage perspectives when nebulizers are used to

* Corresponding author. Tel.: +1 780 492 4707; fax: +1 780 492 2200.
E-mail address: warren.finlay@ualberta.ca (W.H. Finlay).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.09.010

deliver a liposomally encapsulated agent (Taylor et al., 1990). To circumvent these issues, dry powder formulations have been examined. 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 processes. They proposed a solution that utilized spontaneous production of liposomes. It is known that, due to electrostatic interactions, phospholipids will spontaneously encapsulate a particle of suitable charge in an ionic solution, thereby creating a liposomal particle. This process generated promising in vitro results, yet they encountered significant losses in the milling process and observed suboptimal dispersion due to the auto-adhesive properties of the powder.

In this study, we have utilized a spray-freeze-dried (SFD) process that addresses many of the problems encountered by previous formulations and manufacturing methods. We have developed a SFD ciprofloxacin formulation that relies on the spontaneous formation of liposomes. Ciprofloxacin was selected as the active agent because it is a widely used, broad-spectrum antibiotic. In addition, Wong et al. (2003) observed that jet nebulized, liposomal ciprofloxacin provided full protection in mice issued a lethal pulmonary infection of *Francisella tularensis*, whereas free ciprofloxacin was ineffective. SFD produces stable formulations with highly porous particles, which leads to improved aerosolization and dissolution properties compared to other dry powder manufacturing methods (Maa et al., 1999).

In this paper we present a SFD manufacturing method for a ciprofloxacin formulation, the results of a reconstitution study, and the dispersion properties of the powder using a passive inhaler.

The formulation utilized in this study contains dimyristoyl phosphatidylglycerol (DMPG, Genzyme Pharmaceuticals, Cambridge, MA, USA), lactose (Pharmatose 325M, DMV International, Veghel, The Netherlands) and ciprofloxacin (US Biological, Swampscott, MA, USA) in a weight percent ratio 5:17:1, respectively. The formulation forms a smooth suspension upon vortexing (2×30 s to 4×30 s in 1 h) and remains stable at 4 °C for several days. Desai et al. (2002, 2003) utilized a formulation for lyophilization and jet milling with an identical phospholipid-to-ciprofloxacin ration and a slightly different lactose-to-lipid ratio.

Spray-freeze drying was performed with a two-fluid nozzle (Spray System Co., Wheaton, IL, USA), in which compressed nitrogen and a peristaltic pump (ChemTech, Punta Gorda, FL, USA) were used to drive the formulation. The suspension was atomized into a flask containing liquid nitrogen. Following atomization, the remaining liquid nitrogen was allowed to evaporate, and the resulting powder was dried for 48 h in a freeze drier (Labconco Corp., Kansas City, MO, USA). The collector was held at −52 °C while the vacuum was 0.004 mbar. To prevent the powder from melting, the vesicle containing the powder was held at subzero temperature for 7 h. The vesicle was kept at 21 °C for the remainder of the 48 h. The powder was subsequently collected and stored in a sealed vial without desiccant at 4 °C.

The ability of the phospholipids to encapsulate ciprofloxacin before and after SFD was tested by exposure to various liquid media. Prior to SFD, the suspension of lactose, DMPG and ciprofloxacin was tested for leaking. The suspension was centrifuged at 4 °C and 14,000 rpm for 1 h (Allegra 21R, Beckman Coulter, Fullerton, CA, USA). The supernatant and pellet were collected and dissolved separately in methanol. The resulting solutions were analyzed for ciprofloxacin content using UV spectroscopy (UV absorbance at $\lambda = 278$ nm, Diode Array Spectrophotometer, model 8452A, Hewlet Packard, Tulsa, OK, USA). Subsequent to SFD, the powder was reconstituted in various liquid media, including distilled water, isotonic saline solution, bovine mucin type 1 from submaxillary glands (Sigma–Aldrich, St. Louis, MO, USA), porcine mucin (extracted post-mortem using lung lavage) and ex vivo human cystic fibrosis patient sputum (spontaneous expectoration with dental cotton packing between cheeks and gums to minimize admixture with saliva) diluted five-fold with isotonic saline. The suspensions were vortexed 2×30 s and were left to stand at room temperature for 15 min. The procedure utilized to examine ciprofloxacin encapsulation in the drug suspension prior to SFD was also used to evaluate encapsulation efficiency for the powder in the various liquid media. Leaking was also examined for the reconstituted powder in the aforementioned liquids diluted five-fold with distilled water.

The fine particle fraction (FPF), defined as particles with an aerodynamic diameter <5.6 μm and mass median aerodynamic diameter (MMAD) of the

powder were measured using a Mark II Anderson Cascade Impactor (Graseby Anderson, Smyma, GA, USA) with effective cut-off points recalibrated at 60 l/min. Deagglomeration and powder delivery were achieved with a proprietary passive dry powder inhaler that utilizes cyclonic action as well as mechanical impaction to disperse powder particles (Finlay and Wang, 2003). The flow rate was monitored with a pneumotachometer (PT 4719, Hans Rudolph Inc., Kansas City, MO, USA). Prior to testing, the impactor plates were sprayed with a release agent (316 Silicone Release Spray, Dow Corning, Midland, MI, USA). Following impaction, each impactor plate was washed with 5 ml of $CHCl_3$/MeOH/H_2O in a volume ratio of 1.3/2.6/1.1. The lactose was separated from the remaining ingredients using the method described in Bligh and Dyer (1959). The preseparator was washed with 10 ml MeOH and the inhaler with 10 ml MeOH/H_2O, in a volume ratio of 4:1. The resulting solutions were analyzed for ciprofloxacin content using UV spectroscopy following the previously stated procedure and equipment. Five measurements were made using samples from a single powder. To measure liposome particle size, the powder was reconstituted in isotonic saline, vortexed 2×30 s, and equilibrated at room temperature for 15 min, followed by liposome particle size measurement using photon correlation spectroscopy (Malvern Zetasizer 3000, Malvern Instruments, UK). Powder morphology was analyzed with scanning electron microscope (S2500 SEM, Hitachi, Tokyo, Japan; sample coated with gold sputter using a S150B Sputter Coater, BOC Edwards, Crawley, West Sussex, UK).

Bovine mucin, porcine lung lavage and five-fold-diluted cystic fibrosis sputum demonstrated encapsulation efficiencies >70% as shown in Table 1. The reconstituted encapsulation efficiency of the model drug in water was the lowest of all the fluids tested. This is readily explained through the results of Crowell and Macdonald (1999) and Meyer et al. (2001), who found that DMPG formulations require an ionic solution for auto-assembly of lipids.

The highest reconstituted encapsulation efficiency was observed in isotonic saline. Drawing comparison to the three pulmonary liquids, it is possible that the presence of one or more pulmonary surfactants slightly decreases the amount of encapsulation. Dilution of the liquid samples five-fold also caused a reduction in encapsulation efficiency, as shown in Table 1. This is a known and expected result as encapsulation depends on concentration of lipoplexes, ionic strength and the presence of surfactants.

The particles of the powder formulation demonstrated high specific surface area characteristics, indicative of high porosity. A representative SEM image of the powder is shown in Fig. 1. Edwards et al. (1997) examined highly porous particles and acknowledged the benefits as efficient aerosolization of the powder from inhalers, deep lung penetration due to reduced particle mass and a lower likelihood of phyglomatic clearance upon lung deposition due to particle size.

The improved aerodynamic characteristics of the powder relative to the jet-milled powder examined by Desai et al. (2002, 2003), most importantly MMAD, were apparent in deposition testing. The average MMAD was found to be 2.8 μm (standard deviation, S.D. = 1.0 μm, $n = 5$), while the FPF was calculated to be 60.6% (S.D. = 12.2%, $n = 5$). A similar jet-milled ciprofloxacin formulation produced by Desai et al. (2002, 2003) had a FPF of 45%. The average mass of ciprofloxacin in the fine particle fraction per mass of

Table 1
Encapsulation efficiency of the powder reconstituted into various fluids, both undiluted and diluted five-fold with distilled water

	Ciprofloxacin encapsulated		Ciprofloxacin encapsulated after five-fold dilution	
	Average (%)	S.D. (%)	Average (%)	S.D. (%)
Model suspension (prior to SFD)	86.9	9.8	NA	NA
Distilled water	51.0	4.3	NA	NA
Isotonic saline	86.3	6.3	42.1	NA
Bovine mucin	80.1	NA	29.2	NA
Porcine lavage	75.7	3.6	62.5	11.3
CF patient sputum	68.3	6.3	NA	NA

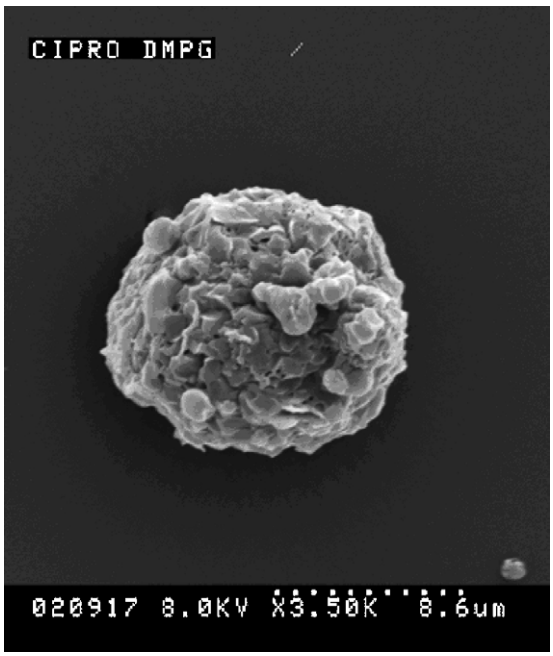

Fig. 1. A representative SEM image showing the morphology of a particle from the model powder.

power was determined to be 20.6 μg ciprofloxacin per mg of powder (S.D. = 5.6 μg/mg, $n = 5$).

Liposome particle size was measured after powder reconstitution in the saline solution. A mean volume analysis showed that 91% of the particles had a diameter <600 nm. The liposome particle distribution is shown in Fig. 2.

The electrostatic properties of DMPG and ciprofloxacin allow the spontaneous production of liposomally encapsulated ciprofloxacin particles in an ionic aqueous media. Ciprofloxacin is also known for electrostatic interactions with phosphatidylglycerols and zwitterionic phospholipids (Vazquez et al., 2001). Indeed, in vitro evidence suggests that these particles will auto-assemble in various liquid media, with encapsulation efficiency depending on surfactant, lipid concentration and ionic strength. Utilizing this property in a powder formulation circumvents many of the problems associated with delivering liposomal particles to the respiratory tract. Droplets created in nebulizers experience shear, as well as shock waves and kinematic discontinuities (Rein, 1993; Yarin and Weiss, 1995) that could impose destructive forces on the liposomes. Finlay and Wong (1998) examined nebulized liposomal ciprofloxacin and found the level of liposome disruption to be dependent on nebulizer design. A model 86111 Sonix 2000 did not disrupt the liposomal particles, but the Permaneb model 700 nebulizer retained only 8% of the ciprofloxacin as encapsulated. Lyophilization and jet milling also lead to harmful effects on the particles, as shown in Desai et al. (2002). Utilizing a dry powder aerosol that relies on lipid formation in the airway surface liquid (ASL) is beneficial because it removes the sensitive liposomal particles from the manufacturing and delivery stages of aerosol delivery.

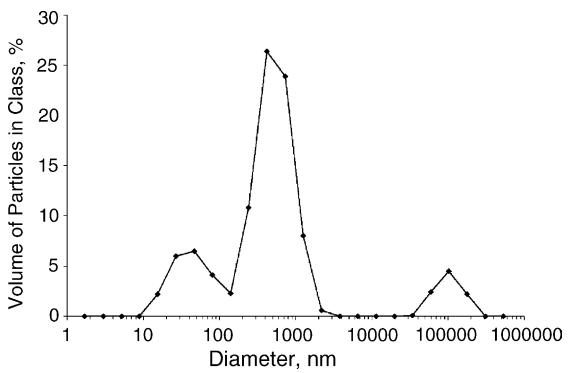

Fig. 2. Volume distribution of lipsome particles upon powder reconstitution in isotonic saline.

Reconstitution of the model powder in various representative pulmonary fluids demonstrated promising liposomal encapsulation. The results suggest that in vivo encapsulation will occur; however, direct evidence has not yet been generated. An experiment similar to Wong et al. (2003) with a suitably chosen bacterial infection would examine the powder efficacy in vivo.

A numerical lung deposition model (Finlay et al., 2000) coupled with an airway surface liquid model (Lange et al., 2001) was utilized to predict ciprofloxacin concentration in the tracheobronchial generations of normal lungs. The lung deposition model utilizes a one-dimensional, Lagrangian approach, with equations from the literature used to predict particle deposition due to diffusion, sedimentation and inertial impaction. In normal subjects, the model matches in vivo data gathered using gamma scintigraphy (Finlay et al., 2000a). The volume of ASL in each generation is predicted using a mass conservation model, with mucous velocity and production rates as independent variables and mucociliary clearance is

modeled as a series of escalators ascending through the tracheobronchial region. Drug concentration in the ASL is calculated utilizing the regional deposition results in conjunction with the regional ASL volume. The aerosol particles are assumed to be homogeneously distributed in the ASL. The model utilized an inhalation flow rate of 60 l/min with a mucous production rate of 10 ml/day and a clearance rate of 10 mm/min, average values used in a previous study (Finlay et al., 2000), as well as the MMAD and geometric standard deviation of the powder measured in this study. Upon administration of a 20 mg powder dosage, the minimum ciprofloxacin concentration is 5 mg/l and occurs in the most distal tracheobronchial generation. This concentration is above the minimum inhibitory concentration (MIC) of many bacteria causing respiratory infection, including *Pseudomonas aeruginosa* (MIC_{90} 4 mg/l), *Streptococcus pyogenes* (MIC_{90} 1 mg/l), *Neisseria gonorrhoeae* (MIC_{90} 0.004 mg/l), *Bacillus anthracis* (MIC 1.6 mg/l) and many other aerobes (Zhanel et al., 2002; Brook, 2002).

The model provides an instantaneous, maximum prediction of ciprofloxacin concentration in the ASL upon inhalation of a given dose. It assumes instantaneous dissolution of the powder and liposome formation. Since the powder contains >70% lactose by weight, and due to the prompt dissolution of lactose in isotonic saline, it is plausible that the powder will dissolve rapidly in the respiratory tract. Further, liposome formation was observed as complete in isotonic saline after 15 min during reconstitution. Consequently, it is reasonable to predict quick dissolution and liposome formation in a respiratory tract. The model provides a first-order estimate of ciprofloxacin concentration in the ASL of a normal adult and demonstrates that liposomal ciprofloxacin may be a possible treatment pathway for numerous bacterial infections.

In this paper, we have presented characteristics of a liposomal ciprofloxacin powder manufactured using a spray-freeze drying process. The powder demonstrated spontaneous in vitro formation of liposomes in aqueous media, with greater efficiency in ionic solutions. Ciprofloxacin demonstrated high encapsulation efficiency in three characteristic pulmonary fluids and also hints at the possibility of in vivo liposomal generation in the respiratory tract. The SFD process produced a powder with an improved mass median aerodynamic diameter and fine particle fraction compared to a previously jet-milled, similar ciprofloxacin powder.

Acknowledgement

This work was funded by the Department of National Defense, Canada.

References

Adjei, A.L., Gupta, P.K., 1998. Inhalation Delivery of Therapeutic Peptides and Proteins. Marcel Decker, New York.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Cdn. J. Biochem. Phys. 37, 911–917.

Bosquillon, C., Preat, V., Vanbever, R., 2004. Pulmonary delivery of growth hormone using dry powders and visualization of its local fate in rats. J. Control Release 96, 233–244.

Brook, J., 2002. The prophylaxis and treatment of anthrax. Int. J. Antimicrob. Agents 20, 320–325.

Crowell, K.J., Macdonald, P.M., 1999. Surface charge response of the phosphatidylcholine head group in bilayered micelles from phosphorus and deuterium nuclear magnetic resonance. Biochim. Biophys. Acta 1416, 21–30.

Desai, T.R., Hancock, R.E., Finlay, W.H., 2003. Delivery of liposomes in dry powder form: aerodynamic dispersion properties. Eur. J. Pharm. Sci. 20, 459–467.

Desai, T.R., Wong, J.P., Hancock, R.E.W., Finlay, W.H., 2002. A novel approach to the pulmonary delivery of liposomes in dry powder form to eliminate the deleterious effect of milling. J. Pharm. Sci. 91, 482–491.

Edwards, D.A., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M.L., Mintzes, J., Lotan, N., Langer, R., 1997. Large porous biodegradeable particles for pulmonary drug delivery. Science 276, 1868–1871.

Finlay, W.H., 2001. The Mechanics of Inhaled Pharmaceutical Aerosols, An Introduction. Academic Press, London.

Finlay, W.H., Lange, C.F., King, M., Speert, D., 2000. Lung delivery of aerosolized dextran. Am. J. Resp. Crit. Care Med. 161, 91–97.

Finlay, W.H., Lange, C.F., Li, W.-I., Hoskinson, M., 2000a. Validating deposition models in disease: what is needed? J. Aerosol Med. 13, 381–385.

Finlay, W.H., Wang, Z.L., 2003. Device and method for deagglomeration of powder for inhalation, US Patent Pending: Ref. 002555-0013.

Finlay, W.H., Wong, J.P., 1998. Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin. Int. J. Pharm. 167, 121–127.

Lange, C.F., Hancock, R.E.W., Samuel, J., Finlay, W.H., 2001. In vitro aerosol delivery and regional airway surface liquid concentration of a liposomal cationic peptide. J. Pharm. Sci. 90, 1647–1657.

Maa, Y.F., Nguyen, P.A., Sweeney, T., Shire, S.J., Hsu, C.C., 1999. Protein inhalation powders: spray drying versus spray freeze drying. Pharm. Res. 16, 249–254.

Meyer, H.M., Richter, W., Rettig, W., Stumpf, M., 2001. Bilayer fragments and bilayered micelles (bicelles) of dimyristoylphosphatidyglycerol (DMPG) are induced by storage in distilled water at 4 °C. Colloid Surf. A: Physicochem. Eng. Aspects 183–185, 495–504.

Oh, Y.K., Nix, D.E., Straubinger, R.M., 1995. Formulation and efficacy of liposome-encapsulated antibiotics for theragpy of intracellular mycobacterium avium infection. Antimicrob. Agents Chemother. 39, 2104–2111.

Rein, M., 1993. Phenomena of liquid drop impact on solid and liquid surfaces. Fluid Dyn. Res. 12, 61–93.

Steckel, H., Eskandar, F., Witthohn, K., 2003. Effect of cryoprotectants on the stability and aerosol performance of nebulized aviscumine, a 57-kDa protein. Eur. J. Pharm. Biopharm. 56, 11–21.

Taylor, K.M.G., Taylor, G., Kellaway, I.W., Stevens, J., 1990. The stability of liposomes to nebulisation. Int. J. Pharm. 58, 57–61.

Vazquez, J.L., Montero, M.T., Merino, S., Domenech, O., Berlanga, M., Vinas, M., Hernandez-Borrell, J., 2001. Location and nature of the surface membrane binding site of ciprofloxacin: a fluorescence study. Langmuir 17, 1009–1014.

Wong, J.P., Yang, H., Blasetti, K.L., Schnell, G., Conley, J., Schofield, L.N., 2003. Liposome delivery of ciprofloxacin against intracellular *Francisella tularensis* infection. J. Control Release 92, 265–273.

Yarin, A.L., Weiss, D.A., 1995. Impact of drops on solid surfaces: self-similar capillary waves, and splashing as a new type of kinematic discontinuity. J. Fluid Mech. 283, 141–173.

Zhanel, G.G., Ennis, K., Vercaigne, L., Walkty, A., Gin, A., Embil, J., Smith, H., Hoban, D.J., 2002. A critical review of the fluoroquinolones: focus on respiratory tract infections. Drugs 62, 13–59.