International Journal of Pharmaceutics 438 (2012) 209–216

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Cyclodextrin as membrane protectant in spray-drying and freeze-drying of PEGylated liposomes

Jolanda M. van den Hoven[a,b,*], Josbert M. Metselaar[b,c], Gert Storm[b,c], Jos H. Beijnen[a,b], Bastiaan Nuijen[a]

[a] Department of Pharmacy & Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands
[b] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands
[c] MIRA Institute for Biomedical Technology & Technical Medice, University of Twente, Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 9 July 2012
Received in revised form 21 August 2012
Accepted 24 August 2012
Available online 31 August 2012

Keywords:
PEGylated liposomes
Freeze-drying
Spray-drying
Lyoprotection
Hydroxypropyl-β-cyclodextrin
Disaccharides

ABSTRACT

In this study it was investigated whether hydroxypropyl-β-cyclodextrin (HPβCD) is able to stabilize the liposomal membranes during drying of long circulating polyethylene glycol (PEG) coated liposomes, as compared to the disaccharides trehalose and sucrose. PEGylated liposomes loaded with prednisolone disodium phosphate (PLP) were dried by spray-drying or freeze-drying. The dried powders were tested on their residual moisture content, glass transition temperature and amorphous character. Upon reconstitution the liposomal size, size distribution and drug retention were determined and the results were compared to the characteristics of the formulation solution before drying. In contrast to the disaccharides, HPβCD stabilizes the liposomal membranes of the PEGylated liposomes during the drying process of both spray drying and freeze-drying when present in a lipid:carbohydrate ratio of 1:6 (w/w). The resulting powder can be stored at room temperature. No changes in size and size distribution were seen upon reconstitution of the HPβCD containing formulations. Drying resulted in a minimal leaking of PLP from the liposomes. Its relatively high T_g' and T_g of HPβCD, as compared to the disaccharides, make HPβCD an excellent membrane protectant for dry PEGylated liposomal formulations.

1. Introduction

Liposomes have proven to be well tolerated drug delivery vehicles that offer the possibility of targeted drug delivery for a wide range of therapeutic agents (Metselaar and Storm, 2005). Physicochemical properties of liposomes can be changed to optimize drug delivery and retention at the target site, thus enhancing their therapeutic efficacy, and to prevent toxicity to non-target tissues (De Silva et al., 1979; Fendler and Romero, 1977; Lopez-Garcia et al., 1993; Metselaar et al., 2002). Furthermore, liposomes can offer a solution in case of formulation problems of the active compound as a result of for instance low aqueous solubility (Barratt and Bretagne, 2007; Chang and Yeh, 2012; Mazerski et al., 1982). However, the phospholipids in the liposomal membrane, especially when dispersed in water, can slowly become oxidized or hydrolyzed (Chen et al., 2010; Crommelin and van Bommel, 1984; Grit and Crommelin, 1992; Zuidam et al., 1995). This could induce fusion of liposomes, leakage of the enclosed drug compound, and structural transformations of the liposomes, which might influence their performance (Ickenstein et al., 2006). Dry products generally show higher stability, and therefore various groups have tried to develop dried liposomal formulations (Chen et al., 2010; Crowe et al., 1985; Glavas-Dodov et al., 2005; Laverman et al., 2000; Ohtake et al., 2006; Skalko-Basnet et al., 2000; van Winden and Crommelin, 1997; van Winden, 2003; Wessman et al., 2010; Wieber et al., 2012). Apart from a stabilization objective, dry liposomal formulations also offer opportunities for routes of administration other than parenteral use only, e.g. as dry powder inhalation.

Commonly applied drying techniques in pharmaceutical manufacturing are spray drying and freeze-drying. Freeze-drying of conventional liposomes (e.g. liposomes without surface modifications) has been well documented in the literature (Chen et al., 2010; Crommelin and van Bommel, 1984; Crowe and Crowe, 1988; Glavas-Dodov et al., 2005; van Winden, 2003). Though less frequently, freeze-drying of long circulating liposomes containing polyethylene glycol (PEG) has also been reported (Hinrichs et al., 2006; Hinrichs et al., 2005; Laverman et al., 2000; Wessman et al., 2010). However, as compared to lyophilization, only a limited number of reports have focused on spray-drying as a method to dry

* Corresponding author at: Department of Pharmacy & Pharmacology, Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, The Netherlands.
Tel.: +31 20 512 47 31.
E-mail addresses: jolanda.vandenhoven@slz.nl, j.m.vandenhoven@gmail.com (J.M. van den Hoven).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.08.046

liposomal formulations (Chougule et al., 2007, 2008; Goldbach et al., 1993; Hauser and Strauss, 1987; Wessman et al., 2010). Most groups tried to spray-dry conventional liposomes, though Wessman et al. (2010) investigated the effect of spray-drying on the structure of PEGylated liposomes. Compared to freeze-drying, spray-drying is much faster, less expensive and more suited for production of defined particles (Ingvarsson et al., 2011). On the other hand, freeze-drying is more suited for the development of sterile drug products. Spray-drying results in a powder mass that requires subsequent handling into the final product format whereas freeze-drying offers the possibility of drying defined volumes of the aqueous formulation in the final product container.

The main issue in drying of liposomal formulations is the stability of the liposomal membranes. These membranes can be easily disrupted during the drying process, for instance due to ice crystals or phase transition of the membranes under influence of temperature, or due to sublimation of water from the liposomal surface (Chen et al., 2010; Ingvarsson et al., 2011; Siow et al., 2007). Therefore, the liposomal membranes need to be protected during the drying process. Cryo- and lyoprotectants that are often used to protect delicate structures like proteins, DNA and liposomes during drying processes are disaccharides like sucrose and trehalose (Crowe et al., 1985; Glavas-Dodov et al., 2005; Hauser and Strauss, 1987; Laverman et al., 2000; Ohtake et al., 2005, 2006). Disaccharides are able to form hydrogen bonds, thereby stabilizing the ordered conformation of the delicate structures upon removal of water molecules (water replacement theory) (Chang et al., 2005; Maitani et al., 2008).

Besides disaccharides, hydroxypropyl-β-cyclodextrin (HPβCD), a cyclic oligosaccharide, has also proven to stabilize proteins during spray-drying (Branchu et al., 1999; Iwai et al., 2007). The exact mechanism is still unknown but might be improved vitrification due to a higher vitrification temperature (the glass transition temperature of maximally cryoconcentrated solutions, T_g') and/or improved water replacement due to its large number of hydrogen donors and acceptors (Abdelwahed et al., 2006; Branchu et al., 1999; Iwai et al., 2007; Serno et al., 2011; Vega et al., 2012). HPβCD has a high aqueous solubility and a safe toxicity profile for a variety of administration routes, including parenteral use (Challa et al., 2005; Loftsson and Duchêne, 2007; Pourmokhtar and Jacobson, 2005). Several products containing HPβCD have been marketed, e.g. Sporanox® and Trisporal® (containing itraconazol) and Indocollyre® (containing indometacin) (Davis and Brewster, 2004).

In this study it was investigated whether HPβCD is able to stabilize the liposomal membranes during both spray-drying and freeze-drying of long circulating PEGylated liposomes, as compared to the disaccharides trehalose and sucrose. The PEGylated liposomes were loaded with the water-soluble drug prednisolone disodium phosphate (PLP) as a model drug. Creating a dry liposomal formulation of a water-soluble drug encapsulated in the aqueous core of the liposome is a major challenge, since the drug can leak out of the liposome during drying (van Winden, 2003). Therefore, drug leakage is a good marker for instability or even rupture of the liposomal membranes during the drying process. From our own experience we know that PLP does not leak out of the PEGylated liposomes in aqueous dispersion (Nanocort; Metselaar et al., 2003). Also, PLP solutions are chemically stable for considerable time. Based on these characteristics, we selected PLP-PEGylated liposomes as a model drug formulation. During drying, the water is removed from both outside and inside the liposomes. Therefore, it might be relevant to protect the liposomal membrane on both sides (Crowe et al., 1985; Ohtake et al., 2005). To evaluate this, liposomal formulations were prepared both with and without lyoprotectant present in the liposome core. Besides drug retention, physicochemical properties and microscopic appearance of the dried liposomal formulations were investigated.

2. Materials and methods

2.1. Preparation of the liposomes

Liposomes were prepared using a film extrusion method (Amselem et al., 1993). Briefly, dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 (DSPE-PEG) (both from Lipoid GmbH, Ludwigshaven, Germany) and cholesterol (BUFA, Uitgeest, The Netherlands) were dissolved in ethanol. A lipid film was created by rotary evaporation at 65 °C. The lipid film was hydrated with a solution containing prednisolone disodium phosphate (BUFA, Uitgeest, The Netherlands) in a concentration of 139 mg/mL. Furthermore, the hydrating solutions contained either 0% or 10% of sucrose (BUFA, Uitgeest, The Netherlands), trehalose (Merck, Darmstadt, Germany) or HPβCD (Roquette Pharma, Lestrem, France) in sterile water for injections (B. Braun, Melsungen, Germany). The resulting coarse dispersion was sized by multiple extrusion steps through polycarbonate filter membranes with a pore size of 100 nm, resulting in liposomes with a diameter of about 100 nm, as was confirmed by dynamic light scattering (DLS). Unencapsulated PLP was removed by dialysis against a 10% solution of sucrose, trehalose or HPβCD using Slide-A-Lyzer dialysis cassettes (Thermo Fisher Scientific, Etten-Leur, The Netherlands) with a molecular weight cut-off of 10 kDa, with repeated changing of the dialysis medium. The lipid content of the liposomal dispersions was determined using HPLC, and the liposomal dispersions were subsequently diluted with their corresponding 10% sugar solutions to a final ratio of sugar:lipid of 6:1 (w/w, dry product), as ratios of 4:1 or higher have shown to protect the liposomes during drying in previous studies (Chaudhury et al., 2012; Crowe and Crowe, 1988; Laverman et al., 2000). The diluted dispersion is used for the drying processes.

All compounds used were of pharmaceutical (Ph. Eur) or highly pure (≥99%) grade and were used without any further purification.

2.2. Spray-drying of the liposomes

The aqueous formulations were spray-dried using a B-290 Mini Spray Drier (Büchi Labortechnik GmbH, Hendrik-Ido-Ambacht, The Netherlands). The spray-drying conditions were selected based on literature (Chougule et al., 2007, 2008; Skalko-Basnet et al., 2000) and were as follows: inlet and outlet temperatures were 100 °C and 68 °C, respectively; airflow rate was 35 m^3/h and the spray gas flow was 670 L/h; with a nozzle size of 0.7/1.5 mm the feed was set at 1 mL/min. The resulting spray-dried powders were kept in closed containers at 2–8 °C prior to characterization and further analysis.

2.3. Freeze-drying of the liposomes

1 mL aliquots of the liposomal dispersions were filled into 8R colorless glass vials (hydrolytic class type 1 Fiolax clear, Aluglas, Uithoorn, The Netherlands). Vials were partly closed using gray bromobutyl rubber lyophilization closures (West Pharmaceutical Services Inc., Lionville, PA, USA) and loaded into the freeze dryer (Model Lyovac GT4, GEA Lyophil GmbH, Hürth, Germany). The lyophilization program was based on the literature (Aso and Yoshioka, 2005; van Winden et al., 1997; van Winden, 2003). Vials were frozen to −35 °C at 0.5 °C/min in two hours. The shelf temperature of −35 °C was maintained for 24 h during the primary drying phase, while a vacuum of 10 Pa was established. At the end of primary drying the temperature was linearly increased to 0 °C in 2 h while the pressure was reduced to 0.9 Pa, to start secondary drying.