International Journal of Pharmaceutics 513 (2016) 175–182

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Effect of ethanol as a co-solvent on the aerosol performance and stability of spray-dried lysozyme

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ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 3 September 2016
Accepted 7 September 2016
Available online 9 September 2016

Keywords:
Spray drying
Lysozyme
Ethanol
Aerosol performance
Protein stability

ABSTRACT

In the spray drying process, organic solvents can be added to facilitate drying, accommodate certain functional excipients, and modify the final particle characteristics. In this study, lysozyme was used as a model pharmaceutical protein to study the effect of ethanol as a co-solvent on the stability and aerosol performance of spray-dried protein. Lysozyme was dissolved in solutions with various ratios of ethanol and water, and subsequently spray-dried. A change from spherical particles into wrinkled and folded particles was observed upon increasing the ratio of ethanol in the feed. The aerosol performance of the spray-dried lysozyme from ethanol-water solution was improved compared to that from pure water. The conformation of lysozyme in the ethanol-water solution and spray dried powder was altered, but the native structure of lysozyme was restored upon reconstitution in water after the spray drying process. The enzymatic activities of the spray-dried lysozyme showed no significant impact of ethanol; however, the lysozyme enzymatic activity was ca. 25% lower compared to the starting material. In conclusion, the addition of ethanol as a co-solvent in the spray drying feed for lysozyme did not compromise the conformation of the protein after drying, while it improved the inhaled aerosol performance.
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1. Introduction

Protein-based therapeutics have gained great interest in human health care. In 2010, there were already about 100 therapeutic proteins approved for clinical use in Europe and the USA, with sales of USD 108 bln (Voynov and Caravella, 2012). Many of the protein therapeutics on the market are used for the treatment of critical diseases, including cancer, immune disorders, and infections. These macromolecule therapeutics may provide hope for some patients with diseases where no small molecule drugs can render optimal treatment. However, protein-based therapeutics are, in general, physically and chemically unstable during manufacturing and long term storage, so they are difficult to formulate (Frokjaer and Otzen, 2005; Leader et al., 2008). During processing, storage and transportation they encounter various known stress factors such as high or low temperature, interfaces, and shearing or stirring, which may accelerate unwanted degradation of the protein (Carpenter et al., 1997; Wang et al., 2010). Stabilization of the protein molecules against this degradation is one of the main tasks in the development of these products.

Commonly used formulation approaches to stabilize protein therapeutics include addition of stabilizing excipients and freeze drying (Abdul-Fattah et al., 2007; Andya et al., 1999; Carpenter et al., 1997; Frokjaer and Otzen, 2005; Randolph, 1997; Wang, 1999, 2000). The latter process is in principle undesirable, as most protein therapeutics are administered by injection or infusion, and thus require the protein to be in a liquid formulation. However, the removal of water is often necessary to obtain a stable product, and many protein therapeutics are therefore marketed as a freeze-dried solid (Frokjaer and Otzen, 2005; Sinha and Trehan, 2003; Wang, 1999, 2000). Recently, protein based therapeutics have also been formulated using the spray drying process (Ingvarsson et al., 2013; Wan et al., 2014b; Yang et al., 2007). This method converts a feed from a liquid state into a dry powder form by spraying the feed into droplets, and subsequently drying the droplets with hot gas medium. Compared to freeze drying, this method is faster, less energy consuming and has potential as a continuous

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http://dx.doi.org/10.1016/j.ijpharm.2016.09.025
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manufacturing platform (Masters, 1979). In addition, spray drying may possess better particle engineering potential than freeze drying (Ameri and Maa, 2006; Cal and Sollohub, 2010).

In the spray drying process, solvent constitutes most of the feed where proteins and excipients are dissolved or suspended. In some cases, a co-solvent may be needed to dissolve both active substance and functional excipients, e.g. in the case of a hydrophilic protein with hydrophobic retarding agents for depot formulation. The composition of feed can be critical in terms of the particle/powder properties as well as the protein stability (Mattos and Ringe, 2001; Wan et al., 2014a). The addition of a second solvent in the spray drying process may alter the drying kinetics of the feed, which will influence the particle formation process, thus affecting the particle properties of resulting powders (Wan et al., 2013). Subsequently, it can have impact on the powder handling and product performance of, e.g. inhaled dry powder (Adi et al., 2008; Chan, 2008; Chew and Chan, 2001).

With regards to the protein stability, an addition of a volatile organic solvent into an aqueous solution facilitates the drying of the droplets, resulting in lower residual solvent in the final product and improved storage stability (Cal and Sollohub, 2010). In addition, compared to pure water based feed, less energy input is required for the evaporation of the volatile organic solvent, which may reduce the thermal burden applied to liable compounds like proteins. Hence, an addition of organic solvent to the feed might not necessarily compromise the protein stability.

In the present study, we used lysozyme as a model protein drug and ethanol as a co-solvent to study how the proportion of ethanol in water solution influenced the conformational stability, enzymatic activity and aerosol performance of inhalable dry powders generated from the spray drying process. Lysozyme was chosen because it is readily available and the methods to characterize its conformation and bioactivity have been well reported in literature (Ógáin et al., 2011; Sassi et al., 2011; van de Weert et al., 2001). Ethanol was chosen as it is among the commonly used solvents in pharmaceutical industry.

2. Materials and methods

2.1. Materials

Chicken egg white lysozyme (Product No.: 62971-F) in the form of crystalline powder (Fluka, Steinheim, Germany), and ethanol (CHROMASOLV®, ≥99.8%; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used. All other chemicals were of analytical grade.

2.2. Preparation of spray dried powder from different water-ethanol feeds

Lysozyme (2.5 g) was dissolved in water (Milli-Q, Waters) before mixing with corresponding volume of ethanol (the mass fraction of ethanol was 0%, 20%, 40%, 60% and 80%; the molar fraction of ethanol was 0%, 8.9%, 20.7%, 37.0% and 61.0%) to prepare different aqueous ethanol mixtures as feeds at a solid concentration of 10 mg/mL. After storage at 4 °C for 24 h, these solutions (LE00, LE20, LE40, LE60 and LE80) were spray dried using a Büchi B-290 Mini Spray Dryer (BÜCHI Labortechnik, Falwil, Switzerland) equipped with an inert loop B-295 under the following process settings: 473 L/h of flow rate (40 mm on spray dryer), ca. 35 m^3/h of drying gas flow rate (95% of Aspirator rate), 2.3–2.6 mL/min of feed rate (10% peristaltic pump rate), and 130 °C of inlet temperature. The outlet temperatures of the spray drying processes from different ethanol-water compositions are listed in Table 1. The spray-dried powders (SDLE00, SDLE20, SDLE40, SDLE60 and SDLE80) were collected into glass vials, sealed with Parafilm and kept in plastic desiccators containing silica gel at room temperature, under vacuum.

2.3. Thermogravimetric analysis

The residual solvent in the spray-dried lysozyme was measured by thermogravimetry (TGA; TA Instruments, New Castle, DE). Around 5–10 mg of dry powders was loaded onto an open platinum TGA sample pans and analyzed in triplicate. The heating program for the measurement was from 25 °C to 250 °C at 10 °C/min.

2.4. Laser diffraction

To obtain the particle size distribution of spray dried powders, a laser diffractometer Mastersizer 2000 (Malvern Instruments, Malvern, UK) was employed. The powders were dispersed with a Scirocco 2000 powder feeder (Malvern Instruments, Malvern, UK) at a dispersive air pressure of 3.0 bar. All the samples were measured in triplicate.

2.5. Brunauer–Emmett–Teller and Barrett–Joyner–Halenda

Nitrogen sorption experiments were performed on a Tristar II 3020™ Surface Area and Pore Analyzer (Micromeritics, Norcross, USA). The samples were degassed in a vacuum at 200 °C under vacuum overnight prior to measurements. The Brunauer–Emmett–Teller (BET) method (Brunauer et al., 1938) was used to calculate the surface area according to the adsorption data in a relative pressure from 0.05 to 0.3 (Leofanti et al., 1998). The pore volume was calculated by using the Barrett–Joyner–Halenda (BJH) model (Barrett et al., 1951).

2.6. Scanning electron microscopy

The morphology and size of the spray dried powders were visually examined by using a TM3030 Scanning electron microscopy (SEM; Hitachi Ltd, Tokyo, Japan) operating under low vacuum (ca. 10^{-3} Pa) at 15 kV. The samples were dispersed onto carbon sticky tabs and sputter coated on a thin layer of gold for 45 s with a Cressington 108 auto Sputter Coater (Cressington Scientific

Table 1
Physicochemical properties of spray-dried protein.

Sample	Ethanol proportion (% w/w)	Outlet (°C)	Yield (%)	Particle size, Dv 50 (μm)	Residual solvent (%)	Surface area (m^2/g)	Pore volume (cm^3/g)
SDLE00	0	70	76	$2.45 \pm 0.01^{*}$	$9.51 \pm 0.77^{\#\#}$	1.21	0.0016
SDLE20	20	69–70	75	2.15 ± 0.13	$5.30 \pm 0.07^{\#,\#\#}$	4.25	0.0111
SDLE40	40	71	69	2.22 ± 0.09	$5.67 \pm 0.18^{\#,\#\#}$	7.45	0.0244
SDLE60	60	73–75	66	2.21 ± 0.03	$4.73 \pm 0.29^{\#}$	5.78	0.0164
SDLE80	80	73–74	64	$2.66 \pm 0.16^{*}$	$4.37 \pm 0.13^{\#,\#\#}$	9.84	0.0246

* $p < 0.05$ compared to the other three samples—SDLE20, SDLE40 and SDLE60.
\# $p < 0.05$ compared to SDLE00
\## $p < 0.05$ compared to the residual moisture of untreated raw material: $4.90 \pm 0.08\%$.

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