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Effect of excipients on encapsulation and release of insulin from spray-dried solid lipid microparticles

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ABSTRACT

The study aimed at investigating the potential of spray drying method for encapsulation of protein drugs into solid lipid microparticles (MP) and evaluating effects of excipients on encapsulation and release of protein from MP. After transformation of model protein insulin to insulin-phospholipid complex, it was dissolved together with lipid excipients in organic solvent, which was spray-dried to form solid lipid MP. Polymeric MP with D, L-lactic-co-glycolic acid (PLGA) were prepared similarly. Around 90% of insulin was encapsulated in glycerol monostearate MP and glycerol distearate MP, whereas the encapsulation efficiency was 60% and 35% for tristearate (TG18) MP and tribehenate (TG22) MP, respectively. The secondary structure of insulin in the spray-dried MP was substantially similar to that of the insulin control solution, suggesting that only minor alterations occurred during the spray drying process. Sustained release of insulin was observed from both TG18 MP and TG22 MP. The burst release of insulin from TG18 MP and TG22 MP was around 30% and 10%, respectively, which was significantly lower than that from PLGA MP (40%). In conclusion, spray drying a solution containing both lipids and protein-phospholipid complex is a promising method for encapsulating protein into solid lipid MP, which can be used for sustained delivery of protein drugs.

1. Introduction

There is an increased interest in therapeutic proteins in the pharmaceutical area, however, the integrity and bioactivity of protein drugs could be lost during manufacturing and storage; therefore stabilization of protein drugs is one of the main tasks in formulation development (Frokjaer and Otzen, 2005; Bilati et al., 2005). Additionally, sustained release formulations are preferred for protein drugs due to short half-lives of many therapeutic proteins (Putney and Burke, 1998; Pisal et al., 2010). Poly (D, L-lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer (Anderson and Shive, 2012), has been used in a number of approved parenteral products for sustained delivery of peptide drugs since 1980s. Lipids are biocompatible materials, solid lipid nanoparticles have shown great potentials for sustained drug delivery via different administration routes (Reithmeier et al., 2001; Schwarz et al., 1994; Borkar et al., 2014; Mu and Holm, 2018). Solid lipid formulations have also drawn great attentions for sustained delivery of protein and peptide drugs such as insulin, thymocartin and BSA, from days to months (Reithmeier et al., 2001; Mu and Holm, 2018; Koennings et al., 2007; Almeida and Souto, 2007; Maschke et al., 2007; Guse et al., 2006).

Spray drying is a continuous process to prepare drug powders, granules or microparticles (MP); the quality of spray-dried product is influenced by several factors, including inlet- and outlet-temperatures and properties of spray drying feed. Hydrophobic drug substances can be efficiently encapsulated into PLGA MP and solid lipid MP via direct spray drying process after dissolving both drug and excipients in organic solvents (Fu et al., 2002; Alvim et al., 2016). However, encapsulating protein drugs into solid lipid MP by spray drying is more challenging because of the incompatibility of hydrophilic proteins and the hydrophobic excipients. To overcome the problem different approaches have been made, such as the use of W/O emulsion or suspension as spray drying feed or using 3-fluid nozzle spray drying technique (Wan et al., 2014; Quaglia et al., 2003). When a W/O emulsion was used in preparing drug-loaded PLGA MP by spray drying, the encapsulation efficiency (EE) of drug in the MP was only around 30% and the burst release of drug from the MP was high (Johansen et al., 2000; Prior et al., 2000). Both the high burst release and low EE of drug could be related to the presence of the aqueous phase in the spray drying feed. Anhydrous particle preparation methods have shown advantages in the preservation of protein stability. For example, tumor necrosis factor-alpha was inactivated to a large extent when the MP

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were prepared by W/O/W or S/O/W method (S: solid) due to the presence of water, whereas its activity was fully preserved when a S/O/O dispersion was used in particle preparation (Iwata et al., 1998). Therefore, spray drying a single phase of organic solution could have the advantage in avoiding the water-organic interface in the feed and reducing the instability of protein drugs during the fabrication process.

Solid lipid MP are usually prepared by solvent evaporation method, hot melting dispersion method, or spray chilling technique (Jaspart et al., 2005; Gamboa et al., 2018). The size of the MP made by hot melting dispersion method or spray chilling technique are typically from 10 µm to 500 µm (Maschke et al., 2007), whereas the MP made by spray drying method are smaller than 10 µm, which is suitable for subcutaneous or intramuscular injections (Reithmeier et al., 2001). Additionally, hot melting dispersion or spray chilling technique usually requires heating to mix lipids and drug substances, which may affect the stability of proteins; whereas the heating process in spray drying only lasts a few seconds (Broadhead et al., 2008). To the best of our knowledge, there was no study reported about protein loaded solid lipid MP made by spray drying method. The aim of this study was to investigate the feasibility of spray drying method for efficient encapsulation of protein drugs into solid lipid MP using an organic solution as the feed and evaluate effects of lipid structure on the release of protein from the MP.

2. Material and method

2.1. Materials

Tribehenate (Dynasan D122, TG22) and tristearate (Dynasan D118, TG18) were a generous gift from IOI Oleo GmbH (Hamburg, Germany). Glycerol monostearate (GMS) and glycerol distearate (GDS) were kindly donated by Gattefosse (St Priest, Cedex, France). PLGA (75/25, RG 753 S, MW around 50 kDa) was obtained from EVONIK (Darmstadt, Germany). Soybean PC (Lipoid S-100, SPC) was purchased from Lipoid GmbH (Ludwigshafen, Germany). Insulin was obtained from Dongbao Enterprise Group Co. Ltd. (Jilin, China). Polyethylene glycol 4000 (PEG 4000), chloroform, dimethyl sulfoxide (DMSO) and acetic acid were bought from Sigma Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Preparation of insulin-phospholipid complex

Insulin-phospholipid complex was prepared according to a method reported previous (Cui et al., 2006) with minor modifications. In brief, phospholipid and insulin, with a weight ratio of 10:1, were mixed with DMSO containing 5% (v/v) of acetic acid under continuously stirring for 2 h at 37 °C until it became a clear solution. The solution was freeze dried and the lyophilized insulin-phospholipid complex was sealed and stored at −20 °C for further uses.

2.3. Spray drying method

The MP were prepared by spray drying according to the formulation composition listed in Table 1. TG22 MP were prepared in chloroform, while other MP were prepared in dichloromethane. Spray drying was carried out using a Buchi 290-Mini Spray Dryer combined with an inert loop B295 (Buchi, Flawil, Switzerland). The operating conditions were as follows: nozzle diameter, 0.7 mm; atomization gas flow rate, 350 L/h; feeding rate, around 2 mL/min; drying gas flow rate, 35 m^3/h; inlet temperature, 55 °C for chloroform solution and 45 °C for dichloromethane solution; outlet temperature, around 42 °C for chloroform solution and around 39 °C for dichloromethane solution. MP powder was collected from the collection jar. The yield of MP was calculated by using the amount of collected MP divided by total amount of excipient.

2.4. Particle size analysis

The particle size and size distribution of spray dried MP were analyzed by laser diffraction principle using a Mastersizer (Mastersizer 2000, Malvern Instruments Ltd., UK) equipped with Hydro 2000S (Malvern Instruments Ltd., UK) in wet dispersions mode. Wet dispersions were prepared by dispersing the MP in Tween 80 solution (0.1%), and the measurements were performed under stirring (1200 rpm) and ultrasound (50% intensity). The obscuration was set between 8% and 12%.

2.5. Encapsulation efficiency

Insulin content in TG18 MP and TG22 MP was measured after dissolving the MP in chloroform, evaporating the organic solvent under nitrogen, and dissolving the precipitated insulin in purified water. Insulin content in the other MP was measured by dissolving the MP in DMSO and precipitating excipients by adding purified water, insulin in the aqueous phase was analyzed after centrifugation. Insulin was quantified by using HPLC (Agilent HPLC system) with a C18 column (4.6 × 100 mm, 5 µm, 300 Å, Waters) at room temperature. A binary solvent system was used at a flow rate of 1 mL/min, and insulin was detected at 220 nm. Solvent A was 0.1% (v/v) trifluoroacetic acid in purified water and solvent B was 0.1% (v/v) trifluoroacetic acid in acetonitrile. A gradient was running for16 min; 0–2 min: 26% B, 2–10 min: 26–38% B, 10–10.2 min: 38–100% B, 10.2–13 min: 100% B, 13–13.2 min: 100–26% B, 13.2–16 min: 26% B. The EE was calculated according to the equation below:

$$EE = (\text{insulin extracted from the MP}/\text{theoretic insulin in the MP})^{*}100\%$$

2.6. Scanning electron microscopy

The morphology of MP was observed by using scanning electron microscopy (SEM) (TM3030, Hitachi, Japan). The samples were coated with gold under an argon atmosphere for 20 s prior to SEM observation and examined under an accelerating voltage of 5 kV.

2.7. X-ray powder diffraction

To elucidate the solid state of lipids in spray-dried MP, X-ray powder diffraction (XRPD) analysis was performed using a PANalytical X'Pert Pro diffractometer equipped with a PIXcel detector (PANalaytical B.V., Almelo, The Netherlands). Measurements were conducted from 5 to 35° (2θ) at ambient conditions by using Cu Kα radiation at 40 mA and 45 kV, with an angular increment of 0.04°/s and count time of 2 s. Data were analyzed by X'Pert HighScore Plus version 2.2.4 (Almelo, The Netherlands).

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements (Perkin Elmer, Shelton, USA) were carried out to determine the thermal behavior of lipids and glass transition point of PLGA. Approximately 3 mg of samples were weighed into DSC pans and crimp sealed. They were heated from 10 °C to 100 °C at a heating rate of 10 °C/min under a flow of nitrogen (20 mL/min). The DSC system was equipped with the software Trios v3.3.1.

2.9. Confocal laser scanning microscopy

Insulin was labeled with fluorescein isothiocyanate (FITC) based on a method described previously (Clausen and Bernkop-Schnürch, 2000). FITC-insulin was encapsulated in PLGA MP (weight ratio of PLGA, SPC and FITC-insulin was: 90:10:1) by the spray drying method described in Section 2.3. Spray-dried MP were suspended in purified water, mounted

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