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Spray freeze drying as an alternative technique for lyophilization of polymeric and lipid-based nanoparticles

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ABSTRACT

The use of nanoparticles for drug delivery is still restricted by their limited stability when stored in an aqueous medium. Freeze drying is the standard method for long-term storage of colloidal nanoparticles; however the method needs to be elaborated for each formulation. Spray freeze drying (SFD) is proposed here as a promising alternative for lyophilizing colloidal nanoparticles. Different types of polymeric and lipid nanoparticles were prepared and characterized. Afterwards, samples were spray freeze dried by spraying into a column of cold air with a constant concentration of different cryoprotectants, and the frozen spherules were collected for further freeze drying. Similar samples were prepared using the commonly used technique, freeze drying, as controls. Using SFD, fast-dissolving, spherical and porous nanocomposite microparticles with remarkably high flowability ($CI \leq 10$) were produced. On the contrary to similar samples prepared using the freeze drying technique, the investigated polymeric and lipid nanoparticles were completely reconstituted (S_f/S_i ratio <1.5) after SFD. SFD proved to be an effective platform for improving the long-term stability of colloidal nanoparticles.

1. Introduction

Nanostructures represent drug delivery systems that can be administered via different routes. Different types of polymeric and lipid-based nanoparticles may act as potential carries for several classes of therapeutic agents such as anticancer drugs, hormones and antihypertensive agents; as well as biological macromolecules such as nucleic acids and antibodies (Hans and Lowman, 2002). Moreover, many novel preparation techniques for drug-loaded nanoparticles are being developed and refined (Abbas et al., 2016; Ali and Lamprecht, 2013; Tran et al., 2012). However, the application of these nanoparticles has not been until now thoroughly exploited due to their poor stability when conserved in aqueous medium for long periods (Coffin and McGinity, 1992).

The problems that limit the use of nanoparticles for drug delivery applications can be either physical instability (aggregation/particle fusion) and/or chemical problems (microbiological contamination, hydrolysis of polymer materials forming the nanoparticles, drug leakage), which are frequently observed when an aqueous suspension of nanoparticles is stored for extended periods (Abdelwahed et al., 2006b; Chacon et al., 1999; Magenheim and Benita, 1991). Therefore, the enhancement of the shelf-life of colloidal nanoparticles is of great interest.

Freeze drying (FD) is the commonly used technique to improve the physical and chemical stability of colloidal nanoparticles by removal of water from the aqueous dispersions to obtain them in a dried form (Abdelwahed et al., 2006b; Franks, 1998). However, FD of nanoparticles requires major investigation of not only the formulation aspects, but also the FD process conditions. The process conditions may have a crucial impact on the stability of nanoparticles during and after FD (Abdelwahed et al., 2006b). In FD, the nanoparticles are subjected to various stresses such as freezing and desiccation stresses, which may be detrimental to the nanoparticle stability. This is usually alleviated by the use of cryo- and lyoprotectants as excipients in the formulation (Lee et al., 2009). The selection of the proper excipients has been largely relied on either experiential approaches or performed by trial and error (Fonte et al., 2016). Moreover, whether the pre-screening tests (Saez et al., 2000; Schwarz and Mehnert, 1997) can improve the FD process by reducing the time of trial and error is still questionable. Nonetheless, high cryoprotectant concentrations and fast freezing rates have been frequently reported to be favoured to achieve a successful FD process of various polymeric

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and lipid nanostructures (Abdelwahed et al., 2006a; Hirsjarvi et al., 2006; Lee et al., 2009; Schwarz and Mehnert, 1997). Therefore, other approaches to improve the current FD process are highly desirable and are being thereby consecutively investigated.

As an alternative, spray freeze drying (SFD) is proposed here for stabilizing and preserving the colloidal nanoparticles in a dried form. In SFD, liquids are rapidly frozen as fine droplets when sprayed into cold air or liquid nitrogen, and the frozen droplets are then dried using a freeze dryer to allow the sublimation of ice and the formation of dried particles. If a colloidal dispersion of nanoparticles is co-sprayed by this method in presence of a cryoprotectant, dried nanocomposite microparticles are ultimately obtained instead of the dried cake in case of FD. It is also worth noting that SFD is proposed here not only to improve the versatility of the current FD process, but also the quality of the final product obtained after drying. Within this context, a good and rapid reconstitution performance of the final product is a main concern to ensure the maintenance of the colloidal characteristics of the nanoparticle dispersions after drying.

In this study, SFD into cold air (Eggerstedt et al., 2012) was investigated as a feasible technique for lyophilization of different types of nanostructures in presence of a fixed concentration of different cryoprotectants. Different types of nanostructures were tested in this approach, including both polymeric and lipid nanoparticles. Furthermore, control samples were also dried using the commonly used technique, FD. Nevertheless, the study took into consideration the reconstitution ability of the dried nanocarriers and the quality of the prepared SFD final products.

2. Experimental

2.1. Materials

Poly (DL-lactide-co-glycolide) (Resomer® RG 502H) and Poly (meth)acrylate (Eudragit® RL PO) were kind gifts from Boehringer Ingelheim and Evonik Röhm GmbH, respectively. Ethyl cellulose (Ethocel standard 4 premium) was purchased from Colorcon, UK. Polyvinyl alcohol (98–99% hydrolyzed) and sodium cholate were purchased from Sigma-Aldrich Chemie GmbH, Germany. Miglyol® 812 (medium chain triglyceride) was supplied by Fagron GmbH, Germany. Soybean lecithin was purchased from Caelo, Germany. Witepsol® H15 was from Sasol GmbH, Germany. Cremophor® A25 and Kolliphor® HS15 were donated from BASF, Germany. Maltodextrin (GLUCIDEX® 19, dextrose equivalent DE of 19) was a gift from Roquette Freres, France. D (−) mannitol (Ph. Eur.) and trehalose (Ph. Eur.) were supplied by VWR International, Netherlands. All other chemicals were of analytical grade or equivalent purity.

2.2. Preparation of nanoparticles

Polymeric nanoparticles were prepared using poly DL-lactide-co-glycolide (PLGA), ethyl cellulose (EC) and Eudragit® RL PO (EDRL) as model polymers. Nanoparticles were prepared by the o/w emulsion solvent evaporation technique (Abdel-Mottaleb et al., 2011; Hoffart et al., 2002), where the polymer is firstly dissolved in dichloromethane, forming the organic phase. This organic solution was then brought into emulsion with aqueous solution of polyvinyl alcohol (PVAL, 0.3% w/v), using ultrasonic cell disruptor (Banoelin sonopuls, Berlin, Germany). Nanoparticles are formed when the organic solvent is evaporated out of the formed emulsion using a Büchi Rotavapor RE 120 (Büchi, Flawil, Switzerland).

Liposomes were prepared by the film hydration method. Briefly, 600 mg of soybean lecithin were dissolved in a 45 ml mixture of chloroform and methanol (2:1 v/v chloroform: methanol). The solution formed was then evaporated under reduced pressure till complete dryness, leaving behind a thin film of lipid. The formed lipid film was subsequently rehydrated with 30 ml of distilled water and sonicated at 60 W for 10 min.

Solid lipid nanoparticles (SLN) were prepared by the hot homogenization technique (Abdel-Mottaleb et al., 2011; Casadei et al., 2006). Firstly, the solid lipid Witepsol (10 g) was allowed to melt by heating to 70 °C and mixed with a pre-heated aqueous solution of 90 ml water, containing 1 g sodium cholate and 2.5 g Cremophor® A25. Homogenization of the hot mixture is subsequently achieved by ultraturrax at 10,000 rpm for 10 min, followed by sonication for 20 min at 70 °C. The hot emulsion formed was then left overnight under magnetic stirring before further analysis.

Lipid nanocapsules (LNC) were prepared by the phase inversion method (Heurtault et al., 2002; Lamprecht et al., 2004). A mixture of 1 g medium chain triglyceride (MCT), 1 g Kolliphor® HS15, 100 mg Sodium chloride, 100 mg soybean lecithin and 3 g distilled water was heated under magnetic stirring up to 85 °C, forming a w/o emulsion. The hot emulsion was then allowed to cool down to 55 °C, where another phase inversion to an o/w emulsion occurs. This cycle of phase inversion was repeated twice before adding 5 ml of cold distilled water (4 °C), followed by stirring for 10 min before further analysis of the particle size.

2.3. Determination of the particle size

The particle size and distribution of the prepared nanostructures were determined by measuring the average volume diameters and polydispersity index (PDI) using particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA) at a fixed angle of 90°. Samples were diluted with distilled water before being analyzed at 25 °C. Three measurements of three different batches for each nanoparticle formulation were used to calculate the average particles size and standard deviation.

2.4. Screening of cryoprotectants

A set of pre-experiments were conducted to investigate the possible selection of the excipients with the highest potential for cryoprotection, using mannitol, trehalose and maltodextrin as model cryoprotectants.

Firstly, all the prepared nanoparticle dispersions were subjected to 48 h incubation with 5% w/v of the investigated cryoprotectants at 4 °C. Secondly, freeze-thaw tests were conducted by freezing the different nanoparticle formulations with 5% of the investigated cryoprotectants in a shelf refrigerator at −30 °C for 24 h and subsequent thawing at room temperature. Control samples without cryoprotection were also investigated. In both experiments, the presence of any aggregates was investigated analytically by particle size analyzer for any increase in particle size and/or polydispersity index.

2.5. Spray freeze drying (SFD)

SFD process was performed basically in accordance with the method described by Eggerstedt et al. (Eggerstedt et al., 2012), but with some modifications. For droplet formation, a monodisperser droplet generator (MTG-01-G2, FMP Technologies GmbH, Erlangen, Germany) with a nozzle plate diameter of 100 μm was used. Nanoparticle dispersions were co-sprayed with a fixed concentration of 5% w/v of different cryoprotectants into a column of cold air (−120 °C), surrounded by a cooling jacket of liquid nitrogen. The droplets are frozen during their flight in the cooled air and the frozen particles were collected after sedimentation in a cooled container, positioned directly at the lower end of the spray column