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Preserving the supersaturation generation capability of amorphous drug-polysaccharide nanoparticle complex after freeze drying

Tie Yi Kiew, Wean Sin Cheow, Kunn Hadinoto *

School of Chemical and Biomedical Engineering, Nanyang Technological University, 637459, Singapore

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ABSTRACT

While the supersaturation generation capability of amorphous nanopharmaceuticals (NPs) in their aqueous suspension form has been well established, their supersaturation generation is adversely affected after drying. Herein we investigated the effects of freeze drying on the supersaturation generation capability of a new class of amorphous NPs referred to as drug nanoplex prepared and stabilized by electrostatic complexation of drug molecules with polysaccharides (dextran sulfate). Using ciprofloxacin as the model drug, two types of freeze-drying adjuvants were investigated, i.e., (1) highly water-soluble excipient (trehalose, mannitol), whose role was to prevent irreversible NPs aggregations upon drying, and (2) crystallization inhibitor (hydroxypropylmethylcellulose (HPMC)), whose role was to suppress crystallization of the dissolved drug and the remaining solid phase. The results showed that dual-adjuvant formulations (i.e. trehalose-HPMC and mannitol-HPMC) were required to preserve the supersaturation generation capability of the drug nanoplex suspension after drying. Freeze drying with only one adjuvant type, or incorporating HPMC as physical mixtures with the freeze-dried nanoplex, were ineffective in preserving the supersaturation. The dual-adjuvant formulations produced prolonged supersaturation levels over 240 min at ≈6–8× of the saturation solubility with comparable area under the curve (AUC) in the concentration versus time plot as that exhibited by the suspension form.

1. Introduction

Amorphous nanopharmaceuticals (NPs) have recently emerged as a promising bioavailability enhancement strategy of poorly-soluble drugs owed to (1) the drug supersaturation generation afforded by the metastable state of the amorphous form (Babu and Nangia, 2011), and (2) the fast drug dissolution rate afforded by the nanoscale formulation, which minimizes the time window for solution-mediated crystallization of the remaining solid phase (Alonzo et al., 2010). Both combine to result in high drug supersaturation, thus high apparent drug solubility, which can lead to enhanced drug bioavailability, provided that the high supersaturation level is maintained over a time period sufficient for the drug absorption across the gastrointestinal lumen (Laitinen et al., 2013). Significantly, the supersaturation generation of amorphous NPs has been shown to be superior in vitro (Matteucci et al., 2007) and in vivo (Dhumal et al., 2008; Miller et al., 2012; Mou et al., 2011) to that generated by microscale amorphous solid dispersions, which represent the conventional supersaturated drug delivery systems.

In contrast to microscale amorphous solid dispersions, whose stabilization strategy relies on the drug molecular mobility restrictions by means of drug dispersions in polymers having high glass transition temperatures (Brough and Williams, 2013), the stabilization strategy of existing amorphous NPs for the most part relies on the occupation of the high energy sites on the nanoparticle surface by polymeric stabilizers, such as hydroxypropylmethylcellulose (HPMC) and polyvinylpyrrolidone (PVP) (Matteucci et al., 2006). Herein the roles of the polymeric stabilizer are multifold, i.e., (1) to inhibit post-nucleation growth of the NPs during their preparation to maintain the nanosize, (2) to suppress the crystallization propensity of the amorphous form during storage, and (3) to inhibit the solution-mediated crystallization of the dissolved drug, which consequently impedes the Ostwald ripening growth of the remaining solid phase undergoing dissolution (Lindfors et al., 2006).

In an earlier study, we developed a new class of amorphous NPs based on the molecular mobility restriction stabilization strategy, via electrostatic complexation of charged drug molecules with

Abbreviations: AU, absorbance unit; AUC, area under the curve; BCS, biopharmaceutics classification system; CIP, ciprofloxacin; DSC, differential scanning calorimetry; DXT, dextran sulfate; FD, freeze drying; HPMC, hydroxypropylmethylcellulose; MW, molecular weight; NP, nanopharmaceutical; PBS, phosphate buffer saline; PTFE, polytetrafluoroethylene; PVP, polyvinylpyrrolidone; SEM, scanning electron microscope; TGA, thermogravimetric analysis.

* Corresponding author. Tel.: + 65 6514 8381; fax: +65 6794 7553.
E-mail address: kunnong@ntu.edu.sg (K. Hadinoto).

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oppositely charged polysaccharides (Cheow et al., 2014). Using itraconazole as the model drug, the nanoscale drug-polysaccharide complex (or drug nanoplex in short) was found to demonstrate more prolonged supersaturation and higher amorphous form stability than conventional amorphous NPs prepared by the aforementioned high energy site occupation stabilization strategy (Cheow et al., 2014). This was attributed primarily to the presence of the polysaccharide stabilizer throughout the nanoplex volume, in contrast to the conventional amorphous NPs, where the stabilizer is only present on the nanoparticle surface.

While the supersaturation generation capability of the drug nanoplex has been well established in its aqueous suspension form (Cheow and Hadinoto, 2012), the same cannot be said upon its dry powder transformation into solid dosage form. For conventional amorphous NPs, the supersaturation generation of the dry powder form was found to be inferior to that of the aqueous suspension form (Matteucci et al., 2007). The inevitable formation of NP aggregates upon drying led to a slower dissolution rate due to the reduction in the surface areas available for dissolution. This was because the NP aggregates typically did not fully disassociate back to primary NPs upon exposure to aqueous medium in the timescale of dissolution (Abdelwahed et al., 2006a; Kho and Hadinoto, 2010). Moreover, the crystallization and growth tendencies of the amorphous NPs were susceptible to rise upon drying due to increased interparticle contacts as the aqueous medium in which the NPs were suspended was removed. The slower dissolution rate and higher crystallization propensity were believed to be responsible for the amorphous NP's diminished supersaturation levels after drying (Matteucci et al., 2008).

Herein we examined the effects of freeze drying – one of the most commonly used drying methods for NPs (Abdelwahed et al., 2006b) – on the in vitro supersaturation generation capability of the drug nanoplex powders. Nanoplex of ciprofloxacin (CIP) – a BCS (Biopharmaceutics Classification System) Class IV drug (i.e., low solubility, low permeability) (Tehler et al., 2013) – was used as the model amorphous NP. The objective of the present work was to develop freeze-drying adjuvant formulations that could produce a high and prolonged drug supersaturation level that was comparable to that generated by the aqueous suspension form of the drug nanoplex. Besides the supersaturation generation, the effects of the different formulations on the physical characteristics (i.e., morphology, amorphous form stability, flowability) of the freeze-dried nanoplex were also examined.

To this end, highly water-soluble excipients (i.e., mannitol, trehalose), which had been proven effective in preventing irreversible aggregations of nanoparticles upon drying via their role as "interstitial bridges" (Kho and Hadinoto, 2010), were used as freeze-drying adjuvants. These excipients, upon their dissolution, facilitate effective dissociations of the nanoplex aggregates back to individual nanoparticles, thus the high dissolution rate of the nanoplex can be maintained. In addition to the highly water-soluble excipients, the effects of HPMC inclusion – a polymeric stabilizer well-known for its crystallization inhibiting property (Tajarobi et al., 2011) – were investigated with the aim of prolonging the supersaturation. Lastly, the present work also investigated the effects of the HPMC's incorporation method, either in the form of (i) its physical mixture, or (ii) freeze dried together with the CIP nanoplex and the highly water-soluble excipients, on the supersaturation generation.

2. Materials and methods

2.1. Materials

Ciprofloxacin (CIP), sodium chloride (NaCl), Pluronic F68, glacial acetic acid, mannitol, trehalose, HPMC, and phosphate buffer saline (PBS, pH 7.4) were purchased from Sigma–Aldrich (USA), while dextran sulfate (DXT, MW 5000 Da) was purchased from Wako Pure Chemical (Japan).

2.2. Methods

2.2.1. Preparation and characterization of amorphous CIP nanoplex

The ionized drug molecules in acid or base were mixed with oppositely charged polysaccharides to form soluble drug-polysaccharide complex in the presence of salt. Owing to the hydrophobic interactions between the drug molecules, the soluble drug-polysaccharide complex formed aggregates, which upon reaching the critical concentration the aggregates precipitated out to form the drug nanoplex, where the critical concentration was governed by the drug hydrophobicity. The amorphous form was produced because the strong drug-polysaccharide electrostatic interaction prevented the drug molecules from assembling into ordered crystalline structures upon their precipitation. The CIP nanoplex was prepared and characterized following the methods presented in Cheow and Hadinoto (2012). Briefly, 10 mg CIP was dissolved in 1 mL 0.2% (v/v) aqueous acetic acid solution and 4.5 mg DXT was dissolved in 1 mL deionized water containing 2.0 mg Pluronic F68 and 5.8 mg NaCl. The CIP solution was added into the DXT solution under gentle stirring after which the mixture was let sit for 3 h in ambient condition. The CIP nanoplex produced was recovered by centrifugation at 13,000 × *g* for 5 min, followed by three cycles of washing before they were resuspended in deionized water.

2.2.2. Freeze drying of amorphous CIP nanoplex

A total of seven freeze-drying adjuvant formulations involving mannitol, trehalose, and HPMC at a constant nanoplex mass were prepared, where the mass ratio of the nanoplex to each excipient was maintained at unity (Table 1). The CIP nanoplex suspension containing the excipients were freeze-dried for 24 h in Alpha 1-2 LD Plus freeze dryer (Martin Christ, Germany). In the last two formulations in Table 1, HPMC was incorporated in the form of physical mixtures with the freeze-dried nanoplex and the water-soluble excipients. The morphology of the CIP nanoplex powders from the different formulations was examined by scanning electron microscope (SEM) model JSM-6700F (JEOL, USA).

Table 1
The freeze-drying adjuvant formulations investigated (FD = freeze drying).

NP (% w/w)	HPMC (% w/w)	Trehalose (% w/w)	Mannitol (% w/w)	Code name
100	–	–	–	FD w/o adjuvant
50	50	–	–	FD w/HPMC
50	–	50	–	FD w/Trehalose
50	–	–	50	FD w/Mannitol
33.33	33.33	33.33	–	FD w/Trehalose & HPMC
33.33	33.33	–	33.33	FD w/Mannitol & HPMC
50	Mixed	50	–	Mixture of FD w/Trehalose + HPMC powders
50	Mixed	–	50	Mixture of FD w/Mannitol + HPMC powders