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Nanonization of curcumin by antisolvent precipitation: Process development, characterization, freeze drying and stability performance

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ABSTRACT

The present work aims to investigate applicability of antisolvent precipitation method for preparation of nanosized curcumin and to control their characteristics by determining the influence of process and solvents on solid-state properties of curcumin nanoparticles. Effects of different experimental parameters on particle size were investigated using dynamic light scattering. Particle morphology was studied using SEM. Drug content in stabilized nanoparticles was determined using HPLC. Residual moisture content after lyophilisation was determined using Karl Fischer method and solid state properties were investigated using DSC, TGA, FTIR and powder-XRD. The resulting product showed a high drug load and contained the drug in amorphous form. The particle diameters of prepared curcumin nanoparticles were found in the range of 100–200 nm. *In vitro* drug release studies indicated a sustained release profile of curcumin from the nanoparticles. Antisolvent precipitation produced amorphous curcumin nanoparticles whose size and morphology could be controlled using gelatine as stabilizer. Lyophilized curcumin nanoparticles with D-sorbitol as lyoprotectant possessed good redispersibility and showed up to 4 times faster *in vitro* curcumin release rate than that of unprocessed curcumin. Stability tests (at 2–8 °C and ambient conditions) indicated that the product was stable for up to 6 months of storage.

1. Introduction

Nanosized particle technology has been the highlight in pharmaceutical field over the past 2 decades. One of its major contributions is the benefit that can be gained by formulating poorly soluble drugs (Chen et al., 2008; Kumar et al., 2009; Pushkar et al., 2006; Shah and Pathak, 2010; Van Eerdenbrugh et al., 2010; Verma and Pathak, 2012). Drug nanoparticles are submicron (100–1000 nm) pure drug particles (amorphous or crystalline) suspended in a dispersion medium (mostly water) stabilized by polymer(s) or surfactant(s) (Chen et al., 2002; Gao et al., 2012; Grau et al., 2000; Kipp, 2004). Both top-down (breaking of large drug particles by mechanical attrition as in milling and homogenization) as well as bottom-up (building up nanoparticles from drug molecules via precipitation) techniques have been utilized to prepare drug nanoparticles (Hu et al., 2014; Li et al., 2007; Liversidge and Cundy, 1995; Müller and Peters, 1998; Van Eerdenbrugh et al., 2008b). The top-down disintegration methods have certain drawbacks such as high-energy input, time consuming, possibility of metal contamination, low particle size uniformity (He et al., 2010; Krause et al., 2000; Van Eerdenbrugh et al., 2008b), which encourage research towards the bottom-up techniques as alternatives for drug nanonization (Sinha et al., 2013). However, bottom-up techniques, such as spray freezing into liquid and supercritical antisolvent precipitation need complex operating conditions and have high production costs because of requirements of high pressure and extreme low temperature (Ali et al., 2011, 2009; Chattopadhyay and Gupta, 2001; Zhao et al., 2007). Antisolvent precipitation is another bottom up technique which is relatively simple, cost effective and easy to scale-up. Ideally, for this technique the drug must dissolve in the solvent, but not in the antisolvent. The drug is first dissolved in a solvent, which is rapidly mixed with a solvent-miscible antisolvent (e.g., water). High supersaturation is thus generated due to the fast diffusion of the drug solution into the antisolvent, precipitation of drug occurs instantaneously by rapid desolvation of drug leading to production

Abbreviations: XRD, X-ray diffraction; DSC, differential scanning calorimetry; TGA, thermal gravimetric analysis; FT-IR, Fourier transform infra-red; SEM, scanning electron microscopy; ANT, antisolvent nanoprecipitation technique; DLS, dynamic light scattering; PDI, polydispersity index; RT, room temperature; RH, relative humidity.

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of submicron particles (Dong et al., 2011; Horn and Rieger, 2001; Matteucci et al., 2006b). Instant and homogeneous mixing of the solvent (drug solution) and the antisolvent is crucial to achieve the particles in the submicron range (Dong et al., 2010, 2009). Although the drug particle formation process is simple but the key challenge is to retain the nanosize of the fresh particles. As smaller particles are more soluble than large ones, material transfer occurs from the fines to the coarse particles driven by a phenomenon called "Ostwald ripening" whereby coarse particles grow at the expense of fine particles re-dissolving (Horn and Rieger, 2001). Some stabilizers cover the nanoparticle surface and retard the growing process of drug nanoparticles for short term but prolonged stabilization can only be achieved by immediate drying such as spray drying or freeze drying (Abdelwahed et al., 2006; Gassmann et al., 1994; Hu et al., 2011; Keraliya et al., 2010; Kesisoglou et al., 2007; Maximiano et al., 2011; Van Eerdenbrugh et al., 2008a). Therefore, the key to producing ultrafine nanosized drug particles by antisolvent precipitation is to create conditions that favor very rapid particle formation and little or no particle growth.

Curcumin is a yellow colored hydrophobic polyphenol derived from the rhizomes of turmeric plant *Curcuma longa* (vernacular name: Haldi). The medicinal use of this plant has been documented in Ayurveda (the Indian system of medicine) for over 6000 years (Aggarwal et al., 2003). It has wide spectrum of biological and pharmacological activities. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (Anwar et al., 2014; Han et al., 1999; Hasan et al., 2014; Kunchandy and Rao 1990; Ranjan et al., 2004, 1999, 1998; Sreejayan Rao 1993; Tønnesen and Greenhill, 1992; Tønnesen et al., 1994). Various animal models and human studies proved that curcumin is extremely safe even at very high doses. Chemically, curcumin is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, (commonly called diferuloylmethane). Being hydrophobic in nature, it is insoluble in water but soluble in ethanol, dimethylsulfoxide, and acetone (Tønnesen and Karlsen, 1985). Despite extensive research and development, poor solubility of curcumin in aqueous solution and rapid metabolism in body remain major barriers for its bioavailability. As a result, the clinical application of this drug is greatly restricted. To increase its solubility and bioavailability, attempts have been made through encapsulation in polymer nanoparticles (Beloqui et al., 2014; Duan et al., 2010; Mulik et al., 2012), solid lipid nanoparticles (Kakkar et al., 2013; Pawar et al., 2012; Sou et al., 2008) or nanogels (Abdelwahed et al., 2006; Adamczyk, 2003; Li et al., 2012; Sun et al., 2014), nanodroplets (Ji et al., 2014), surfactants (Adamczyk et al., 2005; Adamczyk and Warszyński, 1996), microsponges (Arya and Pathak, 2014), bilayers (Adamczyk and Weronski, 1999; Drakalska et al., 2014), phospholipids (Ader et al., 2000; Chen et al., 2009), micelles (Abouzeid et al., 2014; Liu et al., 2013; Song et al., 2014), SMEDDS (Cui et al., 2009) and conjugates (Aggarwal et al., 2003; Ahmad et al., 2009; Maiti et al., 2007; Paradkar et al., 2004).

Kakran et al. have prepared curcumin nanoparticles via antisolvent precipitation as dry curcumin powder for oral administration (Kakran et al., 2012b). The authors have compared two preparation methods: antisolvent precipitation with a syringe pump (APSP) and evaporative precipitation of nanosuspension (EPN). They have shown rod shaped curcumin nanoparticles and reported the length of curcumin nanoparticles. In the present investigation also, antisolvent precipitation process is applied as a simple low-energy bottom-up technique to prepare curcumin nanoparticles but for parenteral administration. A more elaborative study was done in which effect of different process variables on particle size and size distribution of curcumin during particle formation was investigated through dynamic light scattering (DLS). The appropriate solvent and stabilizer were screened by investigating the size of the precipitated particles. Particle morphology was studied by scanning electron microscopy (SEM). Physicochemical properties of the formulated nanoparticles in solid state were characterized by powder X-ray diffraction (powder-XRD), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA) and Fourier transform infrared (FT-IR) spectroscopy. *In vitro* release behaviour of curcumin nanoparticles was studied and the nanoparticles were freeze dried for prolonged stabilization. Stability study of freeze dried curcumin nanoparticulate formulation was done at three different storage conditions

2. Materials and methods

2.1. Materials

Curcumin, crystalline (Himedia, Mumbai, India) was used as active with interest for the use as drug or nutritional supplement; HPMC (Hydroxypropylmethylcellulose) USP grade having viscosity 15 cps (Pharmacoat), manufactured by Shin et Su Japan and supplied by Signet, Mumbai, India. Gelatin powder bacto (SD Fine Chem Ltd., India); methyl cellulose, sucrose, lactose, D-sorbitol, D-mannitol, 2-pyrrollidone and acetonitrile were procured from Loba Chemie, Mumbai, India. Pluronic F-68 (Sigma); dextrose anhydrous and ethyl acetate (Merck, Mumbai). Ethanol obtained from Hong Yang Chemical Corp.; toluene AR obtained from SD Fine Chem. All other chemicals and reagents were purchased from commercial places and used as received.

2.2. Antisolvent nanoprecipitation technique (ANT)

Nanosized curcumin particles were prepared by "bottom-up" antisolvent precipitation technique. Different solvents, antisolvents and their different ratios were screened by investigating the size of the precipitated nanoparticles. In order to achieve an optimal formulation, effects of other variables such as drug concentration in the solvent, effect of temperature, method of addition (slow/rapid) of drug solution to antisolvent, type of addition (whether drug solution to antisolvent or antisolvent to drug solution) have been examined. The organic solvent was chosen based on its safety profile and ability to dissolve curcumin. Briefly, curcumin solution was prepared in acetone and added to antisolvent stirred on a magnetic stirrer. Immediately, nanoparticles were precipitated from the antisolvent and a uniform turbid yellow suspension formed simultaneously whose particle size was analyzed by Zetasizer immediately. Each experiment was performed in triplicate.

2.3. Screening of stabilizers

Different stabilizers were screened to maintain the size of curcumin nanoparticles. A high concentration of stabilizers was used (1% w/v) in order to not make this a limiting factor. For this the general formulation procedure was the same as in Section 2.2 except that 1% aqueous solution of stabilizer was used as antisolvent and the physical stability of nanoparticulate dispersion so formed was checked with time. The selected stabilizer was then screened quantitatively using its different concentrations (0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0% w/v) in order to estimate the minimum concentration of stabilizer necessary to stabilize the nanoparticulate dispersion.

2.4. Characterization of curcumin nanoparticles

2.4.1. Particle size and particle size distribution

Dynamic light scattering (DLS) sometimes referred to as photon correlation spectroscopy or quasi-elastic light scattering, using

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