International Journal of Pharmaceutics 441 (2013) 269–278

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Food proteins as novel nanosuspension stabilizers for poorly water-soluble drugs

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ARTICLE INFO

Article history:
Received 11 August 2012
Received in revised form 22 October 2012
Accepted 18 November 2012
Available online 27 November 2012

Keywords:
Nanosuspensions
Food proteins
Poorly water-soluble drugs
Stabilizers

ABSTRACT

Nanonization of the poorly water-soluble drugs is a promising strategy to improve dissolution and oral bioavailability. To stabilize the drug nanosuspensions, stabilizers are usually used; however, the use of common stabilizers is limited by weak stabilization effect and toxicological concerns for long-term treatment. The present work was to investigate the potential of food proteins as novel safe stabilizers for nanosuspensions using indomethacin as a model drug. The nanosuspensions stabilized by food proteins (soybean protein isolate, whey protein isolate and β-lactoglobulin) were prepared by the nanoprecipitation–ultrasonication method. The particle size could be easily reduced to 100–400 nm with bimodal particle-size distribution through monitoring the preparative variables. The exposure of buried hydrophobic moieties due to heat-denaturation and subsequent adsorption onto the surface of drug particles was assumed to contribute to their efficient stabilization effect. In comparison with conventional stabilizers, food proteins are superior in stabilization efficiency. The dissolution was enhanced significantly owing to particle size reduction. The protein-stabilized nanosuspensions could be easily freeze-fried and reconstituted into nanosuspensions, keeping the original mean particle size and particle-size distribution. It is concluded that the three denatured proteins perform as efficient stabilizers for indomethacin nanosuspensions.

1. Introduction

It is reported that over 40% of the new drug candidates are poorly water-soluble, which limits their absorption in the gastrointestinal (GI) tract and reduces the overall bioavailability (Kohli et al., 2010; Lipinski, 2000). Nano-particulate drug delivery systems such as nanosuspensions, nanoemulsions, polymer micelles and so on have great potential in improving the dissolution rate and oral bioavailability of the poorly water-soluble drugs (Guan et al., 2011; He et al., 2011; Kesisoglou et al., 2007; Qi et al., 2012; Rabinow, 2004). Among them, formulating drugs as nanosuspensions is one of the most promising strategies, owing to its high drug loading, enhanced dissolution rate and saturation solubility, reproducibility of oral absorption, improved dose-bioavailability proportionality and increased patient compliance (Chen et al., 2011; Rabinow, 2004; Shegokar and Muller, 2011).

Conventionally, stabilizers are indispensable in the nanosuspension formulations to prevent them from aggregation and agglomeration (Chen et al., 2011; Kesisoglou et al., 2007; Rabinow, 2004; Verma et al., 2009b, 2011). The commonly used stabilizers include Pluronics, Tweens, D-α-tocopherol polyethylene glycol 1000 succinate (TPGS), polyethylene glycols (PEGs), polyvinyl alcohols (PVAs), polyvinylpyrrolidone (PVP) and other cellulose polymers (Kesisoglou et al., 2007; Rabinow, 2004; Verma et al., 2011). However, the nanosuspensions are not stabilized permanently by these stabilizers and aggregation may occur during storage or when nanosuspensions are being dried. Furthermore, some of the common stabilizers raise toxicity concerns if used in large quantity for a long term, limiting the therapeutic application of drug nanosuspensions (He et al., 2011; Izquierdo et al., 2001; Jiao, 2008; Liu and Zhang, 2010; Rabinow, 2004). For example, Cremophor® EL and Tween-80 are two commercial surfactants that are widely used to solubilize poorly water-soluble drugs, but they also cause serious neuro- and nephrotoxicity as well as acute hypersensitivity reaction (Gelderblom et al., 2001; Hawkins et al., 2008). Thus, there remains to be a demand to find new stabilizers with better stabilizing capacity and less toxicity.

Food biopolymers, especially food proteins, are widely used in formulated foods because they have high nutritional values and are generally recognized as safe (Chen et al., 2006; MaHam et al., 2009). The proteins include soybean protein isolate (SPI), whey protein isolate (WPI), β-lactoglobulin (β-lg) and so on. SPI is a plant protein obtained from an abundant, inexpensive and renewable resource, the soybean. It is composed almost exclusively of two globular

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http://dx.doi.org/10.1016/j.ijpharm.2012.11.033

protein fractions called 7S (*b*-conglycinin) and 11S (glycinin) (Chen and Subirade, 2009). WPI is derived from milk, in which the three most important proteins (β-lg, α-lactalbumin and albumin) account for about 70% of the total weight of whey protein (Perez et al., 2006). β-lg is a globular protein widely used as food ingredient because of its nutritional value. It has a molecular weight of 18.4 kD and five cysteine residues, four of which are involved in disulfide bonds formation in the native protein. These proteins, like traditional surfactants, are amphiphilic. Moreover, it is of interest that non-polar and disulfide bonds buried inside food proteins will be exposed after heat denaturation, which thus enhances the effect of stabilization (Augustin and Hemar, 2009; Chen et al., 2006). Our previous report indicated that the denatured food proteins have better stabilization effect on nanoemulsions than conventional surfactants; and these food proteins have excellent biocompatibility and biosafety; moreover, the food protein stabilized-nanoemulsions can significantly enhance the oral absorption of water-insoluble drugs (He et al., 2011). Chen and Subirade (2006, 2009) also developed a biocompatible vehicle for oral administration of bioactive compounds using WPI or SPI as matrical materials. These previous findings indicate that food proteins have potential to be used for oral drug delivery.

Based on our initial findings and the fact that the food proteins are amphiphilic, we hypothesize that these food proteins can also be used to stabilize nanosuspensions for poorly water-soluble drugs for oral administration. Thus, the aim of our present study was to evaluate the capacity of the three food proteins (WPI, SPI and β-lg) as stabilizers for nanosuspensions. Indomethacin (IND), a poorly water-soluble non-steroid anti-inflammatory drug, was used as the model drug. The nanosuspensions prepared by a precipitation-ultrasonication method, were characterized in term of mean particle size and particle-size distribution, morphology, in vitro drug release, physical state of drug particles and so on. Furthermore, we also used fluorescence emission spectrum to study the interaction between the denatured proteins and drug particles. The primary goal was to achieve efficient nanonization and prevent agglomeration when the nanosuspensions were dried by using these three food proteins.

2. Materials and methods

2.1. Materials

IND was purchased from Sine Pharmaceuticals (Shanghai, China). Whey protein isolate (WPI) was provided by Davisco Foods International Inc. (Le Sueur, MN, USA). Soybean protein isolate (SPI) was obtained from Hufeng Chemical Industry Co., Ltd. (Shanghai, China). β-lg from bovine milk (>90% purity grade) was purchased from Sigma Chemical Co. (St. Louis, MO). Poloxamar-188 was from BASF (Ludwigshafen, Germany). Tween-80 was supplied by Shenyu Pharmaceutical and Chemical Co., Ltd. (Shanghai, China). Poly(ethylene glycol) 6000 (PEG 6000) and Polyvinylpyrrolidone K30 (PVP K30) were kindly provided by China Division, ISP Chemicals Co. (Shanghai, China). Hydroxypropyl methylcellulose (HPMC, 5 cp) was purchased from Feichengruitai Ltd. (Shandong, China). Purified ovolecithin (LIPOID E 80, EPC) was from Lipoid GmbH (Ludwigshafen, Germany). Deionized water was prepared using a Milli-Q purification system (Millipore, USA). Other reagents were of analytical grade and used as received.

2.2. Preparation of nanosuspensions

Aqueous dispersion of the three food proteins was prepared by dispersing 300 mg protein (SPI, WPI, or β-lg) powder into 25 mL water under magnetic stirring for 1 h at 25 °C, and then adjusted to pH 7 using 1 mol/L sodium hydroxide. To denature the proteins so as to expose the nonpolar domains buried in the protein interior, the SPI, WPI and β-lg aqueous dispersion were heated to 105 °C, 85 °C, and 85 °C, respectively, in closed tubes for 30 min. After that, the denatured protein dispersion was cooled down to 25 °C and kept for 2 h before use.

The nanosuspensions were prepared by the precipitation-ultrasonication method (Matteucci et al., 2006; Xia et al., 2010). Briefly, the aqueous dispersion of denatured proteins functioned as the aqueous phase (30 mL), whereas the drug-containing organic phase was prepared by dissolving IND in acetone (2 mL). The organic and aqueous phases were cooled to below 3 °C in an ice-water bath. Then, the organic phase was added to the aqueous phase at a stirring rate of 1200 rpm. After the process of anti-solvent precipitation, the samples were immediately treated with probe ultrasonication (20–25 kHz, Ningbo Scientz Biotechnology Co. Ltd., China). To remove the organic solvent, the obtained nanosuspensions were evaporated under reduced pressure with a rotating speed of 50 rpm at 30 °C for 30 min. The probe with a tip diameter of 8 mm was immersed 1 cm into the liquid, achieving wave traveling downwards and reflecting upwards. The period of ultrasound burst was set to 3 s with a pause of 3 s between two ultrasound bursts. During the process, the temperature was controlled using an ice-water bath.

To compare the stabilizing capacities of the proteins and other stabilizers, IND nanosuspensions stabilized by Poloxamar-188, Tween-80, EPC, PVP K30, HPMC or PEG 6000 were also prepared. The stabilizer concentration in the aqueous solution was 0.8% (w/w). The preparation procedure was similar to that described above.

2.3. Particle size and zeta potential determination

The particle size and particle-size distribution of the nanosuspensions were measured by dynamic light scattering using NICOMP 380 DLS instrument (Santa Barbara, CA, USA). The surface charge of the nanosuspensions was determined by measuring the electrophoretic mobility at 25 °C using NICOMP 380 ZLS (Santa Barbara, CA, USA). Nanosuspensions were diluted by 50-folds in water before measurement.

2.4. Scanning electron microscopy (SEM)

SEM studies of the nanosuspensions and freeze-dried samples were carried out using Philips XL30 instrument (Philips Electron Optics, Amsterdam, The Netherlands). Prior to the examination, the samples were fixed on a brass stub using double-sided tape and gold-coated in vacuum by a sputter coater. The photographs were taken at an excitation voltage of 10 kV.

2.5. Transmission electron microscopy (TEM)

TEM study of the nanosuspensions was carried out on a JEM-1230 transmission electron microscope (Tokyo, Japan). Nanosuspensions were placed on copper grids and negatively stained with 2% (w/v) phosphotungstic acid for 5 min at room temperature.

2.6. UV–vis and fluorescence measurements

UV–vis measurements of dilute protein and nanosuspensions were performed by a UV spectrophotometer (UV2200, Shimazu, Japan) within the wavelength range of 200–900 nm to determine the absorbance of the native and denatured proteins. Intrinsic tryptophan, tyrosine, and phenylalanine fluorescence quenching was