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Solid dispersions containing ursolic acid in Poloxamer 407 and PEG 6000: A comparative study of fusion and solvent methods

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ABSTRACT

Ursolic acid is a molecule with several therapeutic applications not yet commercially explored because of its bioavailability-limiting water insolubility. To avoid this drawback, solid dispersions containing ursolic acid have been employed to increase water solubility. The influence of hydrophilic carriers, PEG 6000 and Poloxamer 407, and the method of preparation, fusion and solvent, were studied for ursolic acid in this work. The solid dispersions and physical mixtures were characterized by particle size, scanning electron microscopy, fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry, hot stage microscopy, water solubility and dissolution profile. Results showed that both methods and polymers used for solid dispersion preparation resulted in homogeneous powders, but the surfactant Poloxamer 407 enhanced the ursolic acid solubility in solid dispersions better than PEG 6000. With Poloxamer 407, both methods enabled complete dissolution of ursolic acid in phosphate buffer; however the solid dispersion showed higher solubility when prepared by the solvent method compared to the fusion method, 689.47 μg/mL and 328.52 μg/mL, respectively. For comparison, the physical mixture solubility was 248.17 μg/mL. The better results achieved with the solvent method were attributed to smaller particle size, amorphic conversion of ursolic acid from its less soluble crystalline state and hydrogen bond formation between drug and carrier, whereas there was a polymorphic change caused by the fusion method. Results indicated that the solid dispersion prepared by the solvent method is an adequate approach to increasing ursolic acid solubility and dissolution, a very important step toward the development of a pharmaceutical dosage form containing ursolic acid.

1. Introduction

Ursolic acid is a pentacyclic triterpenoid that naturally occurs in many medicinal plants and herbs [1]. Several pharmacological effects have been attributed to this drug, including hepatoprotection [2], tumor inhibition [3,4], cardiovascular protection [5,6], enhancement of immune system [7], antioxidant [8], trypanocidal [9], antidiabetic [10–12], anti-inflammatory [13] and osteoblast anabolic activities [14]. However, ursolic acid, a weak acid with pKa 5.29 [15], has a very low water solubility, which limits its bioavailability and therapeutic application [16].

Several techniques can be successfully used to improve the dissolution and bioavailability of poorly water soluble drugs, such as size reduction, use of surfactants, salt formation, pH adjustment, pro-drugs or incorporation of the drug in polymeric or lipid formulations [17]. Regarding the latter alternative, ursolic acid has been formulated with liposomes, hydroxypropyl β cyclodextrin and nanostructured lipid carriers [18–20]. However, none of these approaches demonstrated significant ursolic acid solubility enhancement. Consequently, a novel formulation that enables a greater enhancement of ursolic acid dissolution profile is still necessary.

Solid dispersions can be defined as molecular or amorphous mixtures of poorly water soluble drugs in hydrophilic carriers in which the polymer properties play an important role in the drug dissolution profile. This strategy is one of the most efficacious to improve the bioavailability of drugs with low water solubility [21]. Among the important factors increasing the solubility of drugs in solid dispersions, particle size reduction, reduced agglomeration, improved wettability and solubility, or dispersion of the drug as micro-fine crystals, amorphous materials or in a molecular form must be mentioned [22]. These formulations offer many advantages over others and the most relevant are the lower cost of the adjuvants and the feasible industrial application [23].

Solid dispersions were first described by Sekiguchi and Obi in 1961, who noted that eutectic mixtures improved the release rate of poorly water soluble drugs [24]. Since then, many different techniques and carriers have been introduced. The two main techniques employed to prepare solid dispersions currently are the fusion and solvent methods. In the fusion method, the drug and the carrier are melted together at a temperature above the melting point and then cooled and milled to reduce the particle size. An important prerequisite for this method is that the drug and the carrier must be miscible in the molten form, and a limitation is that the components must be thermostable [25]. Tachibana and Nakumura (1965) were the first to dissolve both the drug and the carrier in a common solvent and then evaporate the solvent to produce

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Table 1
UA content determination (%) of the amount labeled in PMs and SDs.

Product	UA content (%)
Poloxamer 407 PM	98.79 ± 20.54
Poloxamer 407 solvent SD	99.83 ± 2.57
Poloxamer 407 fusion SD	96.57 ± 7.85
PEG 6000 PM	105.74 ± 8.65
PEG 6000 solvent SD	102.85 ± 4.25
PEG 6000 fusion SD	95.64 ± 5.65

a solid dispersion (i.e., solvent method) [26]. Since then, many researchers have used this method, whose main prerequisite is that both carrier and drug must be soluble in the solvent employed. In this method, the decomposition of heat sensitive drugs can be prevented because organic solvent evaporation occurs at low temperatures; however it has the disadvantage of employing organic solvents [21,27]. For the development of a pharmaceutical dosage form based on solid dispersions, both methods, fusion and solvent, must be studied and compared in order to determine the most suitable to the vehiculation of drugs, enabling the highest levels of solubility and dissolution with consequent improvement of bioavailability and therapeutic effect. The literature, on the other hand, is very limited concerning comparative studies between fusion and solvent methods and the most adequate method seems to depend on the carrier and drug characteristics [28–31].

Different carriers can be used in solid dispersion manufacturing and their choice influences the solubility of lipophilic drugs. Among these, polyethylene glycols (PEG), polymers of ethylene oxide with a molecular weight (MW) ranging from 200 to 300,000, are widely used as vehicles for solid dispersion because of their low melting point, rapid solidification rate, ability to form solid drug solutions, low toxicity and low cost [32]. Poloxamers are polyoxyethylene–polypropylene block copolymer nonionic surfactants that can achieve the highest degree of bioavailability for poorly soluble drugs and stabilize solid dispersions, avoiding drug recrystallization [21,33–37]. For some drugs, the improvement in solubility using poloxamers was higher than with other polymers, such as PEG, because of the surfactant characteristics of the former [38]. To date, Poloxamer 407 and PEG 6000 have never been studied as carrier systems in solid dispersions containing ursolic acid.

Previously, our group incorporated ursolic in a solid dispersion formulation prepared with the lipid mixture Gelucire 50/13 and silicon dioxide as a drying adjuvant using the solvent method; however, only a mild enhancement of solubility and dissolution rate was observed [39]. Therefore, it is relevant to investigate other carriers and methods to prepare solid dispersions with ursolic acid, aiming at the greatest enhancement of solubility and dissolution profile. In the present study, we aim to develop and characterize binary solid dispersions containing ursolic acid based on the polymers Poloxamer 407 and PEG 6000 prepared with the solvent and fusion methods. For this purpose, the physical mixtures and solid dispersions were characterized by particle size, scanning electron microscopy, fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry, hot stage microscopy, water solubility and *in vitro* drug release.

2. Materials and methods

2.1. Materials

Ursolic acid (UA) was purchased from Idealfarma (Brazil). PEG 6000 and Poloxamer 407 were obtained from BASF (Germany). HPLC grade acetonitrile and methanol were purchased from Merck (Germany).

2.2. Preparation of solid dispersions and physical mixtures

Solid dispersions (SDs) in 1:10 and drug/polymer ratio were prepared by the fusion and solvent methods. In the fusion method, drug was added to the molten carrier (PEG 6000 or Poloxamer 407) at 300 °C with continuous stirring until the formation of a homogeneous dispersion. The dispersion was allowed to solidify at room temperature and then pulverized with a mortar and pestle [40]. In the solvent method, drug and carrier were solubilized in methanol (10% w/v), followed by evaporation of the solvent at room temperature with stirring. Subsequently, the dispersion was stored in an oven at 40 °C for 24 h to allow the complete evaporation of methanol and then pulverized with a mortar and pestle [41]. The physical mixtures (PMs) were prepared by mixing the drug and carrier and contained UA at the same ratio as the SDs.

2.3. Determination of UA content

Analysis of the drug from the preparations was performed by high performance liquid chromatography (HPLC) using a Shimadzu HPLC system consisting of an LC-10ADVP pump, an SPD-10A VP UV detector (operating at 203 nm), a Rheodyne injector and a model CR6-A integrator. An acetonitrile:water (88:12, v/v) system was used as the mobile phase at a flow rate of 1.0 mL/min with a total injection volume of 20 μL. Separation was performed on a C18 reverse-phase column (LiChrospher® (Merck), 250 × 4 mm (5 μm)) at room temperature (25 °C). Thus, SDs and PMs samples (corresponding to 2.5 mg of UA) were solubilized in 100 mL of acetonitrile and filtered (polytetrafluoroethylene 0.45 μm membrane, Millipore) before HPLC analysis ($n = 3$). This method was validated in our laboratory in accordance with the Q2(R1) ICH Guideline. Thus, specificity, linearity, intraday and interday precision and accuracy, as well as robustness, were all evaluated as described by Eloy and coworkers [42].

2.4. Determination of solubility

The solubility in water of free UA and in PMs or SDs was determined by adding excess drug (10 mg) to 10 mL of distilled water under magnetic stirring (300 rpm) at 25 °C in a temperature controlled water bath until equilibrium was achieved (48 h). The samples were then filtered (polyvinylidene difluoride 0.45 μm membrane, Millipore), suitably diluted with acetonitrile and analyzed by HPLC. Experiments were performed in triplicate.

2.5. Dissolution studies

UA dissolution studies were performed using an SR8 Plus Hanson Corporation instrument (Chatsworth, CA, USA) by incubating a known amount of sample (equivalent to 7.5 mg drug) in 900 mL of 0.1 N HCl solution or pH 6.8 phosphate buffer (37 °C ± 0.2 °C) with stirring from a paddle at 75 rpm. At designated time intervals, 1-mL aliquots were withdrawn and filtered (polyvinylidene difluoride 0.45 um membrane, Millipore), and then the drug was analyzed by HPLC. The volume withdrawn was replaced with the same amount of fresh dissolution medium. Dissolution was performed under sink conditions in triplicate.

2.6. Particle size

Particle size was measured with a Beckman Coulter LS 13 320 laser diffraction particle size analyzer.

2.7. Scanning electron microscopy (SEM)

The surface morphology of UA, carriers, PMs and SDs were examined using a Zeiss® EVO 50 scanning electron microscope (Germany). The powders were fixed on a sample port using carbon double-sided adhesive tape and made electrically conductive by coating with gold (100 s) in a SCD 050 Bal-Tec Sputter Coater (Germany) using voltage from 10 to 25 kV for scanning.