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Enhancement of solubility, antioxidant ability and bioavailability of taxifolin nanoparticles by liquid antisolvent precipitation technique

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ABSTRACT

Taxifolin is a kind of flavanonol, whose antioxidant ability is superior to that of ordinary flavonoids compounds owing to its special structure. However, its low bioavailability is a major obstacle for biomedical applications, so the experiment is designed to prepare taxifolin nanoparticles by liquid antisolvent precipitation (LAP) to improve its bioavailability. We selected ethanol as solvent, deionized water as antisolvent, and investigated primarily the type of surfactant and adding amount, drug concentration, volume ratio of antisolvent to solvent, precipitation temperature, dropping speed, stirring speed, stirring time factors affecting drug particles size. Results showed that the poloxamer 188 was selected as the surfactant and the particle size of taxifolin obviously reduced with the increase of the poloxamer 188 concentration, the drug concentration and the dropping speed from 0.08% to 0.45%, from 0.04 g/ml to 0.12 g/ml, from 1 ml/min to 5 ml/min, respectively, when the volume ratio of antisolvent to solvent increased from 2.5 to 20, the particle size of taxifolin first increased and then decreased, the influence of precipitation temperature, stirring speed, stirring time on particle size were not obvious, but along with the increase of mixing time, the drug solution would separate out crystallization. The optimum conditions were: the poloxamer 188 concentration was 0.25%, the drug concentration was 0.08 g/ml, the volume ratio of antisolvent to solvent was 10, the precipitation temperature was 25 °C, the dropping speed was 4 ml/min, the stirring speed was 800 r/min, the stirring time was 5 min. Taxifolin nanosuspension with a MPS of 24.6 nm was obtained under the optimum conditions. For getting taxifolin nanoparticles, the lyophilization method was chosen and correspondingly γ-cyclodextrin was selected as cryoprotectant from γ-cyclodextrin, mannitol, lactose, glucose. Then the properties of raw taxifolin and taxifolin nanoparticles were characterized by scanning electron microscopy (SEM), fourier-transform infrared spectroscopy (FTIR), high performance liquid chromatography–mass spectrometry (LC–MS), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and thermo gravimetric (TG), and the conclusion was drawn that taxifolin nanoparticles can be converted into an amorphous form but its chemical construction cannot been changed. Furthermore, dissolving capability test, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and reducing power assay, solvent residue test were also carried out. The experimental data showed that the solubility and the dissolution rate of taxifolin nanoparticles were about 1.72 times and 3 times of raw taxifolin, the bioavailability of taxifolin nanoparticles increased 7 times compared with raw taxifolin, and the antioxidant capacity of taxifolin nanoparticles was also superior to raw taxifolin. Furthermore, the residual ethanol of the taxifolin nanoparticles was less than the ICH limit for class 3 solvents of 5000 ppm or 0.5% for solvents and could be used for pharmaceutical. These results suggested that taxifolin nanoparticles might have potential value to become a new oral taxifolin formulation with high bioavailability.

1. Introduction

Taxifolin (3,5,7,3,4-pentahydroxy flavanone or dihydroquercetin, Fig. 1) is a kind of flavanonol (Ma et al., 2012), which was widely distributed in barks of the genus *Pinus* or *Larix* and in the seeds of the genus *Silybum* (Zu et al., 2012). Recent years, taxifolin was also

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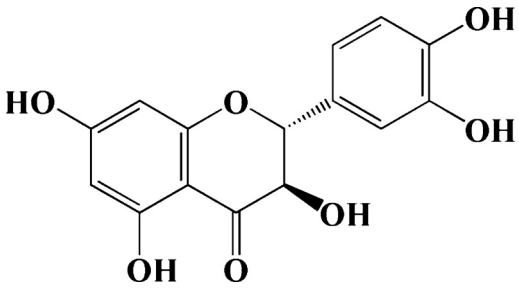

Fig. 1. The chemical structure of taxifolin.

found in fruits, especially grapes, oranges and grapefruit (Abad-Garcia et al., 2009). It played a special role in maintaining normal functions of circulatory system because of its unique antioxidant activity and biological activity (Lee et al., 2012; Liang et al., 2013; Rogovskii et al., 2010), which effectively eliminate excess free radicals in the human body (Teselkin et al., 2000; Vladimirov et al., 2009), improve immune function, and reduce the formation of cancer cells (Weidmann, 2012; Zhang et al., 2013), prevent cardiovascular disease (Wang et al., 2006). At present, because the taxifolin possessed some medicinal properties, such as anti-tumor, anti-virus, antioxidant, it has been widely used in medicine, health care products, food industries (Yang et al., 2011). However, on account of the slight solubility of taxifolin, it was difficult to be absorbed and metabolized by the body, which greatly limited its bioavailability and efficacy (Shikov et al., 2009; Zinchenko et al., 2011; Zu et al., 2012). Solubility of drugs was associated with the specific surface area of materials, along with particle size of drugs reduced, the effective area contacting with media increased, so that the solubility and dissolution rate of drugs will improve (Rasenack and Muller, 2004). Therefore, in order to improve the solubility and bioavailability of taxifolin, it is important to prepare small and uniform amorphous taxifolin nanoparticles by micronization technology.

In general, the micronization technology is mechanical grinding (Rogers et al., 2003), but the method has disadvantages of large energy consumption, low efficiency, wide distribution of particle size of products, etc. In recent years, with the continuous development of nanometer material technology, some micronization preparation methods and technologies of drugs with potential application value such as the liquid antisolvent precipitation (Azad et al., 2013; Dong et al., 2010; Meer et al., 2011; Zu et al., 2013), the supercritical fluid technology (Byrappa et al., 2008; Li et al., 2008; Park et al., 2013), the spray drying method (Hu et al., 2011; Tao et al., 2013; Tshweu et al., 2013), and so on were widely applied in improving the solubility of low solubility drugs. Currently, our group had a related document reported that micronized taxifolin were prepared by the supercritical antisolvent (SAS) technology and its granule morphology was almost needle-like with particle size distributing between 2 μm and 11 μm (Zu et al., 2012), but the technology existed the questions of large equipment investment and lower productivity. This experiment applied the LAP method, according to the dissolved properties of taxifolin, selected ethanol as the solvent and selected water as the antisolvent and engendered precipitations, then prepared taxifolin nanoparticles through lyophilization. The method compared with other micronization technologies, had some advantages including its process is simple, easy to operate, easy to industrialization and lower cost, was expected to realize industrial production (Chen et al., 2004).

The experiment prepared small and uniform amorphous taxifolin nanoparticles by the LAP without changing taxifolin molecule structure. Through the single factor design, the type of surfactant and adding amount, drug concentration, volume ratio of antisolvent to solvent, precipitation temperature, dropping speed, stirring speed, stirring time factors affecting particles size of drugs were mainly investigated, and the appropriate conditions of micronization were obtained. The taxifolin nanoparticles were obtained by lyophilization, and its properties had been characterized by scanning electron microscopy (SEM), fourier-transform infrared spectroscopy (FTIR), high performance liquid chromatography–mass spectrometry (LC–MS), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and thermo gravimetric (TG). Finally solvent residue test, antioxidant test and dissolution test were also carried out to investigate if there was residual ethanol in the process of experiment, and the biological activity and the bioavailability of taxifolin nanoparticles.

2. Materials and methods

2.1. Materials

Taxifolin (FW = 304, purity 98%) was purchased from Nanjing Zelang Medical Technological Co., Ltd. (Jiangsu, PR China); ethanol, acetonitrile, methanol, glacial acetic acid, hydrochloric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, $FeCl_3$, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, poloxamer 188, Tween-80 and γ-cyclodextrin were obtained from Sigma, deionized water was prepared with Hitech-K flow water purification system (Hitech Instruments Co., Ltd., Shanghai, China).

2.2. Preparation of taxifolin nanoparticles

The taxifolin nanoparticles were prepared by the LAP. In short, a certain amount of raw taxifolin were weighed and completely dissolved in a certain volume of ethanol, then the solution was poured into a certain volume of deionized water with a certain amount of surfactants by a peristaltic pump at a certain precipitation temperature under stirring with a certain speed intensity. After a period of time, the ethanol in the solution was removed from the solution by a spin steaming instrument, and then a certain amount of cryoprotectants were added into the solution and stirred evenly through a magnetic stirrer, finally the taxifolin nanoparticles were obtained by the lyophilizer at −50 °C for 64 h. Under the same conditions, taxifolin nanoparticles without cryoprotectants were also prepared. Each experiment was repeated at least 3 times.

2.3. Optimization of the LAP of taxifolin nanopaticles

Single factor method was used to investigate the optimization of operating conditions for amorphous taxifolin nanoparticles by the LAP process. Through preliminary experiments, several main factors influencing the particle size of taxifolin nanoparticles included the type of surfactant and adding amount, drug concentration, volume ratio of antisolvent to solvent, precipitation temperature, dropping speed, stirring speed, stirring time factors, every factor was respectively investigated in order to get the optimal condition, as follows: surfactants were poloxamer 188, carbomer, PEG-6000 and the surfactant concentration was studied at 0.08%–0.45%. The drug concentration was studied at 0.04–0.12 mg/ml. The volume ratio of antisolvent to solvent was studied at 2.5–20. The precipitation temperature was investigated at 5–45 °C. The dropping speed was studied at 1–5 ml/min. The stirring speed was studied at 400–2000 r/min. The stirring time was studied at 1–90 min. Finally, the optimum condition of every factor was obtained based on the smallest particle size of every factor, respectively. The obtained nanoparticles were redispersed in deionized water containing a certain amount of γ-cyclodextrins, prefrozen at −40 °C for 2 h, and subsequently lyophilized at −50 °C