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Preparation and characterization of micronized ellagic acid using antisolvent precipitation for oral delivery

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ABSTRACT

In this work, poorly water soluble phytochemical ellagic acid (EA) was micronized to increase its solubility and thereby the bioavailability during antisolvent precipitation process using *N*-methyl pyrrolidone (NMP) as solvent and deionized water as antisolvent. The micronized EA (m-EA) freeze-dried powder was prepared by the subsequent lyophilization process. The effects of various experimental parameters on the mean particle size (MPS) of m-EA suspension (m-EAS) in the antisolvent precipitation process were investigated. MPS and production efficiency were taken into account comprehensively to obtain the optimum conditions of antisolvent precipitation. Under the optimum conditions, m-EA freeze-dried powder with a MPS of 429.2 ± 7.6 nm was obtained. The physico-chemical properties of m-EA freeze-dried powder were detected by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), liquid chromatography–tandem mass spectrometry (LC–MS/MS), X-ray diffraction (XRD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The results indicated m-EA kept the same chemical structure with raw EA, but the crystallinity was greatly reduced. Furthermore, a comparison of the 50% inhibition concentration (IC_{50}) values revealed that m-EA was more effective than raw EA in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Meanwhile, m-EA also showed higher reducing power. Moreover, the residual amount of NMP was lower than the International Conference on Harmonization limit (530 ppm) for solvents. The dissolution rate of m-EA was approximately 2 times of raw EA. Moreover, the solubility of m-EA was about 6.5 times of raw EA. Meanwhile, the bioavailability of m-EA increased about 2 times compared with raw EA via oral administration.

1. Introduction

Today, it is well known that free radicals and oxidative stress play an important role in many diseases (Scheibmeir et al., 2005) and antioxidants have shown beneficial effects in the therapy of neurodegenerative diseases (Simonian and Coyle, 1996), cancer (Matés and Sánchez-Jiménez, 2000), aging (Mehlhorn and Cole, 1985), diabetes mellitus, reperfusion injury, inflammatory diseases, atherosclerosis and carcinogenesis (Maxwell, 1995). Oxidative stress occurred due to an imbalance between oxidants and antioxidants in favor of the oxidants (Halliwell, 1994). Antioxidants can delay or inhibit oxidation of substrate on which free radicals attack to neutralize free radicals (Young and Woodside, 2001). Many antioxidants as prophylactics and therapeutic agents have been extensively researched and proven to be with lots of pharmacological activities (Ratnam et al., 2006).

Ellagic acid (EA; $C_{14}H_6O_8$; MW: 302.202; 2,3,7,8-tetrahydroxy benzopyrano[5,4,3-cde]benzopyran-5,10-dione; structure shown in Fig. 1), one such polyphenolic phytonutrient found in wide varieties of fruits and nuts (Daniel et al., 1989), has been receiving most attention because possesses a wide array of pharmacological activities like potent antioxidant activity (Çeribaşi et al., 2010), radical scavenging, chemopreventive (Yüce et al., 2007), anticarcinogenic (Wang et al., 2012) and anti-inflammatory (Papoutsi et al., 2008) effects. As a dimeric form of gallic acid, EA contains two lactone groups and four hydroxyl groups that can increase antioxidant activity in lipid peroxidation and protect cells from

Abbreviations: DLLSM, dynamic laser light scattering meter; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DSC, differential scanning calorimetry; EA, ellagic acid; GC, gas chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry; m-EA, micronized ellagic acid; m-EAS, micronized ellagic acid suspension; HPLC, high performance liquid chromatography; ICH, International Conference on Harmonization; MPS, mean particle size; NMP, 1-methyl-2-pyrrolidinone; PSD, particle size distribution; SEM, scanning electron microscope; TGA, thermogravimetric analysis; XRD, X-ray diffraction.

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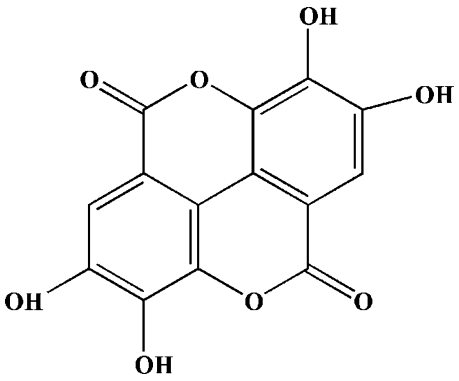

Fig. 1. Chemical structure of EA.

oxidative damage (Hwang et al., 2010). However, the pharmacokinetics study reveals EA for oral use is poorly absorbed, metabolized by intestinal microorganism, rapidly eliminated from the body due to short plasma half life (Teel, 1987) and finally fails in attaining required tissue concentrations (Lei et al., 2003). In Lei's study (Lei et al., 2003), the AUC of EA in rats after oral administration of pomegranate leaf extract was only 838 μg/L h. The low oral bioavailability of EA strictly limits its potential as a systemic antioxidant, which can be attributed to poor aqueous solubility, metabolism in the gastrointestinal tract (Seeram et al., 2004), irreversible binding to cellular DNA and proteins and first-pass effect. The low solubility and permeability make EA fall under class IV of biopharmaceutical classification system (BCS) (Bala et al., 2005b).

Hence, there is a strong need to develop new drug delivery systems to enhance EA bioavailability. Micro- or nano-particles came into view as a rising and promising drug delivery system for oral delivery and succeeded in enhancing bioavailability of poorly water soluble drugs (Hariharan et al., 2006). The oral bioavailability of EA is severely restricted to its low solubility and dissolution rate, which can be increased by small particle size and large surface area of micro- or nano-particles (Kesisoglou and Mitra, 2012; Noyes and Whitney, 1897). Meanwhile, the water solubility also increased (Kesisoglou and Mitra, 2012). Up to now, diverse nanotechnologies were used to prepare EA micro- or nano-particulate systems including microspheres of EA (Jeong et al., 2001; Ogawa et al., 2002), pH dependent microassemblies of EA (Barnaby et al., 2011), amorphous solid dispersions of EA with cellulose ester (Li et al., 2013), EA encapsulated chitosan nanoparticles (Arulmozhi et al., 2013a), poly(lactide-co-glycolide) (PLGA) and polycaprolactone (PCL) (Bala et al., 2005a; Sharma et al., 2007; Sonaje et al., 2007), co-encapsulated antioxidant nanoparticles of EA and coenzyme Q(10) (Ratnam et al., 2009). During above preparation processes of EA micro- or nano-particulate systems, emulsion–diffusion–evaporation (Bala et al., 2005a; Sharma et al., 2007; Sonaje et al., 2007), spray drying (Li et al., 2013), co-precipitation (Li et al., 2013), rotary evaporation (Li et al., 2013) and ionic gelation (Arulmozhi et al., 2013a) methods were utilized. Compared with aforesaid methods, antisolvent precipitation method is rapid and easy to operate and industrialize. Moreover, it has low requirements for the equipment. It has been successfully applied to chemosynthetic and natural medicine including genipin (Zhao et al., 2013), griseofulvin (Beck et al., 2013), artemisinin (Kakran et al., 2013), budesonide (Rasenack et al., 2003) and atorvastatin (Zhang et al., 2009). The drug precipitates just occur by virtue of mixing solvent and antisolvent to form drug supersaturation solutions. The two solvents are miscible and the drug dissolves in the solvent, but not in the antisolvent in the process. The key of this technique is to develop crystal nucleus rapidly and endeavour to prevent their growth.

However, so far there is no report on the antisolvent precipitation for micronized EA (m-EA). In this context, m-EA was prepared by antisolvent precipitation to improve poor properties of EA in this research. This technique was optimized by single factor design. Due to the merits of simpleness and utility, single factor design method is widely used in many engineering fields (Zhao et al., 2014). The effects on mean particle size (MPS) of m-EAS from the experiment parameters such as precipitation time and temperature, the dripping speed of solvent adding into antisolvent, the volume ratio of antisolvent to solvent, concentration of EA solution and stirring intensity were carefully investigated during the optimization process. Then the ultimate m-EA freeze-dried powder was characterized by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), high performance liquid chromatography (HPLC), antioxidant activity and dissolution test. The oral bioavailability of m-EA freeze-dried powder on Sprague-Dawley rats was also evaluated.

2. Materials and methods

2.1. Materials

The EA with a purity of mass fraction of more than 98.5%, maltodextrin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, $FeCl_3$, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$ were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Pomegranate herbal supplement was supplied by GNC (Pittsburgh, Pennsylvania, USA). Pomegranate herbal supplement was available in capsules and each capsule contained 250 mg pomegranate fruit extract which contained 100 mg EA. Acetonitrile and methanol were of high performance liquid chromatography grade. 1-Methyl-2-pyrrolidinone (NMP) (purity > 99.5%) and the other reagents were all analytical grade.

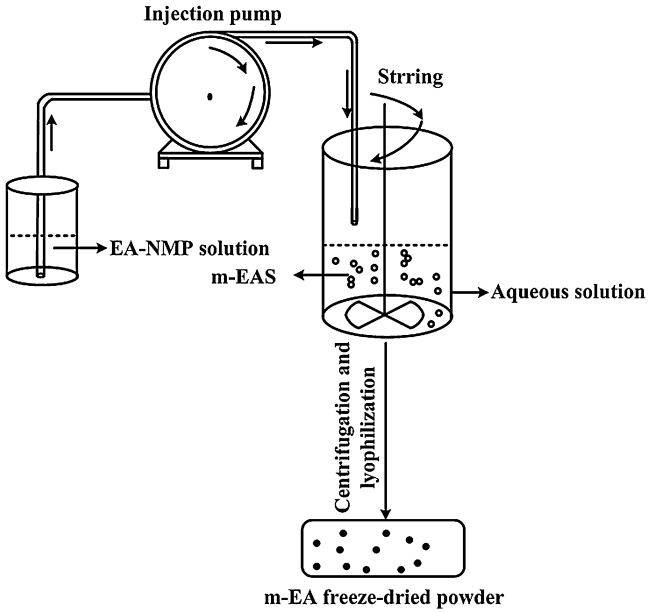

Fig. 2. Schematic description of the preparation procedure of m-EA freeze-dried powder.