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Effects of stabilizing agents on the development of myricetin nanosuspension and its characterization: An *in vitro* and *in vivo* evaluation

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ABSTRACT

Although myricetin has various pharmacological applications, it shows low oral bioavailability (<10%) in rats due to its poor aqueous solubility. To overcome this issue, myricetin nanosuspensions were developed and the effects of stabilizers were investigated. Based on the particle size and zeta potential, stabilizers soya lecithin, TPGS, HP-β-CD, and/or a combination thereof were used. The prepared nanosuspensions were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray powder diffraction (XRD). The resulting myricetin nanosuspensions contained particles in the size range of 300–500 nm and were physically stable. Myricetin was partially transformed from crystalline to amorphous forms in the presence of different excipients after the nanosizing process. The solubility and *in vitro* dissolution of all myricetin nanosuspensions were greatly increased compared with those of the myricetin powder. Consequently, the relative bioavailability in rats were 2.44, 3.57, 1.61, and 2.96 for nanosuspensions stabilized with TPGS, soya lecithin, soya lecithin + TPGS, and HP-β-CD + TPGS, respectively, relative to that of the coarse myricetin. This research demonstrated that nanosuspension is a promising strategy for delivering poor water-soluble drugs such as myricetin and that stabilizers played a critical role in the formulation design of myricetin nanosuspensions.

1. Introduction

Myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone; Fig. 1), a naturally occurring phytochemical, is commonly found in fruits, vegetables, foods of plant origin (Ong and Khoo, 1997), and medicinal herbs, such as grapes, berries, onions, red wine, *Abelmoschus moschatus* (Malvaceae), and *Ampelpsis grossedentata*. As a flavonoid with six hydroxyl groups, myricetin possesses strong antioxidative activity, which is utilized in its various pharmacological applications as an anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, and anti-thrombotic agent (Dajas et al., 2003; Liu et al., 2007; Surh, 2003). However, the applicability of flavonoids as drugs is often impeded by their low bioavailability, which might be related to their low aqueous solubility. For example, the oral bioavailability of quercetin in humans (Hollman et al., 1996) was only 1%, which was ascribed to, at least in part, its low aqueous solubility of 2.84 ± 0.03 μg/mL (Kakran et al., 2012); silybin is an insoluble flavonoid compound (0.4 mg/mL), and its clinical efficacy is discounted by low absorption in the gastrointestinal tract (23–47%) (Woo et al., 2007; Wu et al., 2007); and the uses of baicalein as a pharmaceutical are limited by its low water solubility (approximately 0.1 mg/mL) and poor oral bioavailability (Hsiu et al., 2002). As for myricetin, its low absolute bioavailability (less than 10% in rats) was also attributed to its poor aqueous solubility

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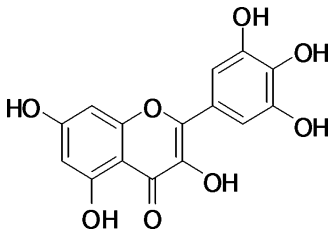

Fig. 1. Chemical structure of myricetin.

(2 μg/mL) (Dang et al., 2014; Yao et al., 2014), which restrained its pharmaceutical development and further clinical application. Therefore, an alternative oral formulation of myricetin with an enhanced solubility and improved bioavailability is highly desired in order to fully realize its therapeutic effects in clinic.

Recently, nanosystems, meaning the design, synthesis, and characterization of particles that have at least one dimension on the nanometer scale, have been used to increase the solubility of poor aqueous soluble drugs and subsequently improve their bioavailability. Gaur et al. (2014) reported that a solid lipid nanoparticle formulation of efavirenz showed mean particle size of 124 ± 3.2 nm and exhibited 10.98-fold increase in area under the concentration–time curve (AUC) in comparison to efavirenz suspension. The plasma level of baicalein, when administering its long-circulating nanoliposomes with an approximately 700 nm particle size, was significantly improved, and the relative bioavailability of baicalein was 452% in mice (Liang et al., 2013). Similarly, the aqueous solubility of kaempferol was increased approximately 139-fold when it was developed into a nanoparticle system, which resulted in a significant improvement in antioxidant activity ($p < 0.05$) compared with the pure drug (Tzeng et al., 2011). However, the formulated composition and processing steps of these nanotechnologies are comparatively complicated such that their difficulties in scale up and reproducibility prevent their commercialization.

A nanosuspension, also called a nanocrystal, is defined as a carrier-free drug delivery system which consists of pure drugs and stabilizers with a mean particle size in the nanometer range, typically between 10 and 1000 nm (Gao et al., 2008, 2012). Compared with other nanosystems, this colloidal system exhibits a number of benefits, including a more efficient particle size reduction, a simple formulation composition, an easier transformation into solid dosage forms, and various administration routes (Agnihotri and Vavia, 2009; Chen et al., 2011; Jacobs and Muller, 2002). Thus, this technology has been quickly adopted by the pharmaceutical industry, and some oral nanocrystal products are now commercially available, *e.g.*, Rapamune®, Emend®, TriCor®, Megace® (Pardeike and Muller, 2010). In recent years, nanosuspensions have also been successfully used to tackle the various problems encountered during the application of flavonoid drugs. Gao et al. (2011) prepared a chemically stable quercetin nanosuspension by an evaporative precipitation process and reported a solubility of quercetin that was enhanced 25.72-fold greater than previously reported. Another study indicated that a vitexin nanosuspension could significantly enhance the *in vitro* dissolution rate compared with an unprocessed vitexin ($p < 0.05$) (Zu et al., 2012). The solubility of baicalin in the form of nanocrystals (495 μg/mL) was much higher than its microcrystals and physical mixture forms (135 and 86.4 μg/mL, respectively) (Jin et al., 2014). However, there have been no reports on the preparation of a myricetin nanosuspension and its evaluation on *in vitro* solubility and *in vivo* bioavailability.

Conventionally, a systematic evaluation on stabilizers is necessary for the production of a nanosuspension because stabilizers play an important role on preventing particle agglomeration. The most common approaches for stabilization are electrostatic and/or steric techniques. Electrostatic stabilization is obtained by adsorbing ionic surfactants (such as soya lecithin and sodium lauryl sulfate (SLS)) onto the particle surface; then, the surface charge and electrostatic repulsion prevents the nanosized particles from agglomerating (George and Ghosh, 2013). Meanwhile, steric stabilization is achieved by adsorbing polymers (such as hydroxypropyl methyl cellulose (HPMC), D-α-tocopherol polyethylene glycol 400 succinate (TPGS), and hydroxypropyl-β-cyclodextrin (HP-β-CD)) or nonionic surfactants (such as Poloxamer 188) onto the surfaces of drug nanocrystals to form a dynamically rough surface to prevent coalescence by repulsive entropic forces (Roux et al., 2002). Among the mentioned stabilizers, HP-β-CD is an excipient with a relatively high water solubility, low economic cost, and low toxicity, and in the pharmaceutical field, it is usually used to enhance the solubility of insoluble compounds by forming a complex (Tang et al., 2013a). It was recently reported that HP-β-CD reduced the surface tension between dissolution medium and drug (de Freitas et al., 2012; Soares-Sobrinho et al., 2012). Thus, it is a potential surfactant. HP-β-CD has not yet been used as a stabilizer in any nanosuspension formulation. It is worthwhile to develop new applications for this excipient because it could increase the diversity of formulations and further develop pharmaceutical technology. Therefore, another purpose of this study was to find potential stabilizers for nanosuspension preparations.

In the present study, four myricetin nanosuspensions with soya lecithin, TPGS, HP-β-CD, and/or a combination of stabilizers were prepared using the precipitation-high pressure homogenization method. Myricetin nanosuspensions were characterized in terms of particle size, zeta potential, morphology, thermal properties, and crystallinity to determine the variation of physicochemical properties. The saturation solubility, stability, *in vitro* dissolution, and *in vivo* pharmacokinetics of myricetin in nanosuspension and as a crude drug were also evaluated. This study provides some guidance to improve the solubility and bioavailability of poorly soluble drugs and also provides some ideas for the formulation design of nanosuspensions.

2. Materials and methods

2.1. Materials

Myricetin at purities greater than 98% was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). TPGS was purchased from Sigma–Aldrich Co. LLC (Shanghai, China). HP-β-CD was purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China). Soya lecithin was kindly donated by Shanghai manshi Bio-Technology Co., Ltd. (Shanghai, China). Poloxamer 188 (P 188) and HPMC E3 were gifted from BASF (Ludwigshafen, Germany). HPLC grade acetonitrile and methanol were purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Ultra-pure deionized water was generated from a Millipore Milli-Q Gradient system (Millipore, Bedford, MA, USA). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of nanosuspension

Two hundred milligrams of myricetin was dissolved completely in 20 mL ethanol as an organic phase. The organic phase was added quickly into the aqueous solution containing 2% (w/v) stabilizers and stirred at 16,000 rpm with a Fluko high shear dispersing emulsifier (Shanghai, China) for 20 min. Afterward, the organic solvent was removed in a rotary evaporator (SB-2000, Shanghai Ailang Instruments Co., Ltd. Shanghai, China) to obtain the coarse