The Journal of Supercritical Fluids 131 (2018) 19–25

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

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

Preparation and characterization of naringenin microparticles via a supercritical anti-Solvent process

Honggang Miao, Zhen Chen, Wenbo Xu, Weiyan Wang, Yaqin Song, Zhixiang Wang*

School of Engineering, China Pharmaceutical University, Nanjing 210009, People's Republic of China

ARTICLE INFO

Keywords:
Naringenin
Supercritical CO_2
Microparticles
SEDS
Dissolution rate

ABSTRACT

Naringenin (NAR) exhibits many biological and pharmacological activities. However, its poor water-solubility and dissolution rate restrict its oral-intestinal absorption rate and bioavailability. In order to improve its dissolution rate, micronized NAR was successfully prepared using a supercritical anti-solvent process. A mixed acetone/dichloromethane solution and CO_2 were used as the solvent and anti-solvent, respectively. The optimal temperature (35 °C), pressure (12 MPa), drug concentration (15 mg/mL), solution flow rate (1 mL/min), and volume percentage of dichloromethane (45%) were determined using a single-factor method. Under optimal conditions, a mean particle size of 611 ± 32 nm was obtained. XRD and FTIR analyses indicated that the unprocessed NAR and micronized NAR using solution-enhanced dispersion by supercritical CO_2 (SEDS) processes exhibited the same crystal structure and chemical structure, and the samples showed similar DSC thermograms. An in vitro dissolution test showed that micronization significantly enhanced the dissolution of NAR.

1. Introduction

Naringenin (NAR) belongs to the class of flavonoids called the flavanones, and it is extracted from citrus fruits [1]. It exhibits anti-carcinogenic [2,3], anti-atherosclerotic [4,5], anti-inflammatory [6], anti-tumor [7], anti-hyperlipidemic [8], anti-oxidant [9] and hepatoprotective activates [10]. However, NAR is poorly soluble in water, which results in low bioavailability [11,12]. This limits its use in medicine, food, and health products.

Many different techniques used to improve the rate of drug dissolution [13–15]. Micronization is an effective method for improving the dissolution rate of poorly-soluble drugs, by forming microparticles that have a higher specific surface area and dissolution rate than the unprocessed drug [16,17]. This can reduce the drug dosage/toxicity and shorten the treatment time [18]. Conventional micronization techniques include spray drying, freeze-drying, and mechanical techniques. However, these techniques sill have several drawbacks, such as relatively large particle size, wide size distribution, and the presence of organic solvent residue in the final products [19]. Thus, several processes based on using a supercritical anti-solvent have been recently introduced to improvedrug micronization [20,21]. The supercritical anti-solvent enables the control of particle size [22] and morphology [23], does not leave behind organic solvent residue [24], and is environmentally friendly [25]. Supercritical carbon dioxide (SC-CO_2) is a commonly used supercritical fluid (SCF) anti-solvent, due to its relatively high supercritical temperature (304.1 K) and pressure (7.38 MPa) [26]. Moreover, CO_2 is inexpensive, readily available, nonflammable, and nontoxic.

In the supercritical anti-solvent processes, a drug is first dissolved in a conventional solvent. SC-CO_2 is then added as an anti-solvent; SC-CO_2 is soluble in a large number of solvents. The dissolution of SC-CO_2 causes the solution to rapidly expand. As the solution density decreases, the solubility of the drug decreases, resulting in the formation of either a supersaturated solution or the precipitation of amorphous particles [27]. Operating parameters such as pressure, temperature, flow rate, and drug concentration have significant influences on the particle size and particle size distribution [28]. While supercritical anti-solvent technologies include the supercritical anti-solvent(SAS) process [29], the aerosol solvent extraction system (ASES) [30], the gas antisolvent (GAS) [31] process and solution-enhanced dispersion by supercritical CO_2 (SEDS) process [32,33]. The SEDS process enables the preparation of smaller particles than the other techniques [34].

In the present study, we demonstrated the first use of SEDS to prepare NAR microparticles. The effects of various process parameters on the morphology and particle size were studied. In addition, the microparticles were characterized before and after SEDS processing using a series of methods.

* Corresponding author.
E-mail address: 1443590147@qq.com (Z. Wang).

http://dx.doi.org/10.1016/j.supflu.2017.08.013
Received 26 May 2017; Received in revised form 24 August 2017; Accepted 25 August 2017
Available online 30 August 2017

Table 1
Maximum solubility of NAR in acetone and DCM (and their mixtures) at 35 °C and atmospheric pressure

Solvent	Solubility (mg/mL)
acetone	110.87
DCM	0.23
acetone/DCM(15% DCM, v/v)	101.24
acetone/DCM(30%DCM, v/v)	80.63
acetone/DCM(45%DCM, v/v)	58.81

2. Materials and methods

2.1. Materials

NAR (≥98%) was obtained from Shanxi Huike Botanical Development Co., Ltd. (Shanxi, China). The NAR standard (≥98%) was purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). High-purity CO_2 (≥99.99%) was purchased from Nanjing Special Gas Co., Ltd. (Nanjing, China).Dichloromethane (DCM, ≥ 99.7%), acetone (≥99.7%), acetonitrile (≥99.5%) and ethanol (≥99.7%) were purchased from Guoyao Co., Ltd. (Shanghai, China). Tests were performed at 37 °C to measure the maximum solubility of NAR in acetone, DCM, and their mixtures (Table 1).

2.2. Preparation of NAR microparticles

A schematic diagram of the SEDS apparatus used to prepare the NAR microparticles is shown in Fig. 1. Firstly, NAR solution was prepared in the solvent mixture. CO_2 was cooled using a low-temperature thermostat and then transferred into a 300-cm^3 crystallization vessel. When the crystallization vessel reached the desired temperature (> 31.26 °C) and pressure (> 7.38 MPa) [35], the NAR solution was passed into the crystallization vessel at high pressure though a coaxial nozzle. At this point, additional CO_2 was pumped into the crystallization vessel. When all of the NAR solution had been pumped into the vessel, CO_2 was continuously flowed into the vessel for a further 60 min (the solvent in the crystallization vessel is not completely drained less than 60 min, while continuously flowing CO_2 more than 60 min wastes CO_2.) to remove the organic solvent residues. Subsequently, the pressure in the crystallization vessel was reduced to 1 atm. Finally, micronized NAR was collected from the crystallization vessel for characterization.

Many factors can influence the mean particle size (MPS) of NAR. In the present study, the effects of NAR solution flow rate (*F*), precipitation pressure (*P*), precipitation temperature (*T*), NAR solution concentration (*C*), and solvent composition (*M*), on MPS were investigated and optimized using a single-factor method.

2.3. Characterization methods

2.3.1. Particle size analysis

The MPS and particle size distribution of the prepared NAR microparticles were measured using a wet laser diffraction method with a Mastersizer 2000 laser particle analyzer (Malvern Instruments Ltd., Malvern, UK).

2.3.2. Scanning electron microscopy (SEM) analysis

The morphologies of the processed and unprocessed NAR particles were determined using SEM (LE1530-SEM, LEO, Germany). Before analysis, NAR particles were transferred onto carbon tape and sputter-coated with a thin layer of gold.

2.3.3. Fourier-transform infrared (FTIR) spectroscopy analysis

The processed and unprocessed NAR particles were diluted to 1% with KBr mixing powder and then loaded onto self-supporting disks. FTIR spectra were obtained using a Nicolet Impact 410 spectrometer (Thermo-Fisher, USA) over a wave number range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} to detect changes in the NAR structure as a result of SEDS processing.

2.3.4. X-ray powder diffraction (XRD) analysis

XRD patterns were collected using an X-ray diffractometer with a rotating anode (Bruker D8, Karlsruhe, Germany) using Cu-Kα radiation generated at 30 mA and 40 kV. The sample holder was filled to the same thickness with either processed or unprocessed NAR particles. The crystalline structures of the samples were analyzed in a 2θ range of 5–45° with steps of 0.05° and a scanning speed of 4°/min.

2.3.5. Differential scanning calorimetry (DSC) analysis

DSC measurements of raw NAR powder and NAR microparticles were performed using a Netzsch DSC 204F1 (Netzsch Geraetabau GmbH, Germany). The NAR samples (5 mg) were placed in aluminum pans. The sample cell was then heated from 50 to 300 °C at a rate of 20 °C/min.

2.3.6. Identification and quantification of residual solvent

In this study, a 6890 gas chromatograph (Agilent Inc., USA) coupled with a 7694E headspace sampler (Agilent Inc., USA) was used to detect the residual organic solvent in NAR samples prepared by SEDS, by using a nonpolar column (30 m × 0.53 mm × 0.5 μm). The following gas chromatography conditions were used: inlet temperature 200 °C; oven temperature 50 °C(4-min hold) to 210 °C(2-min hold) at a rate of 30 °C/min, and nitrogen carrier gas with a flow rate of 1.5 mL/min. The injector was stabilized at 250 °C. The samples were dissolved in dimethyl formamide. The headspace in the vials was maintained at 75 °C for 25 min.

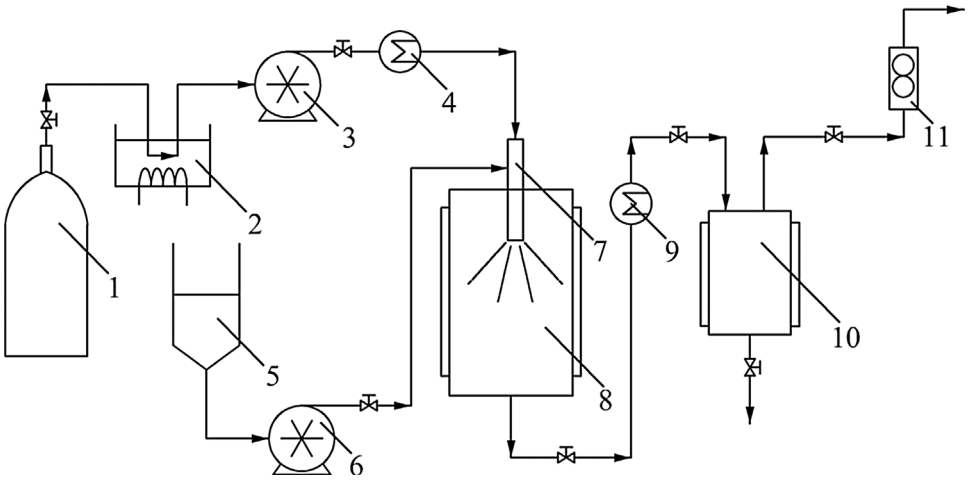

Fig. 1. Experimental apparatus for the SEDS process, (1) CO_2 storage tank, (2) low temperature thermostat, (3) high-pressure pump, (4) CO_2 preheater, (5) liquid storage tank, (6) high-pressure infusion pump, (7) coaxial nozzle, (8) crystallizationvessel, (9) heat exchanger, (10) solvent recovery vessel, (11) rotameter..