J. of Supercritical Fluids 89 (2014) 99–105

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

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

Polymeric coating of fluidizing nano-curcumin via anti-solvent supercritical method for sustained release

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 25 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Supercritical anti-solvent
Fluidization
Coating
Curcumin
PLGA

ABSTRACT

Nano-curcumin was coated by poly(lactic-co-glycolic acid) (PLGA) using a novel fluidization assisted supercritical anti-solvent procedure. PLGA solution was sprayed into supercritical CO_2 media, in which nano-curcumin particles were fluidized by ultrasonic vibration. The influences of process parameters, such as solvent types, solution concentrations, CO_2 flow rates, the ratio of PLGA to curcumin, and ultrasonic power on the particles size and the curcumin loading were investigated. Scanning electron microscopy, laser particle size analyzer, and differential scanning calorimetry were used to characterize as-produced samples in terms of the structure, morphology and particle size distribution. The PLGA-curcumin nano-capsules were obtained with the average size of 63 nm and the loading of 38%, under the ultrasonic power of 210 W, and with the average size of 40 nm and 36% loading, at the ultrasonic power of 350 W. In vitro studies prove that proposed method is successful in preparing sustained release systems.

1. Introduction

Curcumin, derived from the rhizomes of turmeric (*Curcuma longa* Linn.), has been widely used as a coloring agent, food additive, and a famous Chinese medicine for centuries. It has attracted huge interesting recently because of various beneficial pharmacological effects, such as antioxidant, anti-inflammatory, and anticarcinogenic activities. But these effects are mostly limited in clinical trials due to curcumin poor water solubility and rapid hydrolysis [1,2]. Several approaches have been proposed to overcome low bioavailability of curcumin. Among them, physical re-formulation to nano-particles is considered a promising method to improve solubility rate [3]. Coating of nano-curcumin by polymer can protect it against degradation by light and heat. In addition, drug release rate can be controlled by coating polymer [4–6]. PLGA is often used as a drug coating alternative due to its biodegradable characteristic [7–9]. Conventional microcapsulation methods, such as emulsion evaporation, phase separation, spray drying, and freeze-drying need large amounts of organic solvents and other chemicals, further processing for extraction of products from liquid media, and high-temperature operations [10–13].

Supercritical anti-solvent technology (SAS) has been widely used in preparing micro/nanoparticles and micro/nano-spheres because of lower residual solvent in products, simple step, and mild operating conditions [14–16]. Supercritical anti-solvent, assisted with ultrasonic vibration (SAS-EM), has been developed to improve mixing effects. However, aggregation, wide size distribution, and low loading are often the main problems in this technique, especially when it is used for treatment of biopolymers [16–19]. Other modifications, such as supercritical extraction of emulsions, and supercritical coating of suspended drug, also show some drawbacks, such as being involved in liquid media, and nozzle clogging [20–23]. Besides, these methods cannot guarantee to obtain high loaded products. Nano-mixing under sonication in liquid CO_2 has been also used for drug loading purposes, when only simple mixing of drugs with excipients is required [24,25].

In this study, a fluidizing technology based on SAS-EM is developed to prepare PLGA-curcumin nanocapsules with high loading and low aggregation. In this process, PLGA solution is injected into supercritical CO_2 media, in which curcumin nano-particles are pre-loaded and fluidized by ultrasonic power. Fluidizing of nanoparticles not only prevents agglomeration of polymeric nucleases, but also causes high efficient loading due to their uniform suspension in precipitation vessel. A group of initial tests are first carried out, in which pure PLGA is treated by SAS-EM, to obtain suitable operating conditions for producing PLGA particles, and observe the behavior of PLGA under SAS-EM process in the absence of

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http://dx.doi.org/10.1016/j.supflu.2014.02.021
0896-8446/

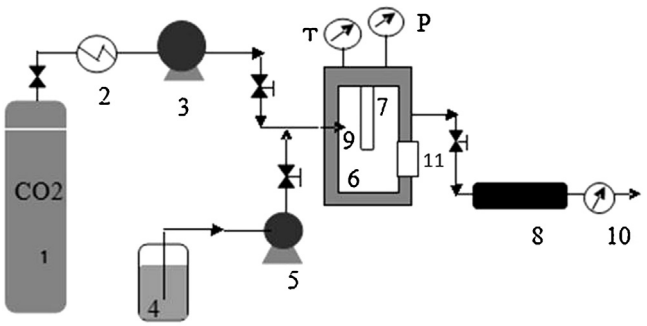

Fig. 1. Schematic of fluidization assisted SAS apparatus: (1) CO_2 cylinder, (2) pre-heater, (3) CO_2 pump, (4) organic solution, (5) HPLC pump, (6) high pressure vessel, (7) ultrasonic vibration device, (8) collector, (9) coaxial nozzle, (10) back pressure regulating valve, and (11) high pressure glass window.

fluidizing particles. The second group of experiments is concentrated on coating of fluidized nano-curcumin by PLGA at different operational condition. Release profile of the coated curcumin is evaluated in vitro, and the release model is obtained.

2. Experimental

2.1. Materials

Poly(lactic-co-glycolic acid) (PLGA, MW = 50,000, 75/25) and pure curcumin (99.9%) were purchased from Sinopharm Company in China. Compressed CO_2 was purchased from China Zhuolo High Pressure Vessel Company. Absolute ethanol (analytical grade), acetone (purity 99.9%) and ethyl acetate (purity 99.8%) were purchased from Shanghai Infeng Chem., Reagents Company.

2.2. Experimental apparatus and procedure

Fig. 1 shows the schematic representation of experimental apparatus. An ultrasonic titanium probe (tip diameter; 1.2 cm) is installed on the top of a high-pressure vessel (stainless steel, 90 mL) with a visible window. Ultrasound field is generated by a 1200 W (max. power) processor (CDS-320, GE Healthcare Co.) with ±10% power accuracy. In the experiments, 80 mg of curcumin nano-particles (mean size: 20 nm), prepared by enhanced mass transfer supercritical anti-solvent method, is pre-loaded into the vessel. Pure liquid CO_2 is then fed into the high-pressure vessel via a heat exchanger (stainless steel, 90 mL) by a syringe pump (CW300, Wuxi Lingjie Co.). Once the desired conditions are attained, CO_2 inlet valve is closed; and nano-particles are fluidized in batch mood for 15 s to ensure being well dispersed. Then, CO_2 is allowed to flow through the chamber, while the system pressure is kept constant by a back pressure regulating valve (FR300, Pressure Tech.), located at the CO_2 outlet. Polymer solution is injected via a co-axial capillary (i.d.: 75 μm, S.S.) into the chamber by a HPLC pump (HBL-1040, Dongtaiyanshan Instrument Co.). Nozzle tip is placed facing the ultrasonic horn at 45° and 2 mm distance. Polymer particles are formed immediately due to the fast mutual diffusion between CO_2 and solution phases, and coat on the fluidized curcumin nano-particles, forming nanocapsule particles. Solvent residuals are carried out immediately from the chamber by CO_2. The influence of ultrasonic power (USP) on the nano-curcumin fluidization and the nanocapsulation process could be observed via the visible window. Ultrasonic works in pulse mode of 1 s (1 s on and 1 s off), during the experiments. An extra collector vessel (i.d.: 16 mm, length: 110 mm), equipped with a 220 nm mesh sieve and paper filters, is placed immediately after the chamber to trap the particles being pulled out by CO_2. After finishing injection of PLGA solution, CO_2 is pumped continually for 40 min to remove the solvent residue. Then, the chamber is depressurized, products are collected from the chamber and collector, and prepared for the later analysis and characterization.

2.3. Characterization and analysis

2.3.1. Size and size distribution

The morphology and particles size of samples are characterized by scanning electron microscopy (SEM, Hitachi S-3400N). Samples are dispersed in distilled water, and sonicated for 3 min in a bath ultrasonic system (Bio-gen pro200, Proscientific). Resultant suspension is dropped on a glass slide, dried under vacuum, and coated with gold–palladium layer.

Size distribution assessment is carried out by a by PDS-Zeta seizer (Malvern Zetasizer Nano-ZS). The aqueous suspension of sample, prepared by the same method as explained for SEM, is added to a Beckman coulter glass cuvette, and located in system cell to analyze.

2.3.2. Differential scanning calorimetry (DSC)

The crystalline structures of the processed and unprocessed samples are analyzed by Differential Scanning Calorimetry (DSC-2200, Analytical Technology). 5 mg of samples was placed in an aluminum sealed pan (50 μL). System was purged with nitrogen to avoid any undesired reactions. Analysis temperature range was from 0 to 200 °C with the rate of 2 °C/min.

2.3.3. Loading assay

The encapsulation efficiency of curcumin in the as-produced samples was checked by UV–visible spectra (Spectrum, 756 PC). A certain amount of the representative samples was taken and put into a vial with 5 mL ethanol. After 3 min the resultant mixture was filtered. The solid part was again dissolved with 5 mL ethanol. The same procedure was repeated three times. Then, the curcumin attached on the outside of the samples was washed out completely, as checked by UV–visible. The curcumin is absorbed at 425 nm. After that, the remained solid part was dissolved entirely with 5 mL ethyl acetate, and detected by UV–visible at 419 nm. Each sample is measured in triplicate to make sure of method accuracy and loading uniformity. Curcumin loading efficiency (CLE) has been calculated by Eq. (1) and shown in Table 2.

$$\mathrm{CLE} = \frac{\text{amount of coated curcumin in sample}}{\text{amount of sample}} \times 100\% \quad (1)$$

2.3.4. In vitro release

After removing the outer curcumin of the sample by ethanol, 0.25 g of encapsulated sample were placed into a vial with 50 mL buffer solution with pH 7.4, prepared based on literature [26]. The suspension was stirred at 50 rpm using a magnetic stirrer, and kept the temperature of 37 °C. The absorbance of the solution taken at equal intervals, in 10 h, was measured at 445 nm.

3. Results and discussion

3.1. Ultrasonic system assessment

In order to take full advantages of ultrasonic in fluidization, dispersion and mixing, we assess firstly our ultrasonic system. Fluidizing status of nano-curcumin in supercritical media is observed at different ultrasonic powers, through visible window of high pressure chamber. Assessment is carried out at 34 °C and 9 MPa while CO_2 flow rate is 10 mL/min. Without ultrasonic vibration, nano-curcumin disperses scarcely in CO_2 flow. Under ultrasonic vibration, particles are uniformly suspended in chamber, and tend