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Continuous nanonization of lonidamine by modified-rapid expansion of supercritical solution process

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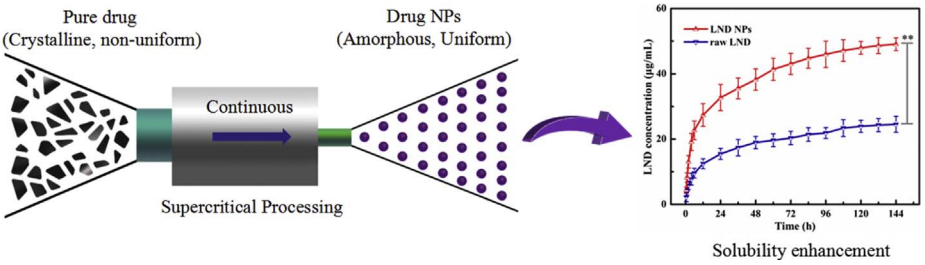
GRAPHICAL ABSTRACT



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ABSTRACT

Despite the significant advancements in the pharmaceutical manufacturing process, the aqueous solubility of most of the potent drugs has remained as an unresolved problem during the formulation development and drug delivery processes. To address this critical issue, we modified the continuous-rapid expansion of supercritical solution (RESS-C) process, which is incessant, stable and well-regulated. Initially, the solubility of lonidamine (LND) in supercritical carbon dioxide (SC-CO_2) was tested using a static analytical method at altered critical conditions (*T*: 308.15-328.15 K, *P*: 10.0-30.0 MPa). Under optimized conditions (LND conc.- 0.5% (w/v), flow rate- 1.0 mL/min, *T*- 328.15 K, *P*- 20.0 MPa and CO_2 flow rate- 30.0 g/min), the modified RESS-C process resulted in nano-sized spheres with a smooth surface and a narrow particle size distribution. Further, the crystal properties of the samples and their molecular interactions were elucidated. The altered physical state of RESS-C processed LND from crystalline to amorphous resulted in the solubility improvement and also enhanced the *in vitro* antiproliferative effects compared to the unprocessed LND, demonstrating the potential of the modified RESS-C process in improving the bioavailability of poorly water-soluble drugs.

1. Introduction

Recently, the supercritical carbon dioxide (SC-CO_2) technology has garnered the enormous attention of researchers widely in the biomedical field due to its environmentally benign nature in various processes and economically promising character [1,2]. Typically, many pharmaceutical processes rely on the use of organic solvents, but this high-pressure technique takes the advantage of CO_2 to replace the organic solvent, thus serving as an alternative in synthesizing drug delivery systems by precipitating the drug alone or in combination with polymer at the micro-/nano- scale, and polymeric scaffolds for tissue engineering, among others [3–5]. This technology is highly

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advantageous due to its mild operating conditions, very low/no organic solvent residue in the end products, efficient separation of active constituents and its ability to overcome most of the drawbacks of the conventional methods such as spray drying and others [1,3,6–8].

Since the first report in 1879 from Hannay *et al.,* SC-CO_2 technology has raised a great interest, following which different processes and their advancements have been made to fabricate the particulate systems. The variants are typically based on the behavior of the SC-CO_2 as a solvent, anti-solvent, solute, and others. Out of all variants of SC-CO_2 technology, RESS is the simplest and an efficient method of particle fabrication as it is easy to operate and yields product with low/no residual organic solvent [6,9,10]. In a typical RESS process, SC-CO_2 acts as a solvent for any solute and is most suitable to fabricate water-insoluble drugs [11,12]. During the particle formation, a series of consequences happen, initially the solution is expanded adiabatically through a nozzle rapidly, the solvent density dramatically decreases resulting in the supersaturation, then the nucleation, and subsequent particle growth *via* condensation and coagulation, leading to the rapid precipitation of the solute from solvent (Fig. S1) [13–15]. Despite the significant advantages of particle formation, RESS process still faces a few obstacles such as limited dissolution capacity, the growth of the particle in the expansion chamber often result in larger agglomerates, long processing time and batch process production, that hinder its potential applicability [6,7,16–18]. To overcome these limitations, a few research groups have modified the traditional RESS processing, one of the advancements include the addition of co-solvent to improve the solubility of drugs in SC-CO_2, in a way, the depressurization of an expanded liquid organic solution (DELOS) process, which involves the crystallization of the compound by using an organic solvent [19]. However, co-solvent selection plays a crucial role in solubilization of drugs and its miscibility with SC-CO_2 [16,20–22]. Polar co-solvents such as acetone, ethanol, dichloromethane (DCM), and others have been utilized so far, but this may result in residual amounts of organic solvent in the end product, which causes product redissolution [23]. Further advancements of RESS process include the rapid expansion of supercritical solution with solid co-solvent (RESS-SC) process and rapid expansion from supercritical solution with a non-solvent (RESS-N), to produce fine-sized particles [9,16,24]. The solid co-solvent utilized in RESS-SC not only enhances the solubility of the drug in SC-CO_2, but also acts as a barrier around the tiny particles to prevent the coagulation/undesirable particle growth in the expansion chamber, and eventually, lyophilization or drying (sublimation) can remove the co-solvent [25]. RESS-N is another interesting variation of RESS that is operated using non-solvents to increase the solubility of the solute in SC-CO_2 during particle fabrication [16,24]. Rapid expansion of a supercritical solution into a liquid solvent (RESOLV) or an aqueous solution (RESAS) were proposed to overcome the excessive coagulation of solute at the expansion stage, where the supercritical solution is expanded into a solvent instead of a gas yielding nano-sized particles in both the cases [26–28]. Meanwhile, the liquid at the receiving end facilitates the redispersibility, and stability of precipitated particles, preventing collision and coalescence between particles in the expansion jet.

Drugs or polymers that are partially soluble in the SC-CO_2 would sufficiently recrystallize neither by the RESS process nor by the supercritical anti-solvent (SAS) process. During the batch processing of such solutes, their poor solubility in SC-CO_2 leads to a low yield in RESS, while there is almost no product in an SAS process [29]. To overcome this issue, we developed an innovative strategy combining the RESS with solution-enhanced dispersion by SC-CO_2 (SEDS) for the continuous processing of partially SC-CO_2 −soluble polymers [20].

Motivated by these astonishing facts, herewith, we amended the experimental-setup of RESS by introducing a few auxiliary tools such as an external injection device for continuous flow, back pressure valve for stabilization of pressure, a mass flow meter to control the flow rate and most importantly, a specially designed expansion vessel for the heat absorptivity of continuous phase transition. To evaluate this new concept, we chose a poorly water-soluble drug candidate, LND, an indazole-3-carboxylic acid derivative, which acts effectively against various cancers (Fig. S2). LND interferes with the mitochondrial function by inhibiting the hexokinase enzyme responsible for ATP production, and interfere with the respiration and maintenance of the mitochondrial transmembrane potential, and prevent the aerobic glycolysis[30]. LND has been used to treat benign prostate hypertrophy and breast and ovarian cancers, among others. However, the poor pharmacokinetics of LND ensued in increased frequency of dosage, which results in severe hepatotoxicity [31,32]. Nanonization of LND could enhance the solubility and eventually improve its bioavailability [1,31–33]. Initially, the equilibrium solubility of LND in SC-CO_2 was measured at different temperatures (*T*: 308.15-328.15 K) in a specific pressure range (*P*: 10.0-30.0 MPa) using a static analytic method coupled with spectrophotometric quantification [34–36]. Further, the LND nanoparticles (NPs) were prepared using the modified RESS-C process, and subsequently, their solubility performance and the antitumor efficiency were evaluated.

2. Materials and methods

2.1. Materials

LND, DCM, methanol (MeOH), N, N- dimethylformamide (DMF) and potassium bromide (KBr) were purchased from Aladdin Co. Ltd. (Shanghai, China). Phosphate-buffered saline (PBS), Fetal bovine serum (FBS), Roswell Park Memorial Institute (RPMI)-1640 medium, Trypsin-ethylenediaminetetraacetic acid (EDTA), Cell Counting Kit-8 (CCK-8), Acridine Orange/Ethidium Bromide (AO/EB) were obtained from Gibco Life Technologies (Carlsbad, USA). A549 cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). CO_2 (99.9% purity) was purchased from the Rihong Air Products Co., Ltd. (Xiamen, China). All other chemicals used in this study were of analytical grade and were used without further purification.

2.2. Methods

2.2.1. Solubility determination

A static equilibrium method was used to measure the experimental solubility of raw LND in SC-CO_2, following the reported procedure [36]. Briefly, a stainless steel equilibrium vessel (internal volume ~30 cm^3) with an attached temperature regulator module to maintain the operational temperature (± 0.1 K) was loaded with the solid solute. Then, the vessel was pressurized using CO_2 regulated with a high-pressure transducer simultaneously and calibrated between 0 and 39.5 MPa. When the desired operating temperature and pressure were reached, the solute + CO_2 mixture was stirred for a short span to attain equilibration and fluid phase saturation. This processing time must be always predetermined to ensure that full saturation has occurred. For LND, the required saturation time was around 30 min, however, we stirred the solution for 40 min and allowed to stay for further 20 min without stirring. Then, an aliquot was drawn by using a six-port sampling valve and a sampling loop of approximately 0.5 cm^3. The sample was depressurized rapidly, and the precipitated solute was collected. The CO_2 in the sample was expanded to a large pre-calibrated volume and calculated based on the resulting sub-atmospheric pressure increment, which measured by a high-precision low-pressure transducer. To ensure that all the solute was collected, MeOH as a cleaning solvent was injected into the sample loop and the content in the expansion lines are recovered in the glass trap.

2.2.2. Modification of RESS-C apparatus

In comparison to the traditional RESS process, the basic approach of this process includes the continuous feeding of the solute with co-solvent, that can enhance the dissolving capacity of SC-CO_2 dramatically