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New polymorphs of 9-nitro-camptothecin prepared using a supercritical anti-solvent process

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ABSTRACT

Recrystallization and micronization of 9-nitro-camptothecin (9-NC) has been investigated using the supercritical anti-solvent (SAS) technology in this study. Five operating factors, i.e., the type of organic solvent, the concentration of 9-NC in the solution, the flow rate of 9-NC solution, the precipitation pressure and the temperature, were optimized using a selected OA_{16} (4^5) orthogonal array design and a series of characterizations were performed for all samples. The results showed that the processed 9-NC particles exhibited smaller particle size and narrower particle size distribution as compared with 9-NC raw material (Form I), and the optimum micronization conditions for preparing 9-NC with minimum particle size were determined by variance analysis, where the solvent plays the most important role in the formation and transformation of polymorphs. Three new polymorphic forms (Form II, III and IV) of 9-NC, which present different physicochemical properties, were generated after the SAS process. The predicted structures of the 9-NC crystals, which were consistent with the experiments, were performed from their experimental XRD data by the direct space approach using the Reflex module of Materials Studio. Meanwhile, the optimal sample (Form III) was proved to have higher cytotoxicity against the cancer cells, which suggested the therapeutic efficacy of 9-NC is polymorph-dependent.

1. Introduction

9-Nitro-camptothecin (9-NC), one of the semi-synthetic and lipophilic camptothecin (CPT) analogues, is a promising anti-cancer agent which has stronger anti-tumor potency in both animal and human trials compared to CPT (Wall et al., 1966; Lian et al., 2014), and has been widely used in the treatment of cancers, such as bladder cancer, advanced pancreatic carcinoma, colorectal cancer, ovarian epithelial cancer and leukemia (Stehlin et al., 1999; Garcia-Carbonero and Supko, 2002; Rivory and Robert, 1995). However, the delivery of the lactone form with the closed E-ring, which is a crucial structure of 9-NC, is quite challenging, since the lactone ring might undergo ring opening hydrolysis and translate to the carboxylate form under physiological conditions, which may lead to low therapeutic efficiency and a number of side effects to normal tissues, such as thrombocytopenia, hemorrhagic cystitis, myelotoxicity and nausea (Wani et al., 1980; Venditto and Simanek, 2010; Saha et al., 2013). Also, due to its poor water solubility, clinical utilization of 9-NC requires a high dose, which might lead to additional toxic reactions. Therefore, the development of a safer, more stable and potent formulation is necessary.

In order to solve the undesired solubility and stability problems of drugs, various drug delivery systems and techniques have been investigated, such as oil/water nano-emulsion (Han et al., 2009), self-emulsifying formulations (Lu et al., 2008), and liposome micelles (Zheng et al., 2011). However, the low loading content and encapsulation efficiency of model drugs limited the cytotoxic activity (Torchilin, 2007). Polymorphism of drugs has also received extensive academic and industrial attention, since different polymorphs may result in critical differences in the physicochemical and biochemical properties, such as particle size, aqueous solubility, dissolution rate, physicochemical stability, bioavailability, etc. (Aguiar et al., 1967; Aldawsari et al., 2013; Brittain, 2009; Kobayashi et al., 2000).

Recently, researchers reported that the recrystallization and micronization of drugs, which were designed for fine particles with different polymorphs, physicochemical or biochemical properties, were successfully conducted using the supercritical anti-solvent (SAS) process (Rossmann et al., 2013; Montes et al., 2011; Park et al., 2007; Yong et al., 2015; Liu et al., 2015). Compared to the conventional crystallization with multi-step operations, which usually leads to a mixture of different polymorphs, the one step SAS process seems preferable to producing fine particles of a pure

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polymorph, which with low solvent residue and desired physical properties, such as particle size, particle size distribution, biological efficacy, etc. (Sun, 2014).

The aim of this study was to search new polymorphic forms and micronize 9-NC by using the SAS process. The operating conditions of the SAS process which influence the mass median diameter (Dp_{50}), particle size distribution (PSD), morphology and crystallinity, were optimized using an orthogonal array (OA) design method. The properties of the raw material and processed samples of 9-NC were characterized by different methods as well as molecular simulation, and the anti-tumor properties of 9-NC polymorphic forms were also evaluated.

2. Experimental

2.1. Materials

9-Nitro-camptothecin (9-NC) (mass purity fraction > 99%) was purchased from Hubei Kangbaotai Fine Chemical Co., Ltd., China. Carbon dioxide (CO_2) with a minimum mass purity of 99.9% was obtained from Guangzhou Shengying Gas Co., Ltd., China. Analytical purity dichloromethane (DCM), ethanol (EtOH), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF) and dimethyl formamide (DMF) were supplied by the Guangdong Guanghua Sci., Tech. Co., Ltd., China, as well as the standard phosphate buffer saline (PBS, pH 6.86). All of these were used directly without further purification. Ultra-pure water was used throughout the study.

2.2. Apparatus and procedure

The equipment used in the automatic semi-continuous SAS process (SAS50-2-ASSY, Thar Technologies, Inc., USA) was employed to carry out the micronization experiments, the same as that reported in our previous work (Jiang et al., 2012; Liu et al., 2013; Wang et al., 2013). As stated in the specifications of the apparatus, the uncertainty of the temperature, pressure and flow rate is ±1% of full-scale temperature, ±1% of full-scale pressure and ±2% for flow rate, respectively.

2.3. Design of experiment

In order to optimize the operating conditions for the preparation of 9-NC micro-particles using the SAS process, a selected orthogonal experimental design OA_{16} (4^5) was adopted. As shown in Table 1, the SAS experiments were carried out with 5 factors, including type of organic solvent (*S*), flow rate of 9-NC solution (*F*), concentration of 9-NC in the solution (*C*), precipitation pressure (*P*) and precipitation temperature (*T*). The range of each level was based on the results of preliminary experiments. And the type of solvents used in this study was selected based on our preliminary experiments and the results of Chen et al. (2009). EtOH, which almost can't dissolve 9-NC, was selected as a nonsolvent. The application of organic nonsolvent in the SAS process was effective in producing smaller particles and reducing the usage of CO_2.

Table 1
Factors and levels of the orthogonal array design.

Levels	Factors				
	S (v/v)	*F* (mL/min)	*C* (mg/mL)	*P* (bar)	*T* (°C)
1	DCM/EtOH (1/4)	0.3	0.5	80	30
2	DMSO/EtOH (1/9)	0.6	0.7	95	35
3	THF	0.9	0.9	110	40
4	DMF/EtOH (1/4)	1.2	1.1	125	45

In this study, according to our studies (Jiang et al., 2012; Liu et al., 2013; Wang et al., 2013) and the results of Reverchon et al. (2010), the steady flow rate of CO_2 was established at 20 g/min to ensure that the overall molar fraction of CO_2 inside the vessel was larger than 0.96 for all of the experiments, which ensure that the SAS operation conditions were run at a CO_2 molar fraction in the single-phase region.

2.4. Characterization methods

A laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK) was used to measure Dp_{50} and PSD of the unprocessed and processed 9-NC particles, where PSD was expressed by Dp_{50} and its standard deviation (SD). Before each measurement, the samples were suspended in pure water and stirred ultrasonically for 15 min in order to disperse effectively, and then two drops of Tween 80 were added into the sample to avoid the aggregation of particles. Each measurement was repeated at least three times.

A scanning electron microscope (SEM) (S-3700N, HITACHI, Japan) was used for imaging the surface and morphology of the particles. When preparing the samples for SEM measurement, particles were spread on an aluminium stub using double-sided adhesive carbon tape, and then coated with a thin layer of gold-palladium alloy in an argon atmosphere using a sputter-coater at room temperature.

An X-ray diffractometer (D8 ADVANCE, Bruker AXS, Germany) with Cu-Ka radiation generated at 40 mA and 40 kV, was used for obtaining the X-ray diffraction (XRD) patterns of products. 10 mg samples of 9-NC particles, forming a weighted dispersion on a glass slide, were filled to the same depth inside the sample holder by leveling with a spatula. All samples were scanned between 5° and 50° (2θ). The diffraction patterns were processed using JADE 5.0 software.

The thermal behaviour of samples was observed by using a differential scanning calorimeter (DSC) (Q200, TA instruments, USA) and thermogravimetric apparatus (TG) (Model TGA Q500, TA Instruments, USA). For DSC analysis, 2–5 mg samples were weighed accurately and sealed in aluminum hermetic pans. For TG analysis, before putting samples into the aluminium pan, the tare should be cancelled. Both DSC and TG analysis were carried out at a temperature heating rate of 10 °C/min under nitrogen purge from 25 °C to 300 °C. Peak temperatures and the enthalpy of fusion were determined using Universal Analysis Software.

A Fourier transform infrared (FT-IR) spectrometer (Nicolet Nexus 670, Thermo Electron Corporation, USA) was used to examine the chemical structure of the processed and unprocessed 9-NC particles. Samples were prepared by dispersing the 9-NC particles (1 mg) in KBr (100 mg) and pressing the mixture into disc form. The scanning range was 400–4000 cm^{-1}, and the resolution was 4 cm^{-1}.

The residual organic solvent in the optimal processed 9-NC sample was measured by Gas Chromatography spectrometry (GC) spectra obtained using a gas chromatograph (GC 4000, Varian, USA). During the measurement, oven temperature was maintained at 100 °C for 2 min initially, then raised at the rate of 2 °C/min to 200 °C. Both the injector and the detector temperatures were set at 240 °C. Peak area percentages were used for obtaining quantitative data.

2.5. Solubility measurement

The in vitro solubility of the unprocessed and processed 9-NC particles was measured as follows (Liu et al., 2013). Excess sample was added into a tube with 10 mL PBS (pH 6.86). The tube was kept at 37 °C using a thermostat water bath (THD 0506, Ningbo Tianheng Co., CHN) and stirred at 100 rpm. After 18 h, a small