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Research Paper

Experimental investigation and oral bioavailability enhancement of nano-sized curcumin by using supercritical anti-solvent process

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ABSTRACT

The biomedical applications of curcumin (CUR) are limited due to its poor oral bioavailability. In this work, CUR nanoparticles were successfully prepared by combining the supercritical anti-solvent (SAS) process with Tween 80 as a solubilizing agent and permeation enhancer. Different processing parameters that can govern the mean particle size and size distribution of nanoparticles were well investigated by manipulating the types of solvents, mixing vessel pressure, mixing vessel temperature, CO_2 flow rate, solution flow rate and solution concentration. Solid state characterization was done by Fourier Transform infrared spectroscopy, differential scanning calorimetry, dynamic light scattering, scanning electron microscopy, and powder X-ray diffraction study. Solubility and dissolution profile of SAS-processed CUR were found to be significantly increased in comparison with native CUR. Further, a validated ultra-performance liquid chromatographic method with quadrupole-time of flight-mass spectrometry was developed to investigate the pharmacokinetic parameters after a single oral dose (100 mg/kg) administration of CUR (before/after SAS-processed) in male Wistar rats. From the plasma concentration vs. time profile graph, oral bioavailability of SAS-processed CUR was found to be increased approximately 11.6-fold ($p < 0.001$) as compared to native CUR.

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1. Introduction

For orally-administered drugs, aqueous solubility and intestinal permeability are the two predominant rate-limiting steps for deciding their bioavailability. Restriction on the use of Biopharmaceutics Classification System (BCS) class IV drug molecules is due to both their poor aqueous solubility and low permeability. Various formulation approaches (for example, polymeric prodrugs [1,2], co-crystallization [3,4], solid dispersion [5–7], salt formation [8,9], cyclodextrin complexation [10,11], micronization [12,13], and nanocrystalization [14,15]) have been studied for this class of molecules to enhance their oral bioavailability. Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a polyphenol extracted from the rhizome of *Curcuma longa*, belonging to the family Zingiberaceae [16]. Apart from its traditional use as a spice, natural coloring and flavoring agent, recent preclinical and clinical studies have proved its therapeutic benefits as an antispasmodic, anticoagulant, antimicrobial, anti-oxidant, anti-inflammatory, anti-tumor, anti-HIV and hypocholesterolemic agent for the treatment of various chronic disorders, such as neoplastic, neurological, hepatic, cardiovascular, pulmonary,

Abbreviations: μm, micrometer; °C, degree Celsius; Ac, acetone; AcN, acetonitrile; AUC, area under curve; BCS, Biopharmaceutics Classification System; CC, calibration curve; CFR, CO_2 flow rate; cm, centimeter; C_{max}, maximum plasma concentration; CO_2, carbon dioxide; CUR, curcumin; DLS, dynamic light scattering; DSC, differential scanning calorimetry; FT-IR, Fourier Transform infrared spectroscopy; g, gram; h, hour; HPLC, high performance liquid chromatography; HQC, high quality control; IS, internal standard; KV, kilovolt; L, liter; LQC, low quality control; Me, methanol; min, minute; mL, millilitre; MPa, millipascal; MQC, middle quality control; ng, nanogram; nm, nanometer; *P*, pressure; pA, picoampere; Pa, pascal; P_c, critical pressure; PDI, poly dispersity index; P-gp, P-glycoprotein; QC, Quality Control; Q-TOF-MS/MS, quadrupole-time of flight-mass spectrometry; RESS, rapid expansion of supercritical solutions; s, second; S.D., standard deviation; S. No., serial number; SAS, supercritical anti-solvent; SC, solution concentration; $ScCO_2$, super critical carbon dioxide; SFR, solution flow rate; SGF, simulated gastric fluid; *T*, temperature; T_c, critical temperature; T_{max}, time to get maximum plasma concentration; UPLC, ultra performance liquid chromatography; USP, United States Pharmacopoeia; V, volt; v, version.

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metabolic and psychological diseases [17–19]. To overcome the above-mentioned bioavailability problems for a BCS class IV molecule, various nanoparticle-based drug delivery approaches for curcumin (CUR) have been reported by research scientists [20–22]. Recently, the use of a supercritical fluid as a solvent (rapid expansion of supercritical solutions; RESS) or an anti-solvent (supercritical anti-solvent; SAS) for poorly-aqueous soluble drugs has been set up as a feasible technique for their size reduction to micro or nanoscale. Supercritical carbon dioxide ($ScCO_2$) is commonly used as a supercritical fluid in SAS and RESS processes due to its advantages, such as mild critical temperature and pressure (T_c = 31.1 °C, P_c = 7.38 MPa), low viscosity, low surface tension, low cost, non-toxicity, non-flammability, strong solvent power and safety in environmental considerations.

Due to low solubility of most drug molecules in $ScCO_2$, use of RESS process is mostly limited, whereas, amorphization [23,24], recrystallization [25,26], micronization [27–29] and nanonization [23,24,30] through SAS process have been successfully achieved. SAS is a one-step efficient process leading to completely dry, smaller particles with narrow size distribution, controlled crystals with preferred morphology and better flow-ability, and organic solvent-free product justifying their industrial applications. By reducing the size and increasing the surface area, SAS process can improve a drug's solubility, dissolution rate, and stability in physiological fluid, which may ultimately reduce the dose, dosing frequency and associated toxic effects along with bioavailability enhancement of the drug. CUR also undergoes photodegradation when exposed to light, in solution as well as in solid form [17], placing difficulty in its handling during formulation development process in an open environment. As SAS process operates in a closed environment, it can solve the above-mentioned formulation problems.

Our research aimed to improve the oral bioavailability of CUR by reducing its particle size to the nanometric range using SAS process, on combining with Tween 80 as a solubilizer and permeation enhancer [31,32]. In this study, the effect of different organic solvents and process variables of SAS process on the particle size and size distribution of CUR nanoparticles was investigated.

2. Materials and methods

2.1. Materials

Curcumin (purity > 99%) was purchased from Sigma–Aldrich, St. Louis, MO, USA. Acetone (LC/MS grade), methanol (HPLC grade), acetonitrile (LC/MS grade), sodium lauryl sulfate (extra pure grade) and Tween 80 (extra pure grade) were obtained from S.D. Fine Chemicals Pvt. Ltd. (Mumbai, India). Carbon dioxide (CO_2) (purity 99.8%) was purchased from Laser Gases Pvt. Ltd. (New Delhi, India). Salbutamol (IS; internal standard) was gifted by Ranbaxy Laboratories Ltd., Gurgaon, India. Solvents used for the ultra-performance liquid chromatographic (UPLC) method were purchased from Merck (Darmstadt, Germany). MS grade ammonium acetate was obtained from Fluka Analytical (Sigma–Aldrich, The Netherlands). Milli-Q water was produced in-house in the laboratory by Milli-Q water purification system (MA, USA). All chemicals were used as received without further purification.

2.2. LC/MS instrumentation and conditions

2.2.1. Ultra-performance liquid chromatographic condition

Ultra-performance liquid chromatography (UPLC) was performed with a Waters ACQUITY UPLC™ system (S. No. F09 UPB 920 M; Model Code# UPB; Waters, MA, USA) equipped with a binary solvent manager, an auto-sampler, column manager, and a tunable MS detector (Synapt; S. No. JAA272, Micromass Ltd., UK). Chromatographic separation was performed on a Waters ACQUITY UPLC™ BEH C18 (2.1 mm × 100 mm; 1.7 μm) column at a temperature of 40 °C. The mobile phase for UPLC analysis, consisting of acetonitrile:10 mM ammonium acetate (90:10 v/v), was degassed and isocratic elution was performed at a flow rate of 0.2 mL min^{-1}. Ten microliter of the sample solution was injected in each run with a total chromatographic run time of 4.0 min. Data acquisition, data handling, and instrument control were performed by Empower® Software v 1.0.

2.2.2. Preparation of stock solution, calibration standards and Quality Control (QC) samples

The required amount of CUR and IS was dissolved in methanol to prepare the standard stock solution having a concentration of 50 μg mL^{-1}. Different working concentrations of CUR were prepared by diluting the stock solutions with methanol. Calibration curve (CC) was prepared by spiking 2% (w/v) aqueous analyte (CUR) in a concentration range of 50 –0.048 μg mL^{-1} in blank plasma (20 μL CUR in 980 μL blank plasma) to yield final concentration ranging from 1000 to 0.97 ng mL^{-1}. Quality control samples were prepared independently in the same way as discussed above at three levels: 500 ng mL^{-1} (HQC, high quality control), 200 ng mL^{-1} (MQC, middle quality control), and 1 ng mL^{-1} (low quality control (LQC)). All the solutions were stored at −20 °C until analysis.

2.2.3. Plasma sample preparation

A fixed concentration (50 ng mL^{-1}) of IS (50 μL) was mixed with 500 μL of plasma sample and vortexed with 200 μL of 10 mM ammonium formate solution at 300 rpm for 5 min. To this mixture, 5 mL of extraction mixture (ethyl acetate:chloroform: 9:1 (v/v)) was added and kept in a reciprocating shaker for 20 min at 100 rpm. Further, it was centrifuged at 1500 rpm for 10 min at 4 °C. Supernatant (4 mL) was carefully transferred into a glass test tube and evaporated to dryness using a centrifugal evaporator (Model MV100, S. No. 026258, Tomy Medico Co., Ltd., Japan). Then, the dried residue was reconstituted with 500 μL of mobile phase and a 10 μL aliquot was injected into the LC/MS system for the quantification of CUR.

2.2.4. Quadrupole-time of flight-mass spectrometry (Q-TOF-MS/MS) conditions

MS detection was performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. The nebulization gas was set to 500 L h^{-1}, the cone gas was set to 50 L h^{-1} and the source temperature was set to 100 °C. The capillary voltage and sample cone voltage were set to 2.5 KV and 50 V, respectively. The Q-TOF Premier™ was operated in V mode with resolution over 32,000 mass with 1.0 min scan time, and 0.02 s inter-scan delay. Argon was employed as the collision gas at a pressure of 5.3×10^{-5} Torr. Quantitation was performed using Synapt Mass Spectrometry (Synapt MS). The transitions occurred at m/z values of 367.0 → 217.1 and 240.1 → 148.0 for CUR and salbutamol (IS), respectively. The optimum values for compound-dependent parameters such as trap collision energy and transfer collision energy were set to 17 and 4 V, respectively, for fragmentation. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using the MassLynx v 4.1 software.

2.3. Supercritical anti-solvent process

The schematic presentation of SAS process (Waters SFC, USA (412) 967-5655, S. No. 3636971) is shown in Fig. 1(A). It consists of a heat exchanger (TharSFC, USA, model 4.2A, 1000VA, S. No.

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