

## Effect of Operating Parameters on Yield and Anti-Oxidative Activity of Puerarin in Supercritical Process

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**Abstract:** Puerarin particles were prepared in the process of solution-enhanced dispersion by supercritical CO<sub>2</sub> (SEDS), where the dichloromethane was employed as an organic non-solvent to increase the saturation ratio of the initial puerarin solution in ethanol. A 2<sup>3</sup> factorial experiment was designed to investigate and identify the relative significance of the processing parameters on the yield of puerarin. In the range of the parameters studied, the yield increased with an increasing non-solvent/solvent ratio, and decreasing puerarin concentration or flow rate. The anti-oxidative activities of puerarin prepared under different parameters were also investigated, and the results show that there is no significant difference. The results indicate that the SEDS process combined with non-solvent could produce puerarin nanoparticles with a higher yield.

**Keywords:** Puerarin, yield, anti-oxidative activity, supercritical CO<sub>2</sub>.

### 1. Introduction

Bioavailability of poorly water-soluble pharmaceutical compounds is limited by their solubility and dissolution rate. The dissolution rate of these drugs can be improved by decreasing particle size to increase the surface area, and/or decrease crystallinity [1-4]. Another field where small drug particles are required is the pulmonary drug administration [5-7].

Various micronization methods have been developed to produce drug - micro-/nano-particles. Traditional micronization processes such as crushing, grinding, milling and spray drying can result in wide size distribution, thermal denaturing, excessive surface change or roughness and hence limit good control of properties of the active ingredients as well as the physical chemistry [8-10].

Supercritical fluids (SCF) have revealed great potential in particle engineering. The application of supercritical CO<sub>2</sub> to micronize pharmaceutical compounds has proved to be an effective route due to its mild critical conditions (T<sub>c</sub>=304.1K, P<sub>c</sub>=7.38 MPa), non-toxicity, non-flammability, and lower price [11-15]. Especially, the Supercritical Anti Solvent process (SAS) can be considered as a most suitable one for pharmaceutical compounds, since it combines the high power solubility of the organic solvents and the low solubility of the drugs in supercritical CO<sub>2</sub>, to

determine the precipitation of drugs from their organic solutions. It avoids most of the drawbacks of conventional micronization techniques and presents many advantages for drugs, like the mild operating temperature, very low/no solvent residue, efficient separation and being environmentally benign [16-20].

In a SAS process, generally the drugs were dissolved in organic solvents, and then contacted with supercritical CO<sub>2</sub>. Consequently the drugs are precipitated due to which a higher super-saturation is produced by the mutual diffusion between organic solvents and supercritical CO<sub>2</sub>.

Puerarin is an isoflavone isolated from a Chinese herbal medicine, Radix Puerariae Lobatae which is the dried root of *Pueraria labata* (Willd.) Ohwi. It has been reported to have comprehensive pharmacological action in treatment of diabetes and cardiovascular diseases, but its clinical applications have been hindered by its low solubility and dissolution rate [21, 22].

In our previous study [23], an organic non-solvent, dichloromethane was employed in SEDS process to prepare puerarin fine particles, the effect of operating parameters on the surface morphology, particle size and particle size distribution were investigated. In this study, we attempt to study the effect of operating parameters on yield of puerarin in SEDS process, and also investigate the anti-oxidative activities of puerarin prepared under different conditions.

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## 2 Experimental Part

### 2.1. Materials

Standard puerarin in HPLC grade (purity  $\geq 99.0\%$ ) was purchased from the International Laboratory (USA). Puerarin, with a purity of 99.3%, was purchased from the Nanjing TCM Institute of Chinese Medical Material (Nanjing, China). CO<sub>2</sub> with a purity of 99.9% was supplied by Hong Kong Specialty Gases Co., Ltd. (Hong Kong). All other compounds were of analytical purity.

### 2.2. Methods

#### 2.2.1 Micronization of Puerarin by SEDS Process

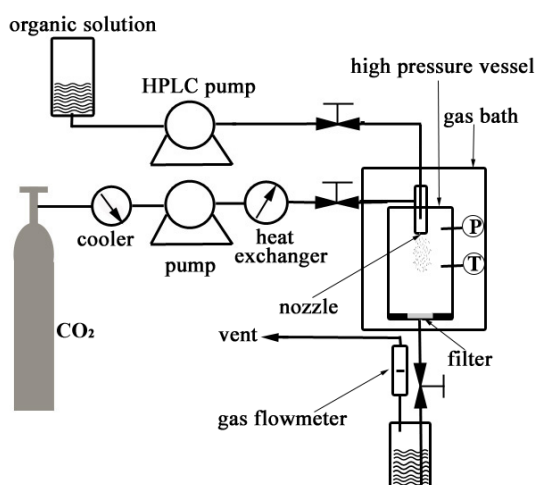


Figure 1 Schematic diagram of Apparatus for SEDS process

Puerarin was dissolved in a certain volume of ethanol, and an organic non-solvent of puerarin, DCM, was added to the solution of puerarin in ethanol to obtain a homogeneous solution with a higher saturation ratio.

Figure 1 shows a schematic diagram of the apparatus used for the SEDS process, which consists of three major components: a CO<sub>2</sub> supply system, an organic solution delivery system and a high pressure vessel with a volume of 1000 ml.

In the SEDS process [24-31], the CO<sub>2</sub> fed from a CO<sub>2</sub> cylinder was cooled down to around 0 °C by a cooler in order to ensure liquefaction of the gas and also to prevent cavitations. A high pressure meter pump was then used to deliver liquefied CO<sub>2</sub> to the high pressure vessel, and a heat exchanger was used to preheat the liquefied CO<sub>2</sub> to the desired operating

temperature after it left the pump head. The high pressure vessel was incubated in a gas bath to keep the temperature constant during the experiment. When the desired pressure in the vessel was reached, a steady flow of CO<sub>2</sub> was maintained, and the system pressure was controlled by adjusting a downstream valve and monitored by a pressure gauge to keep the pressure constant. When the desired pressure and temperature were stabilized, the puerarin solution was delivered into the high pressure vessel through a stainless steel coaxial nozzle using an HPLC pump. During the process, the pressure, temperature and flow rate of CO<sub>2</sub> were kept at 12 MPa, 306 K and 25 standard liters per hour (NL·h<sup>-1</sup>), respectively. When the spraying was finished, fresh CO<sub>2</sub> was used to wash the products for about 30 minutes in order to remove the residual organic solvent. During the process of washing, the operating conditions of the system were maintained as described before. After washing, the high pressure vessel was slowly depressurized and the products were collected for characterization.

#### 2.2.2 Experimental Design

Level	Code	A	B	C
High level	+1	2:1	1.0	1.0
Centre point	0	1:1	0.75	0.75
Low level	-1	1:2	0.5	0.5

Where A: Non-solvent/solvent (DCM/ethanol, v/v); B: Final puerarin concentration (%); C: Flow rate of puerarin solution (ml·min<sup>-1</sup>).

By analyzing the SEDS process, the key variables were identified as follows: ratio of non-solvent/solvent, puerarin concentration and flow rate of the solution. To investigate the influence and significance of the three variables and their interactions on the surface morphology and particle size, a 2<sup>3</sup> factorial experiment was designed and conducted, as shown in Table 1. The levels of the variables were determined on the basis of pilot experiments. In addition, three experiments at the central point were carried out to estimate the variances in the process. Analysis of variances was applied to the experimental data using the MINITAB software version 15. Additional experiment was carried out to confirm the experimental conditions that generated superfine particles identified in the above factorial experiments.