# A STUDY ON MICRONIZATION OF PHYTOSTEROL BY THE RESS TECHNIQUE WITH SUPERCRITICAL $CO_2$

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**Abstract**

The bioavailability of pharmaceutical substances can be improved by micronizing the particles. After micronized the particles have greater specific surfaces which lead to better solubility.. In this paper, the micronization of phytosterol particles is researched by the RESS process(the Rapid Expansion of Supercritical Solutions). The relationships between processing parameters and the final size of the particles were studied. The impact of the preexpansion pressure ($P_o$=15, 20 and 25 MPa), preexpansion temprature ($T_o$= 318 and 333K), nozzle temperature ($T_n$=398, 423 and 448K ) and nozzle dimension on the particle size of precipitated phytosterol were studied. The morphology and size of particles were studied through SEM. Amorphous particles ranging from 1 to 20 μ m were obtained by RESS.

***Key Words:*** Phytosterol; RESS; micronization; supercritical $CO_2$

## 1.Introduction

Phytosterols, in particular their fatty-acid mainly derived from phytoesters, are known for their blood cholesterol lowering effect. They are used widely in atherosclerosis treatments or sometimes as an additive for cosmetic active substances[1]. However, some functions of phytosterols are often limited because of their poor solubility in aqueous media. Reducing the particle size by the rapid expansion of supercritical solutions (RESS) can enhance the solubility and so improve the bioavailability of phytosterols[2].

The RESS process involves a rapid volume expansion[3]. A supercritical fluid (usually $CO_2$) saturated with the substrates is depressurized through a heated nozzle into a low pressure chamber. Then an extremely fast expansion of the solution composed of the supercritical fluid and solute leads to high supersaturation, and subsequently causes an extremely rapid nucleation of the solute in form of very small particles. Advantages of the RESS process include mild operating conditions and the production of pure fine particles which are solvent free [4].

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The aim of this work is to analyze the influence of operating variables on the phytosterol particles size. The particle morphologies and sizes were studied through SEM. In addition, before and after RESS-processing, dissolution rates of phytosterol particles in aqueous media were researched and the bioavailability of RESS processed microparticles was compared to usually available phytosterol.

## 2.Experimental techniques

### 2.1 Apparatus and procedure

A multifunctional equipment has been devised on the base of a HA121-50-01 Supercritical Fluid Extraction Equipment (Haian Supercritical Fluid Extraction Ltd., Nan Tong, P. R. China). It can realize some microparticles preparation process including the RESS and the SAS(Supercritical Antisolvent) processes. In this paper, only the RESS part was used.

Fig. 1 shows schematically the apparatus used for the RESS. Carbon dioxide was liquefied in a cooling device, and pressurized to the desired pressure and preheated to extraction temperature through a heat exchanger. Then the supercritical $CO_2$ was charged into a stainless steel extraction autoclave packed with the solute phytosterol. Here the supercritical $CO_2$ was saturated with phytosterol. Then the saturated supercritical solution was expanded through a thermostated capillary nozzle with an inner diameter of 40 μ m to atmospheric conditions (298.1k, 0.1MPa ). The nozzle must be preheated to avoid plugging by solutes precipitation. Since the pressure was decreased sharply, fine particles of even particle size were formed.

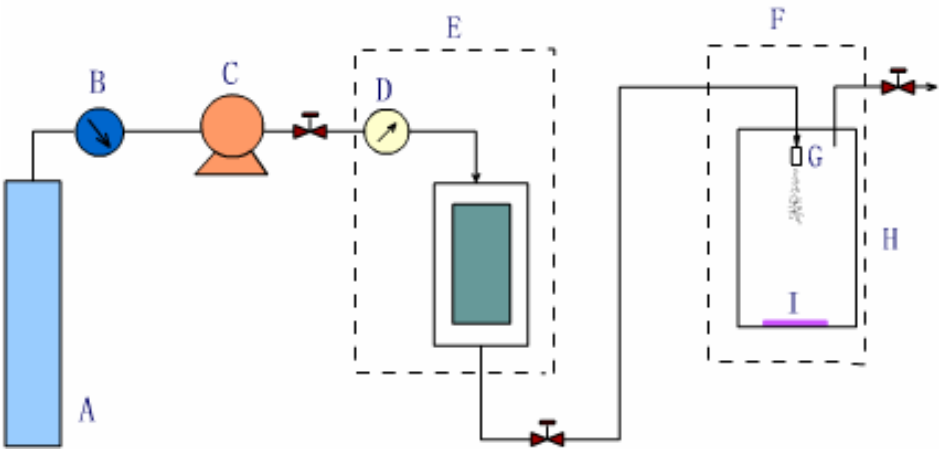



Fig.1. the Ress equipments. A, $CO_2$; B, cooling device; C, pump; D, heat exchanger; E, extraction unit; F, precipitation unit; G, thermostated vessel and capillary nozzle; H, low pressure expansion chamber; I, particles acceptor.

### 2.2 Analytical procedures

The morphologies and particle sizes of phytosterol particles were studied by the photomicrographs taken by a HITACHI Ltd. S-2150 Scanning Electron Microscope (SEM). Samples were sputter-coated for 60s with gold-palladium.

The dissolution rates of phytosterol particles treated und untreated with the RESS

process were studied through their respective UV absorbancy differences with time when dissolved in distilled water. Absorbancy is determined by a TU-1901 Spectrophotometer produced by General Analysis Instruments Ltd., Beijing (P.R.China).

### 2.3 Reagents and materials

Phytosterol with a minimum purity of 95% was obtained from Tian Wei Medicine Industry Ltd., Shan Xi (P. R. China). Carbon dioxide (99.99%) was provided by Minhang Gas Supply Station, Shanghai (P. R. China).

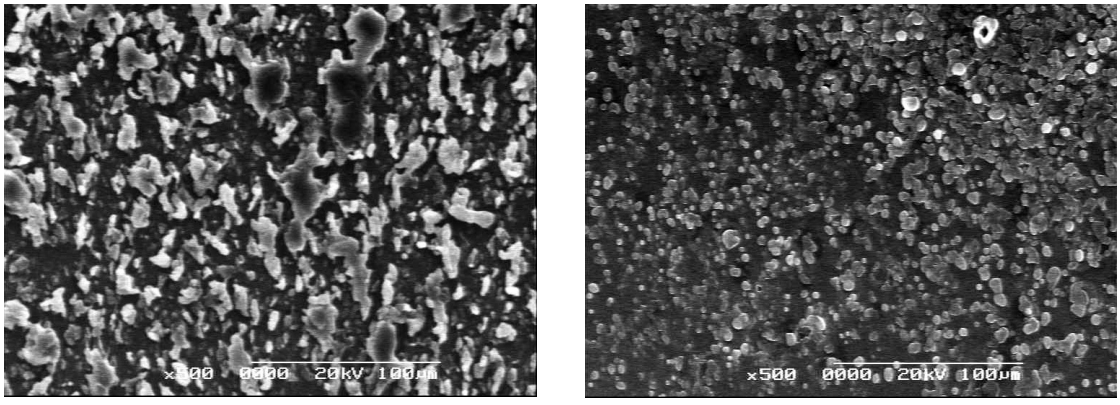
## 3.Results and discussion

A series of experiments was performed at different processing conditions in order to investigate the influences of operating variables on particle size. The main factors include preexpansion conditions and nozzle dimension.

In this paper, RESS experiments were performed at two different preexpansion temperatures ($T_o$= 318 and 333 K) and three different preexpansion pressure ($P_o$ = 15, 20 and 25 MPa). To avoid carbon dioxide liquefying at the entry of the capillary nozzle, the nozzle temperature must above 368.2 K [5]($T_n$ = 398, 423 and 448 K). These preexpansion conditions were selected to prevent particle precipitation inside the capillary nozzle, and subsequently clogging the nozzle. The extraction temperatures and pressures equaled respectively to the preexpansion temperatures and pressures in all experiments. The $CO_2$ flow rate is kept at 10-20 kg/h.

### 3.1 Selection of nozzle size

As illustrated in Fig. 2a and 2b, a change could be seen in the morphology of the particles and also a decrase in the size of the phytosterol particles with decrased the inner diameter of nozzle (from $D_n$ = 60μ m to $D_n$ = 40μ m). So in the following studies $D_n$= 40 μ m was chosen.




(a) (b)

Fig.2. SEM micrographs of phytosterol particles at 15 MPa, $T_e$ = 318 K, $T_n$ = 423K.
(a) $D_n$= 60μ m; (b) $D_n$ = 40μ m.

### 3.2 Effect of preexpansion pressure

Experiments were carried out: at $P_o$=15, 20 and 25 MPa, at $T_o$= 318 K, $T_n$ = 398 K. Figs. 3 and 4 show the SEM micrographs of the phytosterol particles obtained at 15 and 25 MPa, respecrively. There was a significant decrease of particle size from 5 to 10~15μ m when the preexpansion pressures were reduced from 25 to 15 MPa. In all cases, the particles were amorphous with a narrow distribution of particle size. Especially when the extraction pressure was fixed at 15 MPa, narrow particle size distribution of phystерol with a average particle size of about 5μ m was obtained.

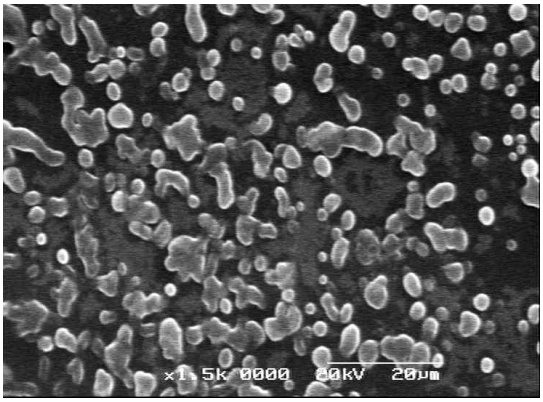



Fig.3. SEM micrographs of phytosterol particles at 15 MPa ($T_e$ = 318 K, $T_n$ = 398K,$D_n$ = 40μ m ).

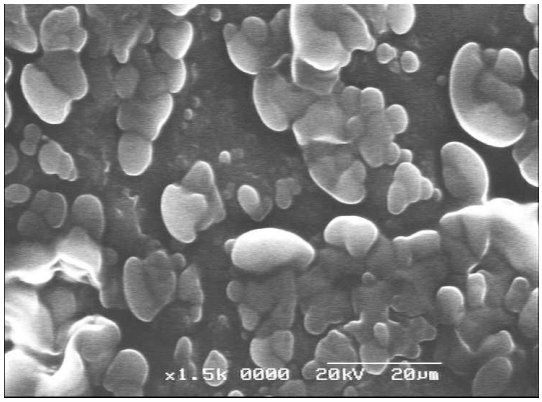



Fig.4. SEM micrographs of phytosterol particles at 25 MPa ( $T_e$ = 318 K,$T_n$ = 398K,$D_n$ = 40μ m ).

Preexpansion pressure not only influences the flow situation of the supercritical solution, but also effects directly the concentration of phytosterol in the supercritical $CO_2$. At the same extraction temperatures, lower preexpansion pressure implicates higher supersaturation of supercritical solution when expanding at the exit of nozzle. Therefore, smaller particles were produced at lower pressure.

### 3.3 Effect of preexpansion temperature

Some RESS experiments were performed at different temperatures: 318 and 333 K and the other process parameters were fixed ( $P_o$=25MPa, $T_n$ = 423 K). The increase of the preexpansion temperature from 318 to 333 K produced a a large decrease of particle size and a narrowing of particle size distribution. At preexpansion temperature 333K very small particles with mean particle size about 1μ m were obtained. This effect is illustrated in Figs. 5 and 6.

With respect to temperature effect, it can be seen that for the same preexpansion pressure and other processing variables, a higher extraction temperature means a smaller size of the obtained particles. It might be due to a more unfavorable dissolution of phytosterol in supercritical $CO_2$ at higher temperature. Higher temperature leads to higher supersaturation and higher nucleation speed, and then more crystal nucleus. Smaller size particles are formed consequently.

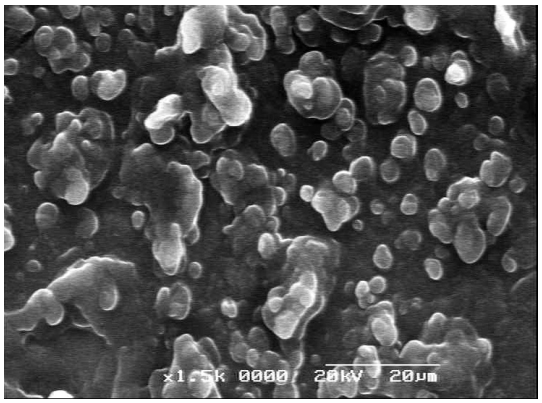



Fig. 5. SEM micrographs of phytosterol particles at $T_o$=318K ( 25MPa, $T_n$ = 423K, .$D_n$ = 40µ m )

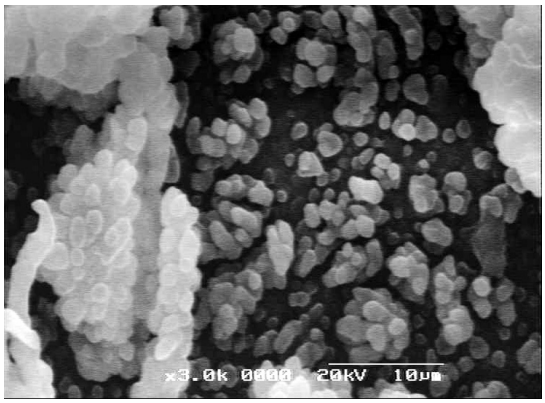



Fig.6. SEM micrographs of phytosterol particles at $T_o$=333K ( 25MPa, $T_n$ = 423K, .$D_n$ = 40µ m )

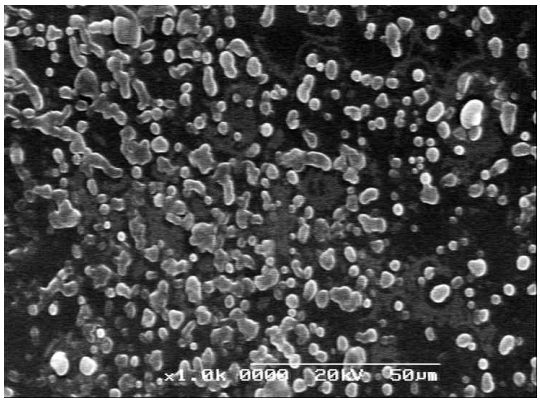



Fig. 7. SEM micrographs of phytosterol particles at $T_n$=398K ( 20MPa, $T_o$ = 333K, .$D_n$ = 40µ m )

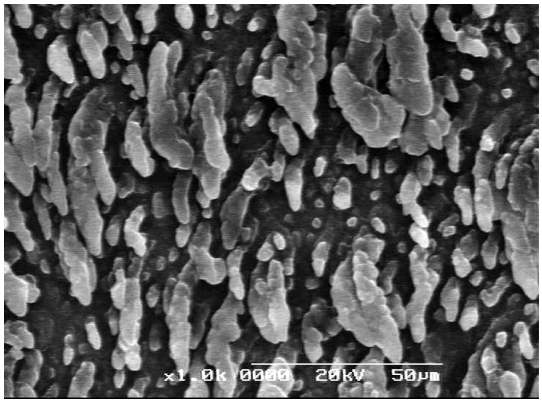



Fig. 8. SEM micrographs of phytosterol particles at $T_o$=423K ( 20MPa, $T_o$ = 333K, .$D_n$ = 40µ m )

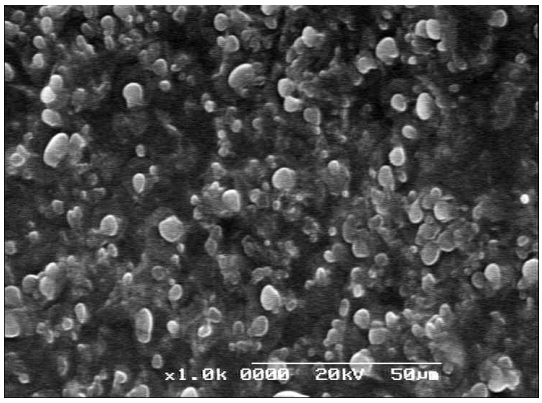



Fig. 9. SEM micrographs of phytosterol particles at $T_o$=448K ( 20MPa, $T_o$ = 333K, .$D_n$ = 40µ m )

### 3.3 Effect of nozzle temperature

A series of experiments was carried out keeping the nozzle at different temperature: 398, 423 and 448K. Figs. 7, 8 and 9 show the SEM micrographs of phytosterol obtained at different nozzle temperatures. There is no regular connection between the particle size and nozzle temperature. But it is necessary to ensure the

temperature over a certain value to prevent the precipitated particles clogging inside the nozzle.

### 3.4 The improvement of bioavailability

Dissolution comparison experiments between RESS-processed and unprocessed phytosterol have been carried out to verify the improvement of bioavailability of the RESS-produced phytosterol. 0.3 g each kind of phytosterol particles were added respectively into 200ml distilled water in a thermostated vessel (308k). Every 15min samples were tanken out of the vessel and replaced with an equal amount of distilled water. The samples were assayed photometrically at 200 nm. The absorbancy means the solubility of particles. As shown in Fig.10, the dissolution rate of the RESS-processed phytosterol is much higher and results in a noticeable higher concentration in comparison with the RESS-unprocessed particles. Particles produced by RESS realized the dissolution equilibrium 3 hours early than those unprocessed. This is an imposing demonstration that RESS-processed particles effectively improve the dissolution rate and the bioavailability in biological systems.

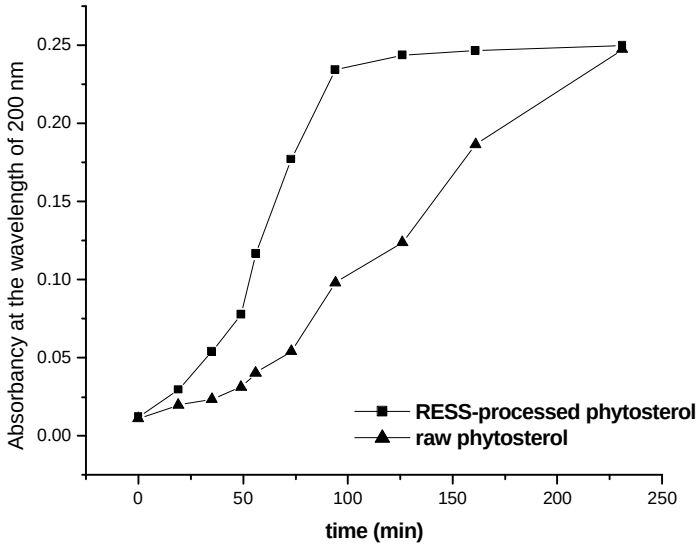



Fig.10. Effect of RESS-produced particles on the dissolution rate of phytosterol

### 4. Conclusion

Dry and fine particles of phytosterol, with size ranging from 1-20μ m were successfully produced through the RESS process.

The study of the operating parameters effect on the particle size shows that both the decrease of preexpansion pressure and increase of preexpansion temperature lead to a higher supersaturation of phytosterol in supercritical $CO_2$ and smaller particles were formed. The results presented in this paper show that the RESS processing of phytosterol leads to a significantly better dissolution rate resulting in an improved bioavailability. Thus, the RESS process is a promising method to improve the low bioavailability of insoluble or poorly soluble pharmaceuticals.

**Acknowledgements**

The authors gratefully acknowledge the financial support of the Advancement Center Shanghai of Nano-project.

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