O2-1 **Preparation of Lutein-loaded Zein Nanospheres by Supercritical CO2 Antisolvent**

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INTRODUCTION AND OBJECTIVES

Lutein is widely used as a bioactive ingredient in pharmacy and function food. However, the poor water solubility of lutein has made its use problematic in food formulation (Miguel F. et al. 2008). Moreover, low water solubility of it may be prone to reduced bioavailability. Nanosphere of lutein is a good form to improve its solubility and increase bioavailability. Zein is one of natural biological macromolecules, with excellent biocompatible and biodegradable properties. It was reported to be used as a carrier material for encapsulating, protecting, and delivering active ingredients (Lingyun C. et al. 2006). Supercritical CO_2 Antisolvent Precipitation (SAS) is a newly developed technique to make nanoparticles, which is widely used to encapsulate bioactive substances because CO2 is non-toxicity, non-flammable, and its critical temperature and pressure is low, and products are free of organic solvent remains (Martın A. et al. 2007). The purpose of this study is to prepare lutein-loaded zein nanospheres using SAS techniques, as well as to study the properties of the prepared lutein-loaded zein nanospheres.

MATERIALS AND METHODS

Materials and solvent

Food grade Zein, lutein and CO2, together analytical grade acetone, DMSO were purchased from the SJTU Shop.

Methods and characterization

SAS procedure The schematic diagram of the experimental apparatus used in this work is shown in Fig. 1.

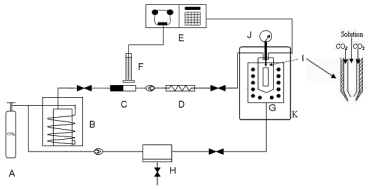

Fig.1 The flow chart of SAS equipment

A-CO_2 cylinder; B-cooling system(refrigerator); C-piston pump; D- heat exchanger; E-HPLC pump; F-solution; G-high pressure vessel(precipitator); H-separator; I-coaxial nozzle; J-pressure meter; K-gas bath

The apparatus includes three parts: a sample delivery unit, a precipitation unit and a separation unit.The sample delivery unit is composed of two pumps: one for CO2, the other for solution. It has entryways from which CO2 and liquid solution can be pumped into the vessel, separately. The inner structure of a nozzle is coaxial shown in Fig.1 B. The solution and CO2 are sprayed into the vessel through the inner tubule (diameter is 0.2mm) and outside part (diameter is 1mm), respectively. The precipitation unit is a 200ml vessel with view windows. The vessel is heated by the air bath, the temperature of which can be measured and controlled to within ±0.1 K by a temperature sensor. The vessel pressure is measured to within ±0.1 MPa by a pressure sensor. The separation unit is consisted of a separator (300ml) and a wet gas meter. An organic solvent can be separated from SC-CO2 in the separator because of lower pressure. Volumetric flow rate of CO2 can be measured by the wet gas meter.

Lutein loading Lutein content was analyzed by Ultraviolet Spectrophotometry Detector (765PC, Shanghai Spectrum Instruments, Shanghai, China). Lutein loading was calculated by the equation as follows:

Loading=lutein amount/total amount

Entrapment efficiency The entrapment efficiency of lutein was determined by analyzing the free state of lutein in the Zein nanospheres, which was calculated as follows:

Entrapment efficiency=(1 - surface lutein amount/total amount of lutein in zein nanospheres)×100%

Particle size The particle size and size distribution of samples were measured by Dynamic Light Scattering instrument (DLS, Zetasizer Nano ZS90, Malvern, Britain)

Particle morphology The particle morphology of samples was observed with a FEI SIRION 200/INCA OXFORD Field Emission Scanning Electron Microscope (Jeol Jem-7401F, SEM).

XRD analysis Samples were analyzed by X-ray diffractometer (XRD, D/max-2200/PC,Japan Rigaku Corporation, Japan) with a rotating Cu anode. The Cu Ka radiation was generated at 20 mA and 40 kV and monochromatized by a nickel filter. Diffraction patterns recorded the X-ray intensity as a function of 2θ angle covering from 2.0°to 50.0°. The scanning rate was 6°/min.

RESULTS AND DISCUSSION

The experimental conditions and results are listed in the table 1.

Effects of temperature on the loading, encapsulation ration, size of particle, and recovery can be seen from No1, No2 and No3. SEM images are also shown in Fig 2. It can be seen that the shape of the particle is sphere and particle size is around 100nm which is less than that of DLS.

Experimental conditions and results of SAS process

No	Zein concentration w/v%	Ratio of lutein and zein	Pressure MPa	Temperature ℃	Flow speed of solution ml/min	Results			
						Recovery%	Loading %	Encapsulation ratio %	Size nm
1	2.0	1：24	10	32	1	60.5	2.14±0.09	75.22±1.25	244
2	2.0	1：24	10	40	1	56.0	2.36±0.08	73.65±1.54	299
3	2.0	1：24	10	45	1	58.8	2.98±0.15	67.71±0.70	355
4	2.0	1：24	12	45	1	63.8	2.58±0.13	69.30±2.38	255
5	2.0	1：24	15	45	1	67.2	1.58±0.12	83.15±1.48	243
6	1.5	1：18	10	45	1	51.7	5.87±0.10	60.38±1.24	205
7	1.0	1：12	10	45	1	45.1	8.07±0.14	41.34±1.97	206
8	1.0	1：12	10	45	0.5	42.8	7.59±0.12	46.38±2.01	198
9	1.0	1：12	10	45	1.25	49.0	7.01±0.19	34.44±0.98	Aggregate

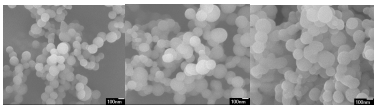

Fig.2 SEM images of nanocapsules at various temperatures 10MPa, 2%, 4kg/h, 1ml/min (A)32□; (B)40□; (C)45□

Effects of pressure on the loading, encapsulation ration, size of particle, and recovery can be seen from No3, No4 and No5. SEM images are also shown in Fig 3. It can be seen that the shape of the particle is sphere and particle size decreased from 355nm to 243nm.

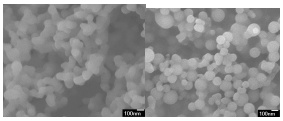

Fig.3 SEM images of nanocapsules at various pressure 45□, 2%, 4kg/h,1ml/min (A)12MPa; (B)15Mpa

Effects of the concentration of zein solution on the loading, encapsulation ration, size of particle, and recovery can be seen from No4, No5 and No6. SEM images are also shown in Fig 4. It can be seen that the shape of the particle is sphere. However, the particles are aggregated when the concentration is 1.0mg/ml.

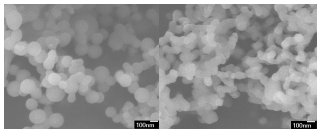

Fig.4 SEM images of nanocapsules at various zein concentration 10MPa, 45□, 4kg/h, 1ml/min (A) 1.5%; (B) 1.0%

Effects of the solution speed on the loading, encapsulation ration, size of particle, and recovery can be seen from No7, No8 and No9. SEM images are also shown in Fig 5. It can be seen that the shape of the particle is sphere. However, the particles are severely aggregated when the solution speed is 1.25ml/min.

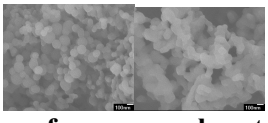

Fig.5 SEM images of nanocapsules at various sulution rate 10MPa, 45□, 1%, 4kg/h (A) 0.5ml/min; (B) 1.25ml/min

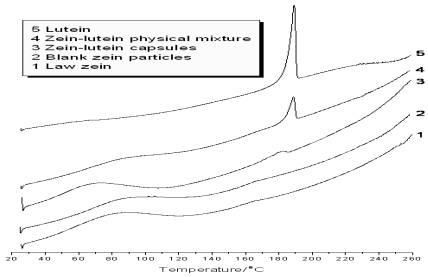

Fig.6 DSC thermogram of law materials and products (1-law zein; 2-blank zein particles; 3-zein/lutein nanocapsules; 4-zein/lutein physical mixture; 5-lutein)

Fig.6 shows the DSC pictures of different samples. We can infer that lutein may be existed in crystal form in the zein nanospheres.

Fig.7 shows the XRD pictures of different samples. We can know that lutein was embedded in the zein nanosphers.

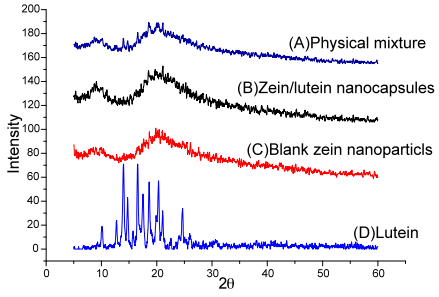

Fig.7 XRD patterns of (A) physical mixture of zein nanoparticles and lutein, (B)zein/lutein nanocapsules, (C) blank zein nanoparticles, (D)lutein

CONCLUSION

Lutein-loaded nanospheres were prepared by SAS technique. Temperature, pressure, ratio of lutein and zein, solution flow rate can influence the size, drug loading and encapsulation efficiency of the lutein/zein nanospheres. Zein nanospheres with drug loading of 5.87% and encapsulation efficiency of 60.38% were prepared under the temperature of 45□, pressure of 10MPa, ratio of lutein and zein of 1: 18, solution flow rate of 1mL/min. The average particle size was 205nm. The DSC thermogram and the XRD analysis showed that lutein was in the form of crystal in the lutein nanoparticles, and was embedded in the lutein/zein nanosphere.

REFERENCE

- Miguel F. et al. (2008) *Precipitation of lutein and co-precipitation of lutein and poly-lactic acid with the supercritical anti-solvent process.* Chemical Engineering and Processing, 47(9): 1594-1602.
- Lingyun C. et al. (2006) *Food protein-based materials as nutraceutical delivery systems*. Trends in Food Science and Technology, 17(5): 272-283.
- Martın A. et al. (2007) *Co-precipitation of carotenoids and bio-polymers with the supercritical anti-solvent process*. J. Supercritical Fluids, 41(1): 138-147.