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Production of natural carotene-dispersed polymer microparticles by SEDS-PA co-precipitation

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Abstract In this work, composite microparticles of poly (ethylene glycol) (PEG) loaded with natural carotene were successfully produced using solution enhanced dispersion by supercritical fluids through prefilming atomization (SEDS-PA) process. The characteristics of dispersion of carotene in the polymeric drug carrier were analyzed by color difference between carotene and PEG using optical micrographs. The morphologies and particle sizes (PSs) of the composite microparticles were studied by SEM micrographs. The precipitates were amorphous particles of PEG embedded with flake-like natural carotene crystals. The carotene degradation of the carotene/PEG composite microparticles was substantially lessened under the protection of PEG carrier in comparison with that of carotene microparticles. With the increase of the carotene content added in solution, the PSs of carotene crystals dispersed into the PEG carrier increase, the carotene loading yields in the composite microparticles (W_{c2}) obviously increased, whereas PSs of these composite particles slightly decreased. Higher operating temperature resulted in formation of bigger coalesced composite particles and the substantial increase of W_{c2}. With increase of operating pressure PSs decreased, whereas no clear dependence of W_{c2} was obtained on it.

Introduction

Research, development, and sales of drug delivery systems (DDSs) are increasing at a rapid pace throughout the world [1]. These systems can improve the drugs' therapeutic efficacy, in vitro and in vivo stability, bioavailability, targetability, and biodistribution to reduce toxicity [2]. The delivery systems of composite particles include particles of the polymeric drug carrier with the drug dispersed within it or adsorbed on its surface and drug particles coated with the polymeric drug carrier [3]. The polymer carrier protects the labile drug molecules from degradation in the gastrointestinal tract and helps in targeting therapeutic agents to the infected sites. The carrier system acts as a long circulating drug reservoir from which drugs can be slowly released over a prolonged period.

Microparticle formulations with a controlled particle size (PS) and particle size distribution (PSD) are important in developing the DDSs. Conventional pharmaceutical methods for production of the DDSs include emulsion and double-emulsion solvent extraction, liquid antisolvent, spray drying, and freeze-drying. All of these techniques are associated with the use of organic solvents which lead to high residual contents of toxic solvent in the final product and low loading efficiencies due to the partition of pharmaceuticals between the two immiscible liquid phases [4]. Besides freeze-drying produces particles of a broad-size distribution, spray-drying can result in significant loss of biological activity due to high temperature.

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Supercritical fluid precipitation (SFP) technologies that take advantage of the characteristics of supercritical fluids (SFs) to form microparticles have received increasing attention and been widely used to produce high quality particulate pharmaceuticals [5–9]. SFP technologies can precipitate micro- or even nanoparticles with a narrow PSD, reduce the residual solvent in the product to very low concentration and largely condition the final results. Of the many possible SFs, carbon dioxide is the most widely used. It has low critical points ($T_c = 304.1$ K and $P_c = 7.38$ MPa), and as an environmentally benign process solvent offers the additional benefits of being non-toxic, non-flammable, inexpensive, and can be used at a mild critical temperature suitable for the processing of thermally labile compounds.

Among all the SFP technologies, the Gas (or supercritical fluid) Anti-Solvent (GAS or SAS) process is the most often referred to. The process exploits the ability of gases or SFs to dissolve in organic liquids and to lower the solvent power of the liquid for the solid in solution, thus causing the solid to precipitate. In a general SAS/GAS process, an organic solution of solute is atomized through a nozzle into a high pressure vessel containing a near critical or supercritical fluid, causing intimate mixing of the solution and the fluid and resulting in liquid expansion and particles precipitation [10–14]. One of the key procedures in SAS/GAS process is droplet formation due to jet breakup at the exit of the atomizer [15, 16]. Smaller liquid droplets allow for an increase in the two-way mass transfer rates between the solvent and antisolvent, resulting in larger nucleation rates and smaller particles. Solution enhanced dispersion by supercritical (SEDS) fluids is a novel SFP technology developed based on the principle of SAS. In this process, a nozzle with two coaxial passages allows introducing supercritical CO_2 (SC-CO_2) and a solution of active substance(s) into the particle formation vessel where pressure and temperature are controlled. The high velocity of SC-CO_2 allows breaking up the solution into very small droplets. Moreover, the conditions are set up so that SC-CO_2 can extract the solvent from the solution at the same time as it meets and disperses the solution. SF antisolvent precipitation processes (including SEDS process) have been used for the processing of diverse materials including low molecular weight substances, proteins and polymers [17–21]. Moreover, addition of a carrier (often a polymer) to the active solution can lead to the formation of active substance-loaded micro-/nano-sized composite particles [4, 22–25].

In our previous work [26], based on the mechanisms of atomization a prefilming twin-fluid atomizer was designed and applied to the SEDS process to optimize the atomization and mixing of SC-CO_2 and solution through the impingement of dense gas (SC-CO_2) on the liquid film and the use of swirl. Ephedrine, bixin and natural carotene were successfully micronized by the SEDS through prefilming atomization (SEDS-PA) process [26–28].

In the present work, composite microparticles of PEG loaded with natural carotene were produced by the SEDS-PA co-precipitation with the aim of evaluating the efficiency of the SEDS-PA process in producing active substance-loaded polymer microparticles, examining the effect of polymer carrier on carotene degradation of carotene/polymer composite microparticles, and studying the influence of the carotene content added in solution (represented by W_{c1}, the ratio of the amount of added carotene to the total amount of added polymer and carotene, i.e. carotene/(polymer + carotene) w/w), operating temperature and pressure on the characteristics of dispersion of carotene in the polymeric drug carrier and on the yield of carotene-loading in composite microparticles (represented by W_{c2}, i.e. carotene/(polymer + carotene) w/w).

Materials and methods

Materials

Natural carotene with purity higher than 98% was separated from Dunaliella Salina of Inner Mongolia Jilantai (China) in laboratory. Dichloromethane (DCM) in analytical grade was used as the solvent. Carbon dioxide in food grade (99.97%) was used as the SF, and poly (ethylene glycol) (PEG, MW 4000) as the carotene carrier.

Methods

Preparation of particles

As indicated in Fig. 1 [26], composite microparticles of PEG loaded with natural carotene were produced using the SEDS-PA process from homogeneous PEG/carotene DCM solution. SC-CO_2 was pumped to the top of the particle formation vessel through the inner capillary of the atomizer by a diaphragm pump. Once the particle formation vessel reached steady state (temperature and pressure), the solution was introduced into the vessel by an HPLC pump through the coaxial annular passage of the atomizer. Meanwhile the SC-CO_2 continued to flow through the vessel to maintain the steady state. Precipitated composite microparticles were collected on a filter at the bottom of the vessel. The fluid mixture (SC-CO_2 plus solvent) exited the vessel and flowed to a depressurization tank where the conditions (temperature and pressure) allowed gas–liquid separation. After the delivery of liquid solution (about 100 mL) to the particle formation vessel was interrupted, pure SC-CO_2 continued to flow through the vessel for

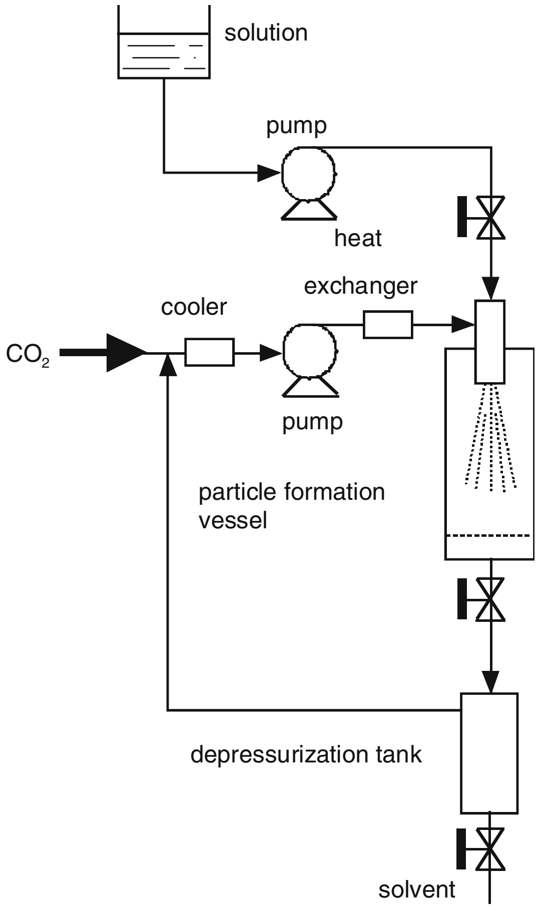

Fig. 1 Experimental facility

further 60 min to remove residual solvent from the particles. In all experiments performed the liquid flow rate was fixed at 4.0 mL/min, the CO_2 flow rate at the standard sate ($T = 273$ K, $P = 101.325$ kPa) was fixed at 45 mL/min, and the concentration of solutes (polymer plus carotene) in solution was 6 g/L.

Natural carotene is a highly unsaturated hydrocarbon compound mainly composed of β-carotene. It is easily degraded by light, heat or air. The carotene crystals unprocessed and micronized were collected in small bottles full of N_2 and stored in refrigerator to prevent from degradation.

Particle characterization

The color of PEG (transparent gray) is obviously different from that of carotene (purple), so we analyzed the characteristics of dispersion of carotene in the polymeric drug carrier by color difference between carotene and PEG using optical micrographs.

The morphologies and mean PSs of the precipitates were studied by scanning electron microscopy (SEM) (Cambridge S250-MK2). Before SEM analysis, the microparticle sample was manually dispersed onto an aluminum stub with a thin self-adhered film, and then the sample was coated with a thin layer of gold using a sputter coater.

Analyses of the carotene loaded and residual DCM in the microparticles

The yield of carotene loaded in the carotene/PEG composite microparticles was analyzed by spectro photometry. The optimal absorption peak of the natural carotene is at 450 ± 10 nm. The yield of carotene loading (W_{c2}) can be calculated by testing the absorbency of the sample solution of the carotene/PEG composite microparticles at the carotene ultraviolet wavelength of maximum absorption based on the state standard of P.R. China [29].

Solution preparation

(1) The carotene/PEG composite microparticle sample (about 60 mg) was weighed accurately, then dissolved into a little of DCM in a 100 mL brown calibrated flask and diluted to volume with cyclohexane to form the homogeneous solution 1.
(2) 5.0 mL solution 1 was transferred into a 100 mL brown calibrated flask and diluted to volume with cyclohexane to form the homogeneous solution 2.
(3) 5.0 mL solution 2 was dripped into a 50 mL brown calibrated flask and diluted to the mark with cyclohexane to obtain the homogeneous solution 3.

Calculation of the carotene loading yield

Based on the UV absorbency of the solution 3 examined at the 450 nm wavelength, W_{c2} was calculated by

$$W_{c2} = \frac{A}{W \times (V_2/V_1) \times 2500} \tag{1}$$

where A is UV absorbency of the solution 3 tested at the 450 nm wavelength, W mass of the microparticle sample (g), V_1 product of the diluted volumes of the sample (mL), V_2 product of the solution volumes transferred (mL) and 2,500 coefficient.

The residual DCM in the SEDS-PA samples was quantified using headspace gas chromatography.

Results and discussion

Characteristics of dispersion of carotene in PEG and morphologies of composite microparticles

The separated carotene used in this work has good crystallizability. Its optical micrograph presents purple flakelike

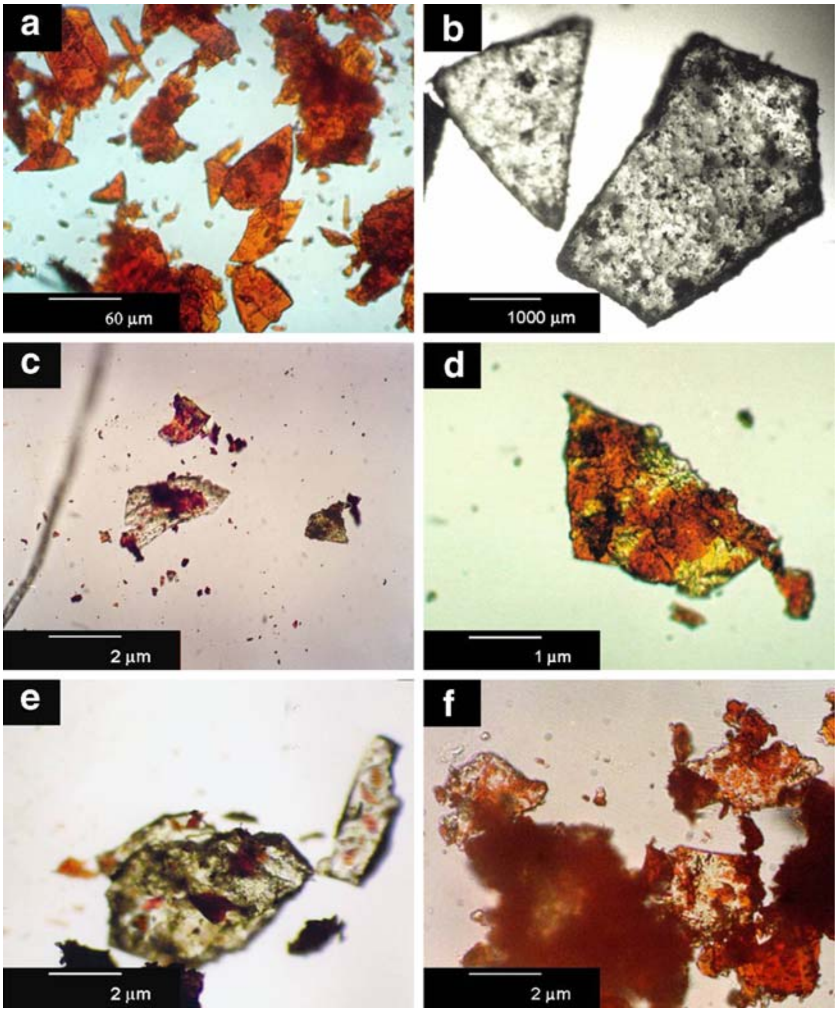

Fig. 2 Optical micrographs of unprocessed carotene crystals (**a**), unprocessed PEG4000 particles (**b**) and carotene-loaded microparticles of PEG4000 obtained by the SEDS-PA process (**c**–**f**) (**c**) $T = 308$ K, $P = 20$ MPa, $Wc1 = 50\%$; (**d**) $T = 308$ K, $P = 16$ MPa, $W_{c1} = 60\%$; (**e**) $T = 308$ K, $P = 16$ MPa, $W_{c1} = 33\%$; (**f**) $T = 308$ K, $P = 16$ MPa, $W_{c1} = 25\%$

crystals of size 20–70 μm (see Fig. 2a). Unprocessed PEG powders (Fig. 2b) are gray and transparent, and their sizes range from 1 mm to 4 mm. The optical micrographs (Fig. 2c–f) display the dispersion characteristics of carotene in the carotene/PEG microparticles obtained by the SEDSP-PA process. The SEM micrographs (Fig. 3a–d) show morphologies and the change in sizes of the composite microparticles. All the experiment results were summarized in Tables 1 and 2.

From Fig. 2c–f, it can be observed that the carotene could be successfully charged into PEG using the SEDS-PA co-precipitation and also presents flake-like crystals in the carotene/PEG composite particles. The SEM micrograph analyses demonstrated that the sizes of the composite particles obtained at the temperature lower than 313 K were substantially reduced as compared with that of unprocessed carotene (or PEG), the mean particle sizes range between 1 μm and 6 μm and the corresponding particle size distributions are narrow (see Fig. 3a–c). From Fig. 3a–d, it can also be noted that the PSD broadens with increasing PS. Due to the non-crystallizability of PEG, the composite particles tend to be amorphous that they give a variety of configurations, such as flake-like, bar-like and agglomerated, etc. (see Fig. 3a–d).

Generally most polymers could absorb a large concentration of CO_2 (about 10–40 wt%) that either swells the polymers or melts them at a temperature much below theirs melting/glass transition temperature (about 10–50 K) [30–32]. PEG used in this work has low melting/glass transition temperature (323–327 K), so its precipitates gained at a higher temperature (≥313 K) tend to be soft, sticky and easily agglomerated resulting in formation of bigger coalesced composite particles with a broad PSD, as showed in Fig. 3d.

The concentration of carotene added was found to be an important factor that influences the dispersion characteristics of carotene in the PEG microparticles. With decrease of W_{c1} the sizes of carotene crystals dispersed into the PEG microparticles decrease (see Fig. 2d–f).

The residual amounts of DCM for a selected number of the processed samples were measured and found to be less than 30 ppm in all cases.

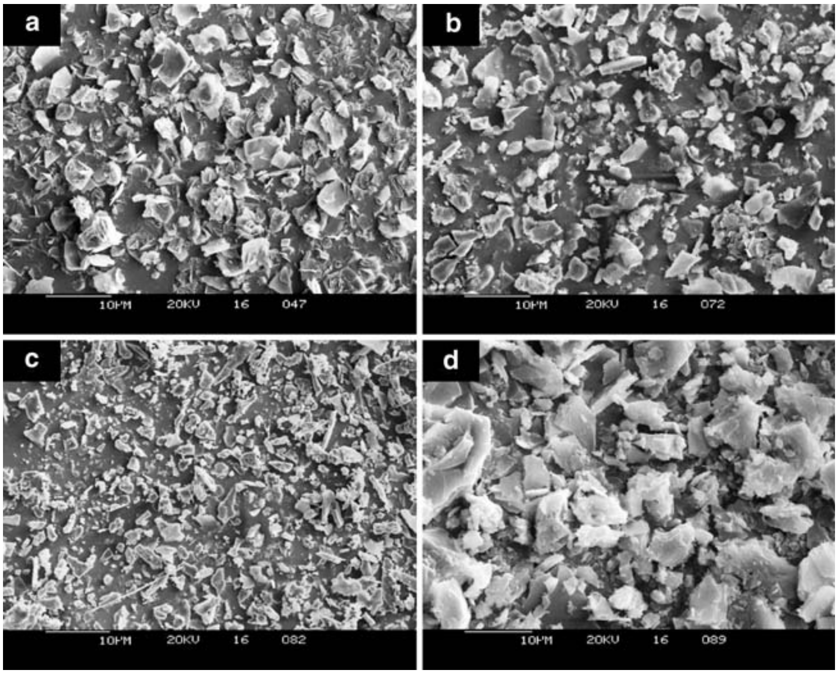

Fig. 3 SEM micrographs of carotene/PEG composite microparticles obtained by the SEDS-PA process. (**a**) $T = 308$ K, $P = 16$ MPa, $W_{c1} = 20\%$, PS = 2–6 µm; (**b**) $T = 308$ K, $P = 16$ MPa, $W_{c1} = 33\%$, PS = 2–5 µm; (**c**) $T = 308$ K, $P = 20$ MPa, $W_{c1} = 33\%$, PS = 1–3 µm; (**d**) $T = 318$ K, $P = 16$ MPa, $W_{c1} = 20\%$, PS = 3–10 µm

Table 1 Carotene loading yields and particle sizes of composite particles

T (K)	W_{c1} (%)	W_{c2} (%)				PS (µm)			
		10	14	16	20 (MPa)	10	14	16	20 (MPa)
308	17	18.2	15.2	16.8	15.7	2–10	2–8	2–6	2–4
308	20	20.3	17.8	17.2	16.0	2–10	2–7	2–6	2–4
308	25	24.8	22.7	21.5	23.2	2–8	2–7	2–5	1–4
308	33	27.3	23.7	24.9	27.9	2–7	2–6	2–5	1–3
308	50	36.3	36.0	32.3	40.4	2–7	2–5	1–4	1–3
308	60	50.8	46.3	43.0	48.9	2–6	2–5	1–4	1–2

Table 2 Effect of temperature

T (K)	P (MPa)	W_{c1} (%)	W_{c2} (%)	PS (µm)
308	16	20	17.2	2–6
313	16	20	20.3	2–8
318	16	20	21.4	3–10
323	16	20	23.4	–

Effect of PEG carrier on carotene degradation

In order to investigate the effect of PEG carrier on carotene degradation of carotene/PEG composite microparticles, we put carotene/PEG composite microparticles and carotene microparticles obtained by the SEDS-PA process into a closed dark box, used an ultraviolet lamp fixed in the box to irradiate these samples, shaked these sample bottles a moment once an hour to insure these microparticle samples degraded evenly by the ultraviolet light; and then inspected color variation of these samples and analyzed carotene contents in microparticles according the method described in section ''Analyses of the carotene loaded and residual DCM in the microparticles.'' Table 3 demonstrates the variation of carotene contents in carotene microparticles (represented by CMP) and carotene/PEG composite microparticles (represented by CCMP) with time (represented by τ) under the irradiation with an ultraviolet lamp. From Table 3 it could be noted that carotene, a highly unsaturated hydrocarbon compound, was easily degraded by ultraviolet light. But after coated with PEG, the degradation of carotene induced by ultraviolet light was substantially lessened.

The phenomenon further argued that carotene could be effectively coated with PEG carrier, and it was under the protection of the carrier that the carotene was prevented for degradation and kept active for a longer time.

Table 3 Carotene contents in microparticles irradiated by an ultraviolet lamp

τ (h)	Contents (%)		Content variation (%)	
	CMP	CCMP	CMP	CCMP
0	88.8	63.0		
30	70.6	59.8	18.2	3.2
70	58.2	54.3	12.4	5.5
140	33.7	38.6	24.5	15.7
240	14.0	25.2	19.7	13.4

Influence of the content of carotene added in solution on carotene loading yield and PS

The operating conditions of experiments to study the effect of the content of carotene added in solution (W_{c1}) were, $T = 308$ K; $P = 10$, 14, 16 and 20 MPa, respectively. From Table 1 it was observed that the influence of W_{c1} on W_{c2} was remarkable, but on PS was trivial (see Fig. 3a, b). With an increase of W_{c1} the carotene loading yields obviously increased, whereas PSs of carotene/PEG composite particles decreased slightly.

Influence of temperature on yields of carotene loading

In order to explore the effect of temperature we performed experiments in the range of 308–323 K fixing pressure at 16 MPa and W_{c1} at 20%. The results were listed in Table 2. From Table 2 and Fig. 3a, d, it could be noted that the influence of T on W_{c2} and PS was remarkable. With the increase of temperature, both W_{c2} and PS increase. As described in section ''Characteristics of dispersion of carotene in PEG and morphologies of composite microparticles,'' the melting/glass transition temperature of PEG used in this work is near to the oprating temperature. So with the increase of temperature the precipitates of PEG tend to be softened due to the effect of SC-CO_2 on it, i.e. it becomes difficult for PEG in the droplets to be hardened in the extraction of the solvent by SC-CO_2 from droplets that some of the PEG flow with SC-CO_2 into the depressurization tank resulting in the increase of carotene loading yield in the precipitated composite particles. Meanwhile the seriouse agglomeration results in precipitation of big coalesced composite particles. In experiment we indeed found that the microparticles of PEG could be hardly obtained at the temperature of 323 K; the precipitates were generally an even wax-like lamella sticking on the wall of the particle formation vessel.

Influence of pressure on the carotene loading yield and PS

The effectiveness of the SAS/GAS/SEDS techniques is connected to the solubility of the carrier solvent in the supercritical antisolvent. A measure of the liquid solvent solubility is given by the volumetric expansion of the liquid phase in the presence of the antisolvent at a given value of temperature and pressure. As CO_2 has high solubility in DCM, the volumetric expansion of DCM in SC-CO_2 could rapidly increases with increasing operating pressure at a given temperature; i.e. increasing quantities of SC-CO_2 are rapidly solubilized in it [33], which causes a significant reduction in partial molar volume and cohesive energy density of DCM, lowing its solvent power for the solid solute [34] and thus causes higher supersaturation of the expanded liquid solution resulting in precipitation of smaller particles (see Table 1 and Fig. 3b, c). But from Table 1, it can be observed that W_{c2} did not seem to depend on pressure in all the experiments performed.

Conclusions

This work has shown that the SEDS-PA process can be successfully utilized to co-precipitate composite microparticles of PEG loaded with natural carotene. The products were amorphous particles of PEG embedded with small flake-like natural carotene crystals. At the operating temperature lower than 313 K, the mean particle sizes obtained range from 1 μm to 6 μm and the corresponding PSDs are narrow. The carotene degradation of carotene/PEG composite microparticles was substantially lessened under the protection of PEG carrier in comparison with that of carotene microparticles. The concentration of carotene added to the formulation was found to be an important factor that influences the dispersion characteristics of carotene in the PEG precipitates. With decrease of W_{c1} the sizes of carotene crystals dispersed into PEG microparticles decrease. With increase of W_{c1} the carotene loading yields obviously increased, whereas PS slightly decreased. With the increase of temperature the precipitates of PEG tend to be softened due to the effect of SC-CO_2 on it, and the PEG in droplets becomes difficult to be hardened resulting in formation of big coalesced composite particles and the substantial increase of W_{c2}. Within the experiments performed, no clear dependence of W_{c2} can be obtained on pressure.

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