

SUPERCRITICAL ANTI SOLVENT PRECIPITATION OF CAROTENOIDS FROM AN EMULSION

F. Mattea, A.Martín, C. Azpeleta, A. Serna, M.J. Cocero

High Pressure Process Research Group, Department of Chemical Engineering and Environmental Technology, University of Valladolid, 47011 Valladolid, Spain.

fmatea@gmail.com

ABSTRACT

Carotenoids have been already precipitated with the aid of supercritical fluids with successful results. The use of these carotenoids in most industrial or pharmaceutical processes requires an improvement on their dissolution rate in water, thus the particle size must be lowered to the submicro or nano size. In this work the feasibility of the supercritical anti-solvent (SAS) process to obtain β -carotene particles from an o/w emulsion consisting in: β -carotene – dichloromethane solution as the dispersed phase and water as the continuous phase is studied.

First, in order to prepare the emulsion two different surfactants were used: a combination of Span 20 and Tween 20 and a modified non-ionic starch, the o/w ratio was varied from 15% to 50%. The mean droplet size of most of the emulsion was below one micrometer. Afterwards the supercritical antisolvent process was applied to the system and the resulting suspension was analyzed. The concentration of dichloromethane in the product and the particle size distribution were measured. Also the stability of the emulsion was measured at three different temperatures and pressures to ensure the precipitation of the particles inside the droplets. The particles of β -carotene were measured by means of dynamic light scattering techniques and their mean diameter was between 200 and 600 nm.

INTRODUCTION

The main applications of carotenoids are as precursors of vitamin A and antioxidants in human diet, and as natural colorants in food, cosmetic and pharmaceutical products. Because of the nature of these applications there is a growing interest on enhancing the dissolution rate of these substances in aqueous solutions, and enable the use of them in several industrial processes. One way to enhance the dissolution rate of these particles is to increase the superficial area of the particles, by lowering their particle size.

The precipitation of carotenoids by means of supercritical fluids present several advantages over conventional techniques, regarding on its possibility to obtain relatively small particles sizes in the range of micrometers using mild-low temperatures and an inert atmosphere. However, it is not possible at the present to obtain carotenoids particles with nanometer or sub-micro particle sizes with a supercritical antisolvent process. For example, lutein and lycopene particles were precipitated in a supercritical antisolvent [1-2] process (SAS) resulting in needle type particles with a mean particle size between 5 and 80 μm . β -carotene was precipitated in gas antisolvent process [3], the particles obtained presented a platelet morphology with a particle size between 5 and 10 μm .

The use of emulsion-based techniques to precipitate natural substances, especially substances with pharmaceutical applications has also been studied intensively. Füredi-Milhofer et al. [4] crystallized aspartame by slow cooling to 5°C a w/isooctane/AOT microemulsion prepared at high temperature. The resulting particles exhibited a new crystalline form with enhanced dissolution rates and a mean size below 100nm. Trotta et al. [5] studied the precipitation of mitotane, an anticancer drug by a solvent diffusion method. In their study they used different organic solvents and the influence of emulsion droplet size on the size distribution of the drug nanoparticles. The resulting

suspension of mitotane presented particle sizes below 100nm. They also formed griseofulvin nanosuspensions with the solvent diffusion method obtaining particle sizes below 100nm and enhancing the dissolution rate of the drug in water [6].

Shekunov et al. [7] proposed the use of a combination of supercritical fluids and emulsion precipitation to take advantage of the enhanced particle size reduction, crystallinity and surface properties of the emulsion based precipitation techniques and the efficiency of large scale, continuous extraction with supercritical fluids. Their study focused on the precipitation of three substances: cholesterol acetate, megastrol and griseofulvin obtaining nanosuspensions with a mean particle size of 200nm, 200nm and 550nm respectively.

The aim of this work is to apply a process similar to the one presented by Shekunov et al. [7] to form nanosuspensions of β -carotene with a submicro or nano mean particle size. As a first stage, the formation of a sub-microemulsion with β -carotene dissolved in the organic phase was studied, where a food accepted surfactant was used and compared with a blend of two well known surfactants: tween 20 and Span 20 in order to obtain a stable emulsion with the desired droplet size. Afterward the SAS process was applied to the emulsion and the product obtained was analyzed with a dynamic light scattering technique. Also the residual solvent in the product was analyzed to observe the efficiency of the process.

MATERIALS AND METHODS

Materials.

Crystalline β -carotene with a minimum purity of 99% was kindly provided by VITATENE León (Spain), Dichloromethane purchased from Panreac Química (Spain) was used to prepare the emulsions. Tween 20 and Span 20 were purchased from Aldrich Chemical Co. Hi-Cap® 100 was provided by National Starch Food Innovation. CO₂ at 99,95% was delivered by Carburos Metálicos S.A. (Spain).

Preparation of emulsions.

β -carotene was dissolved in dichloromethane to form a solution with the desired concentration (between 0.1 and 0.25 %w/w). This solution was dispersed into an aqueous solution of the surfactant. Two main surfactants were employed, a modified starch (Hi-Cap® 100) with a 3% w/w concentration in the water phase and a blend of tween 20 and span 20. In the former, a thoroughly study of the effect of the different concentrations of each surfactant was performed in order to obtain the smaller particle size with a relatively good stability. The hydrophilic-lipophilic balance (HLB) value of the blend was varied between 8.6 and 13 and the particle size together with the change of the particle size distribution was considered as a stability proof. The emulsion was prepared mixing both solutions together with a high speed stirring mixer (IK labor pilot 2000/4) at 13750 rpm for 3 minutes to form an emulsion with a final mean droplet size between 200 and 800nm with an oil/water phase ratio between 0.2 and 1 in volume.

Precipitation process

A schematic diagram of the pilot plant used for the precipitation process is shown in Figure 1. Two diaphragm pumps (Dosapro, Spain) are used to feed the SC-CO₂ and the emulsion into the vessel. The precipitator is an isolated and jacketed AISI 316 stainless steel vessel of 1.5 L of volume. This precipitator is equipped with a concentric tube nozzle for the injection of the solution and CO₂, and is provided with a baffle plate to avoid the bypass of the emulsion and to work as a separator vessel. The pressure in the precipitator is controlled by two back pressure regulator valves placed in parallel for safety reasons. A vessel is used to achieve the separation of solvent and CO₂ after pressure release. Other elements in the pilot plant are the heat exchangers required to cool CO₂ before pumping it and for achieving the operating conditions, safety devices (safety valve and rupture disc) and instrumentation.

A typical experiment starts by pumping pure CO₂ into the precipitator. When the desired operating conditions (temperature, pressure and flow rate) are achieved and remain stable, the emulsion is fed to the precipitator. When the desired amount of emulsion has been injected, the liquid pump is stopped and only pure CO₂ is fed. The flow of CO₂ is maintained during a period long enough for the complete removal of solvent from the precipitator. After the decompression, a sample of the suspension can be sampled from the bottom of the precipitation vessel. For the precipitation process 0.5 liters of the emulsion was processed with the above described apparatus, the pressure and temperature were 100 bar and 35 °C which are usual values for a SAS process. The flow rates employed were from 0.3 to 1 Kg/h for the emulsion and 4 Kg/h for the carbon dioxide.

Emulsion analysis

The emulsion drop mean size and distribution was measured with a dynamic light scattering (DLS) instrument (Autosizer Lo-C, Malvern Instruments Ltd.). This instrument is equipped with a 4mW Diode laser (λ : 670 nm). The system detects particles sizes ranging from 0.003 to 3 μ m. Three measurements were done for each sample, half an hour after the emulsification to minimize any creaming or coalescence effect, then 3 and 6 hours after emulsification to observe the evolution and stability of the droplet mean size with time. The same evolution was studied at temperatures from 20°C to 30°C.

Suspension analysis

To evaluate the feasibility of the SAS process with an emulsion, the organic solvent content of suspension obtained from the precipitation process was measured. The suspensions obtained from the precipitation vessel were filtered with a 0.1 μ m pore size membrane (JV Omnipore™ membrane filter) and the filtered liquid was then analyzed for residual dichloromethane using gas chromatography (GC). The GC was made by means of a HP 5890A chromatograph and a Teknokroma Hayesep P column (3 m 6 1/80, 60–80 mm) with a He flow rate of 30 ml/s and an inlet temperature of 200°C. The temperature was increased from 55°C to 180°C at 8°C/min. The components were detected by TCD at 200°C.

The volume-weighted and number-weighted mean diameter of the particles in the produced suspensions was measured with the DLS instrument. The concentration of the measuring cell in the DLS instrument was adjusted by dilution with deionized water.

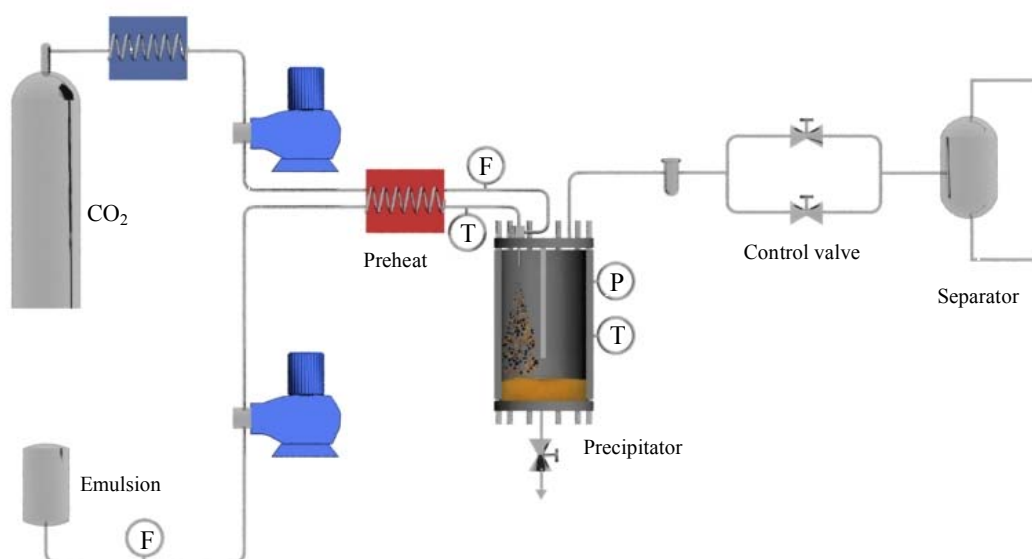


Figure 1. Precipitation process scheme. (F) flow meter (P) Pressure meter (T) Thermocouple

RESULTS AND DISCUSSION

Emulsion Formation

Two types of surfactant were tested to form a suitable emulsion for the precipitation process: a modified starch and a blend of Tween 20 and Span 20, in both cases the conditions for the preparation of the emulsion were based on the minimum final droplet size and the short-term stability of the emulsion. The different blends of Tween 20 and Span 20 are depicted in Table 1. These blends were used as surfactants in an emulsion containing deionized water and of dichloromethane with an organic phase percentage of 30% v/v. The characteristics of the resulting emulsions can be seen in Figure 2.

Table 1. Used Surfactants

Tween 20 %w/w	Span 20 %w/w	HLB
0	100	8.6
87.6	12.4	9.6
75.3	24.7	10.6
62.9	37.1	11.6
50.6	49.4	12.6
38.3	61.7	13.6

Only the emulsion prepared with with an HLB value of 12.6 presented the droplets sizes below 300 nm and none above 600nm. The objective of the whole process is to obtain nanoparticles inside the organic phase of the emulsion. Therefore, the blend of surfactants with HLB of 12.6 is the most suitable for the precipitation process.

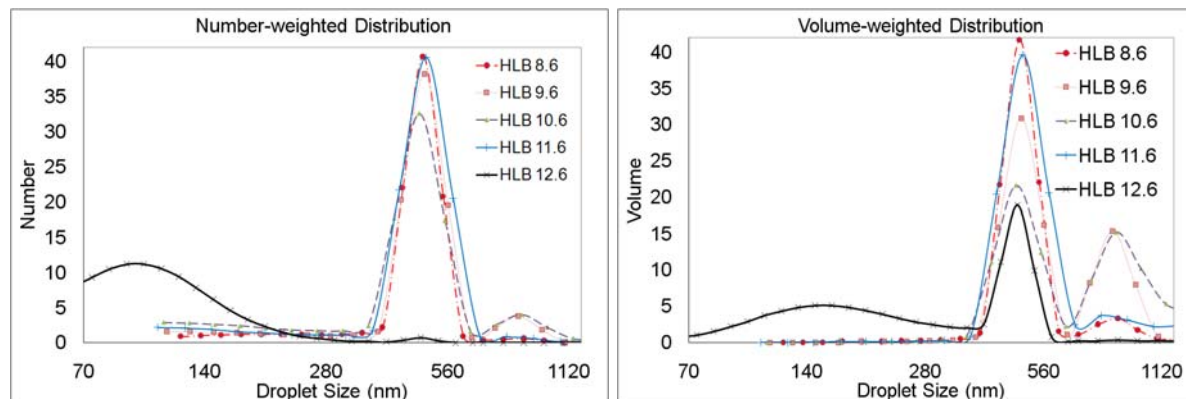


Figure 2. Number and number-weighted droplet size distribution of the different emulsions.

Emulsion Stability

Since the time to process the desired amount of emulsion is not greater than 2 hours the stability of the different emulsions was analyzed for a period of 3 hours. The droplet mean size and distribution of the emulsion were measured at 3 hours after the emulsification and the results are shown in Figure 3.

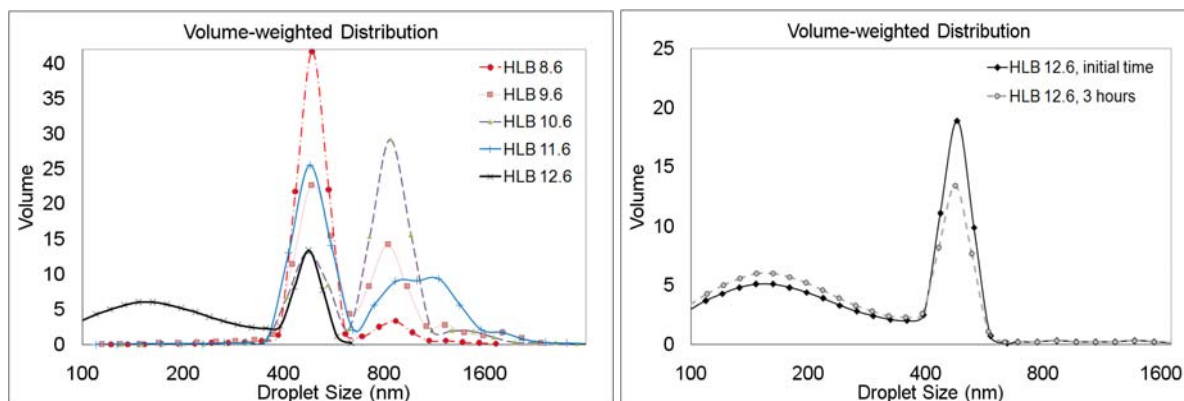


Figure 2. droplet size distribution change in 3 hours.

The droplet size distribution of the emulsion prepared with an HLB of 12.6 after 3 hours of emulsification didn't show any significant change ensuring the stability during the precipitation process time.

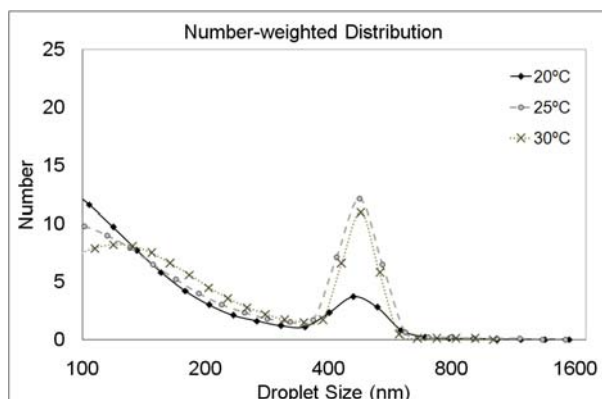


Figure 3. Effect of Temperature on droplet size distribution

The stability with temperature was also studied. The emulsion was kept at 20, 25 and 30°C for half an hour and then analyzed at the same temperature with the DLS instrument. Figure 3 shows the results of the analysis. The results indicate that an increase in temperature produces a relatively small increase in the mean droplet size. However, the mean droplet size of the emulsion at 30°C is still between 200 nm and 600 nm.

SAS Precipitation

Only the experiments carried out with an emulsion flow rate of 0.3 Kg/h produced suspensions with a concentration of DCM below 0.2%. The supersaturation of the solution representing the organic phase of the emulsion is slower than the one usually achieved in SAS experiences, mainly due to the additional diffusion of the carbon dioxide through the water phase. Thus, the mixture of the carbon dioxide and the emulsion is a very important step of the process. The correct carbon dioxide/emulsion flow rates ratio is a key factor in the precipitation of the β -carotene particles inside the emulsion droplets and to ensure the minimal content of the organic solvent.

The suspensions obtained from the precipitation processes were analyzed with the DLS instrument. Figure 4 shows the particle size distribution of a typical suspension obtained from the precipitation of an emulsion prepared with the modified starch surfactant.

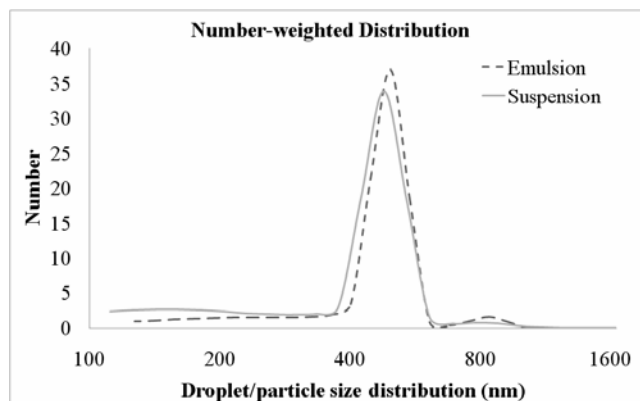


Figure 4. emulsion and suspension size distribution

CONCLUSIONS

The precipitation of sub micro particles of β -carotene inside the organic phase of an o/w emulsion, using supercritical CO₂ as an antisolvent was studied. The particles obtained have a particle size between 200 and 600nm which is lower than the particle size obtained in regular SAS processes. However, a suspension is obtained instead of dry particles so an additional step is required. The main objective of the study was to improve the dissolution rate of the carotenoid particles in water, therefore the use of a suspension would not be a complete drawback.

The stability of the emulsion containing the carotenoids was studied, and with the emulsification method proposed the emulsion resulted stable for the requisites of the precipitation process, regarding to the process time and used temperatures. With respect to the particles characterization, it is still desired to observe the physicochemical properties of the product. The liofilization of the produced suspension followed by SEM, XRD, and dissolution tests will be performed in a following study to compare the obtained particles with the original ones.

REFERENCES

- [1] Miguel F., Martin, A., Gamse, T., Cocero, M.J., *J. Supercrit. Fluids*, 36, **2006**, p. 225.
- [2] Miguel F., Martin, A., Mattea, F., Cocero, M.J., *J. Supercrit. Fluids*, 41, **2007**, p. 138.
- [3] Cocero, M.J., Ferrero, S., Miguel, F., *Proc. 4th Int. Symp. High Pressure Process Tech and Chem. Eng., Venice*, 90, **2002**, p. 22.
- [4] Füredi-Milhofer, H., Garti, N., Kamyshny A., *J. Cryst. Growth*, 198/199, **1999**, p 1365.
- [5] Trotta, M., Gallarate, M., Pattarino, F., Morel, S., *J. Controlled Release*, 76, **2001**, p 119.
- [6] Trotta, M., Gallarate, M., Carlotti, M.A., Morel, S., *Int. J. Pharm.*, 254, **2003**, p 235.
- [7] Shekunov, B.Y., Chattopadhyay, P., Seitzinger, J., Huff, R., *Pharm. Res.*, 23, **2006**, p. 196.