Precipitation of Lycopene by Continuous Supercritical Antisolvent Process.

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The re-crystallization of Lycopene from dichloromethane solutions has been studied in a continuously operating antisolvent apparatus. Influence parameters like pressure, temperature, solute concentration in the feed solution, solution to CO_2 flow ratios and mixing nozzle were tested. Successful re-crystallization of Lycopene resulted in particle sizes smaller than 1 micron achieved at 90 bar, independent from the other operation conditions. Temperature was limited with 45°C because of degradation of Lycopene at higher values.

INTRODUCTION

Carotenoides are wide spread in nature with a yearly production of 10^8 tons per year with around 600 substances of different structures. The widest used carotenoid is β Carotene. It is mainly used as natural pigment and colorant for the food and beverage industry. Another natural pigment from this carotenoid group that presents a higher antioxidant activity than β Carotene is Lycopene (see **figure 1**), whose molecular weight is 536.88 g/mol.

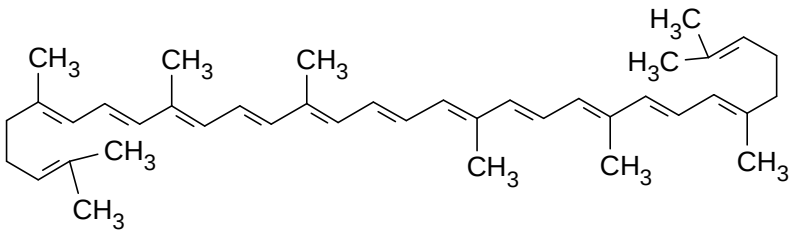

Figure 1: Lycopene structure ($C_{40}H_{56}$)

The red coloured pigment Lycopene is present in high concentrations in tomatoes, pink grapefruits, water melons, haws and certain kinds of olives. Lycopene is extremely lypophilic and has therefore the tendency to aggregate and crystallize in aqueous media. In tomatoes Lycopene is deposed in crystalline form. For industrial purposes, it is important to obtain Lycopene in micron sizes, in order to control its solubility in water and also to make its dosing much easier by co-precipitation in a polymeric matrix.

For the production of particles in micro and nano scale supercritical fluids becomes more and more important. Different processes are developed depending on the material to be powdered. The aim of this research is the study of the antisolvent process for the re-crystallisation of Lycopene from dichloromethane solutions using supercritical CO_2 as antisolvent. Beside a lot of other substances this process has already been successfully applied for particle formation of β-carotene [1-4], another main carotenoid.

I - EXPERIMENTAL

I.I. REAGENTS AND MATERIALS.

Crystalline Lycopene with a minimum purity of 99.5% was obtained from ANTIBIOTICOS, S.A. (Spain). Dichloromethane (99.5%) was provided by PANREAC QUIMICA, S.A. (Spain). Carbon dioxide (99.99%) was supplied by Carburos Metálicos, S.A. (Spain).

I.II. EXPERIMENTAL SETUP

For the re-crystallization a continuous pilot plant is used, which has been developed previously [3, 5]. The flow diagram of the continuous GAS plant is presented in **figure 2**.

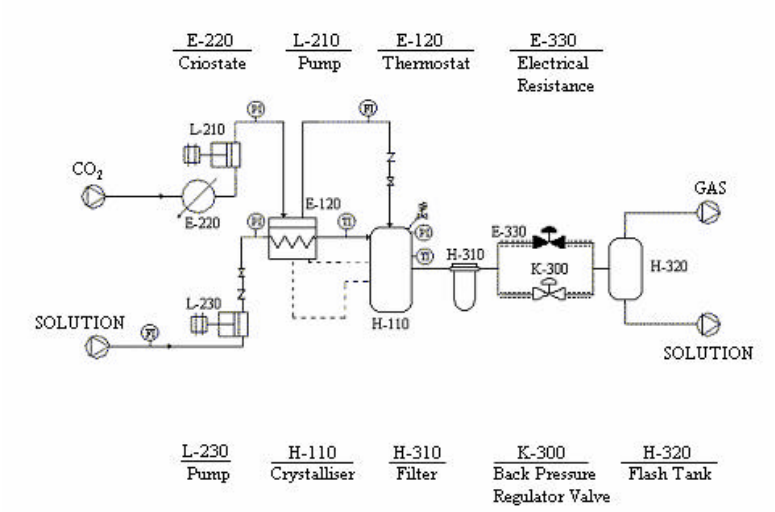

Figure 2: Flow diagram of the continuous GAS plant

The equipment uses two diaphragm pumps (Dosapro, Spain), one for CO_2 (L-210) and another one for the solution (L-230). An isolated and jacketed AISI 316 stainless steel crystallizer (H-110) with a metal porous frit (1 µm) at the exit is used to keep the pressure and perform the crystallization. Beside the porous frit at the outlet of the crystallizer also an external stainless steel filter (H-310) with a pore size of 1 µm from Headline filters (UK) is installed. A separation flask (H-320) is used to achieve the separation of the solvent and the CO_2 after the pressure release valve. The other elements are flow, pressure and temperature meters as well as heat exchangers to achieve the operating conditions for the pilot plant.

Two different mixers (see **figure 3**) were used. On one side a concentric tubes nozzle is used consisting of an outer tube of 3.2 mm (i.d.) and one inner tube of 1 mm of internal diameter (i.d.). For realizing an intensive mixing of the two fluids the inner tube ends 10 mm before the outlet of the outer tube into the crystallizer. On the other side a commercial diffuser was installed. The diffuser is an atomizing nozzle from Spraying Systems Co. (Spain). It is a stainless steel 1/4J SU 22 spray nozzle mounted inside the upper closure of the crystallizer.

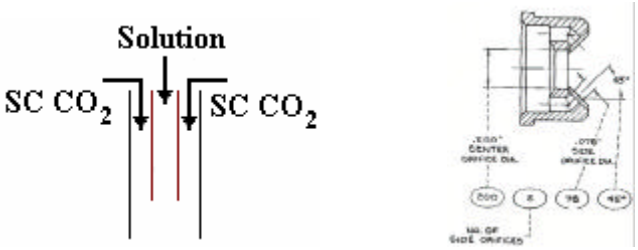

Figure 3: Concentric tubes nozzle (left) and commercial nozzle (right)

During all the experiments the GAS plant worked accurately without any plugging of the nozzle or the filter, so we can state that it presents a strong and versatile design.

I. III. ANALYTICAL METHODS.

The concentration of solutions and purity of crystals was analyzed by HPLC, using a Waters 996 Photodiode Array Detector as detection system and a Waters 600 Controller as pump. The eluent was detected at 445 nm for dichloromethane. The column was C-18, Nova-Pack, 60Å, 4 μm, 3.9×300 mm. As mobile phase 90/10 methanol–chloroform, 1 ml/min isocratic, at 293 K was used.

The photomicrographs of the crystals have been taken by a JEOL JSM-820 Scanning Electron Microscope (SEM).

I. IV. EXPERIMENTAL CONDITIONS.

Pressure and temperature: 7, 9, 12, 15 MPa and 308.2 and 318.2 ºK.
Solvent flow rate: 0.485, 0.530 kg/h.
Lycopene feed concentration: 125, 250 and 500 mg/L.
Solvent: Dichloromethane.
Mixers: 1 mm inner duct and commercial diffuser (1/4J SU 22, spraying systems)

II. RESULTS AND DISCUSSION.

Effects of pressure, temperature, initial solution concentration and mixing nozzle, have been studied. The experiments performed and its operation conditions are presented in **table 1**. SEM figures of the obtained product are presented and discussed afterwards.

II.1. EFFECT OF PRESSURE.

For studying the influence of pressure in particle size and morphology, pressure is varied at two different temperatures keeping constant the others variables. These temperatures are 308.15 and 318.15 K. For both temperatures, it was used the commercial nozzle.

Lycopene is usually precipitated into long needles or into leaves-like shape.

Nº	P **bar**	T **ºC**	Nozzle*	$Flow_{solution}$ **mL/h**	C_0 **ppm**	$Flow_{CO2}$ **Kg/h**	$\%CO_2$	Lmin **mm**	
N1	90	308.15	CN	365	500	4.3	89.9	2	Fig. 4
N2	90	308.15	CN	365	250	4.3	89.9	<1	
N3	90	308.15	CN	365	125	4.3	89.9	1	
N4	120	308.15	CN	365	500	4.3	89.9	2	Fig 5
N5	150	308.15	CN	365	500	4.3	89.9	2	Fig 6
N6	90	308.15	CN	398	500	3	85.0	<1	
N7	150	308.15	CN	398	500	3	85.0	3	
N8	70	308.15	CN	398	500	3	85.0	1	
N9	120	308.15	CN	365	500	3	86.1	3	
N10	150	308.15	CN	365	500	4.3	89.9	1	
N11	120	308.15	CN	365	125	4.3	89.9	2	Fig 13
N12	120	308.15	CN	365	250	4.3	89.9	1	Fig 12
N13	90	318.15	CN	365	500	3	86.1	1	Fig 7
N14	120	318.15	CN	365	500	3	86.1	1	Fig 8
N15	150	318.15	CN	365	500	3	86.1	1	Fig 9
N16	90	318.15	CTN	365	500	3	86.1	<1	Fig 10
N17	90	308.15	CTN	365	500	3	86.1	<1	Fig 11

Table 1. Experimental conditions and figures.
*(CN = Commercial Nozzle, CTN = Concentric Tube Nozzle)

At 308.15 K the experimental values for the variables kept constant are: 500 ppm for the Lycopene concentration in the solution, 365 mL/h for the solution flowrate and 4.3 kg/h for the CO_2 flowrate.

In **figure 4, 5** and **6** the particles obtained increasing the pressure from 90 to 120 and 150 bar are presented. As it can be seen, average particle size increases with pressure from 90 to 120 bar, and from 120 to 150, its increase is not so important. Same behavior for Nalmefene Hydrochloride is described by Adami [6], for cholesterol by Subra [7] and for paracetamol dissolved in acetone by Kröber [8], increasing particle size with pressure.

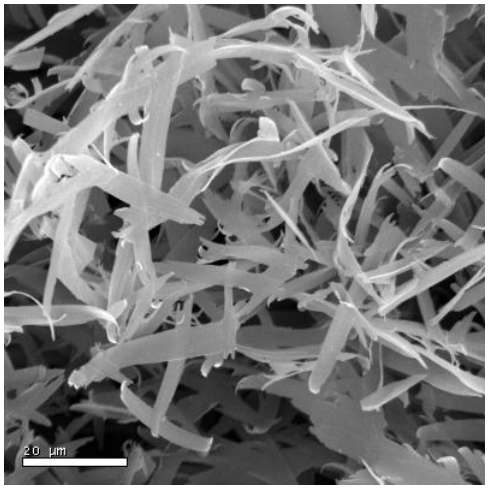

Fig. 4. N1. r = 20 microns. 750x.
dp_{mean} = 15 – 20 microns

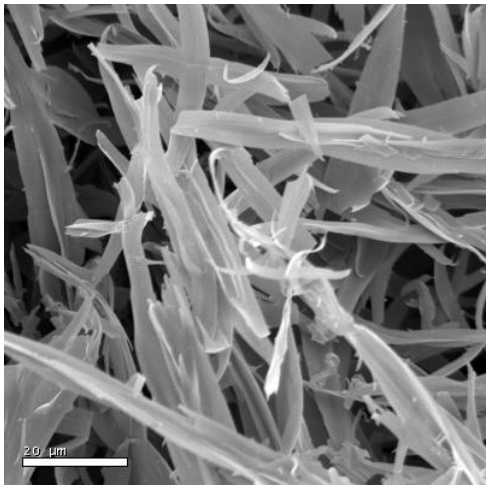

Fig. 5. N4. r = 20 microns. 750x.
dp_{mean} = 50 microns

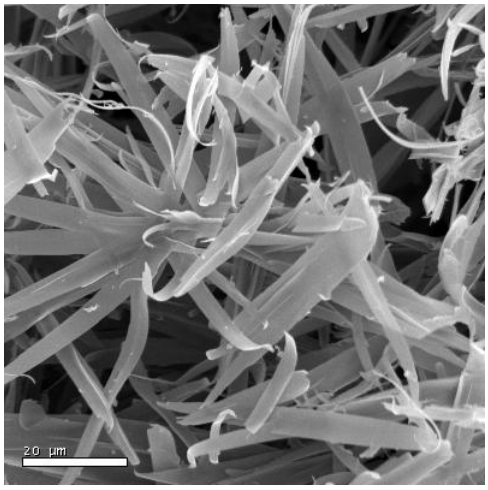

Fig. 6. N5. r = 20 microns. 750x. dp_{mean} = 50 microns

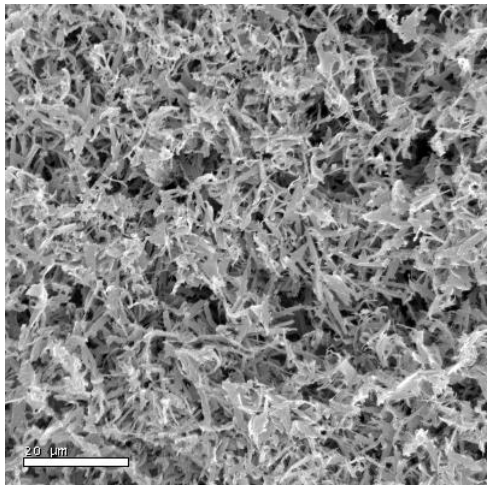

Fig. 7. N13. r = 20 microns. 750x. dp_{mean} = <10 microns

At 318.15 K the experimental values for the variables kept constant are: 500 ppm for the Lycopene concentration in the solution, 365 mL/h for the solution flowrate and 3.0 kg/h for the CO_2 flowrate.

In **figure 7**, at 90 bar and 318 K, particles smaller than 1 micron are obtained, with an average particle size close to 10 micron. In **figure 8**, at 120 bar and 318 K, particle size is increased to 60 microns, but a lot of smaller particles are present attached to the larger needles. **Figure 9** at 150 bar is similar to figure 8 at 120 bar, but particles are longer, up to 80 microns, with a higher amount of small particles attached to these needles. At 120 and 150 bars, submicronic particles are obtained.

This behaviour suggests the presence of two phases. One phase, with presents a high solvent concentration, in which, big crystals are produced due to that the growing mechanism is acting as the controlling one. The other phase is rich in antisolvent (SC CO_2) and hence, the controlling mechanism is the nucleation. As the nucleation is the controlling mechanism,

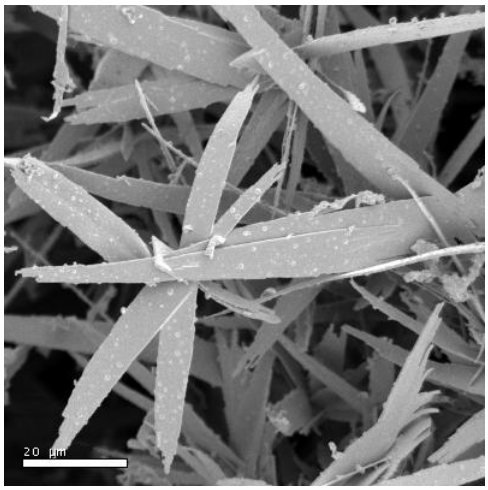

Fig. 8. N14. r = 20 microns. 750x. dp_{mean} = 60 microns

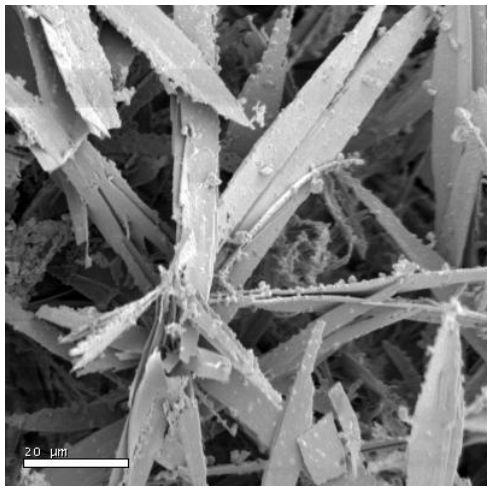

Fig. 9. N15. r = 20 microns. 750x. dp_{mean} = 80 microns

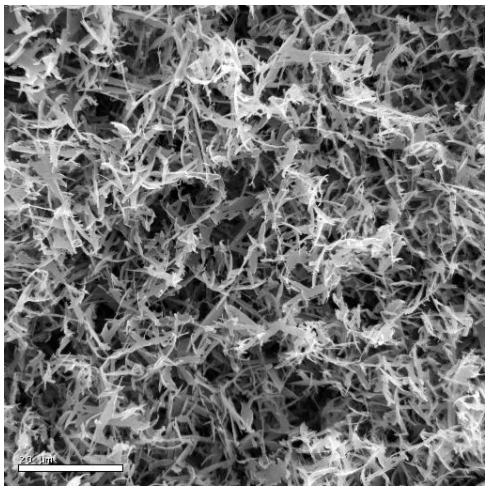

Fig. 10. N16. r = 20 microns. 750x. dp_{mean} = 10 microns

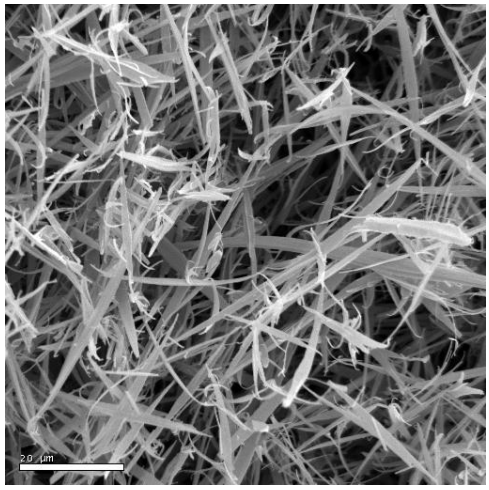

Fig. 11. N17. r = 20 microns. 750x. dp_{mean} = 30 microns

particles are not able to grow and then they present a smaller size. The same behaviour is described by Amaro- González [9] for lobenzarit and Cocero [3] for β carotene when changing the nozzle. This might suggest that at these higher pressures the precipitation time is smaller than complete mixing time and then the precipitation happened in two phases.

II.2. EFFECT OF TEMPERATURE.

Temperature influence in particle size is studied at 90 bar, 500 ppm concentration of Lycopene in the solution, 365 mL/h of solution flowrate together with 3.0 kg/h of CO_2. In this case, the concentric tubes nozzle is used. Temperature is varied from 308.15 to 318.15 K.

In **figure 10**, particles at 318.15 K are obtained. As it can be seen, particles of 10 microns and even smaller are obtained. In contrast, in **figure 11**, bigger particles with sizes close to 30 microns are obtained at 308.15 K. So increasing temperature decreases particle size of the obtained product. Temperature has no been further increased, to avoid the thermal degradation of Lycopene, as it is a thermolabile compound. Same behaviour is described for Nalmefene Hydrochloride by Adami [6], and by Kalogiannis [10] for paracetamol, decreasing particle size with increasing temperature.

II.3. EFFECT OF SOLUTE CONCENTRATION.

The influence of the Lycopene concentration in the particle size of the obtained product is done at 120 bar, 308.15 K, 4.3 kg/h of CO_2, 365 mL/h of solution and with the commercial nozzle as contacting system. Concentration is decreased from 500 ppm to 250 and 125 ppm in the initial solution.

Figure 5 presents the particles for the highest concentration of 500 ppm. As it can be seen, the mean particle size is 50 microns. In **figure 12**, concentration is decreased to 250 ppm, presenting a slight reduction in the particle size to 40 microns. This particle size decrease is more obvious decreasing the Lycopene concentration to 125 microns as shown in **figure 13**,

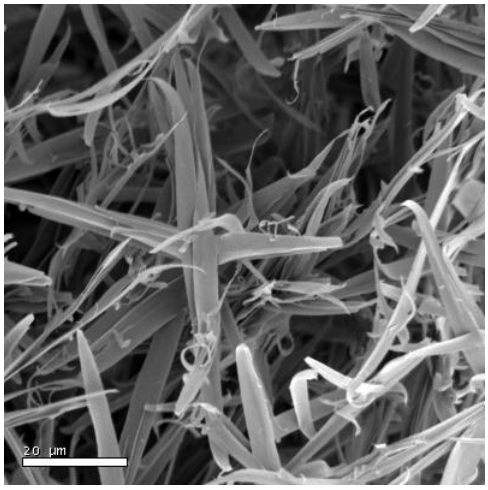

Fig. 12. N12. r = 20 microns. 750x. dp_{mean} = 40 microns

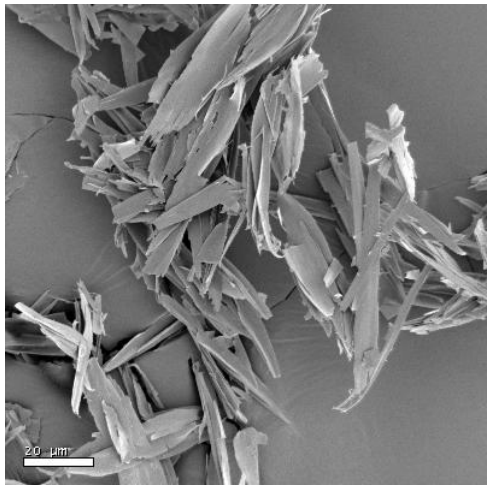

Fig. 13. N11. r = 20 microns. 750x. dp_{mean} = 20 microns

where the particle size is reduced to 20 microns. So, particle size of Lycopene decreases with decreasing concentration.

Most authors presents a different behaviour of the particle size with the concentration of the solute in the initial solution [10], [11], but others authors like Foster [12] describe the same behaviour for the batch precipitation of trobamycin, decreasing particle since when decreasing the solution concentration in the initial solution.

II.4. EFFECT OF NOZZLE DESIGN.

For this variable, crystallization is performed at 90 bar, 318 K, 500 ppm of Lycopene in initial solution, with a solution flowrate of 365 mL/h and a CO_2 massrate of 3.0 kg/h.

In **figure 7** it is presented the Lycopene which was obtained with the commercial nozzle. Particles present sizes smaller than 10 microns. In **figure 10**, it is presented the Lycopene precipitated by the concentric tubes nozzle, presenting a mean particle size of 10 microns. So, from this point, it has to be said that there is no neat influence of the nozzle in the particle size in the precipitation of Lycopene. From the observation of the crystallizer, with the concentric tubes nozzle it is achieved a higher mixing degree, as the product is homogeneously distributed all over the height of the crystallizer.

CONCLUSION

Lycopene was successfully crystallized from Dichloromethane solutions by continuous GAS process. Lycopene increases its size, when increasing the pressure from 90 to 150 bar; decreases its size, when increasing temperature from 308.15 to 318.15 K; decreases its particle size when decreasing the solute concentration in the initial solution from 500 to 125 ppm. There is also no clear influence of the nozzle in the particle size of the obtained product, but the concentric tubes nozzle increases the homogeneity of the crystallization.

Fig. 14. N16. Lycopene on the inner wall of the crystallizer

Fig 15. N16. Recovered Lycopene.

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