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Preparation of stable aqueous nanodispersions of β -carotene by supercritical assisted injection in a liquid antisolvent

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Abstract

In food industry, nanodispersions of water-insoluble active compounds are attractive food ingredients as they improve food formulations and the bioavailability of the active compounds. β -carotene, is a natural colorant used in food industry and, besides its excellent colorant properties, it has been employed as precursor of retinol and retinoic acid which have an important role in human health as vitamin A precursor and as cellular regulatory signal, respectively. Carotenoids are practically insoluble in water; it is possible to enhance their dissolution rate by lowering the particle size. For these reasons, precipitation processes are needed for the production of stabilized nanodispersion of β -carotene, taking into account that carotenoids are unstable compounds, very sensitive to oxidation processes. Supercritical fluid precipitation techniques are exceptionally suitable for labile products. The precipitation process can be performed at mild temperature conditions, on an inert atmosphere, particle sizes in the micron and sub-micron range can be obtained. Recently a new supercritical assisted process was developed for the direct production of stabilized nanoparticles suspensions. The process, named Supercritical Assisted Injection in Liquid Antisolvent (SAILA), produces stable nanoparticle suspensions in water. In this work SAILA process was successfully applied for the production of β -carotene nanoparticle water suspensions. Nanoparticles in the range 50-150 nm were produced varying the process conditions. The produced nanodispersions produced were characterized by high Zeta-potential values and maintain their stability over months.

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Keywords: Nanoparticles, Supercritical CO₂, Expanded liquid Injection Liquid antisolvent, beta-carotene

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1. Introduction

Carotenoids are compounds constituted by eight isoprene units joined in a head to tail pattern, most of them have 40 carbon atoms. They can be classified in two different ways, by their chemical structure or by their functionality. They can be classified by their structure as Carotenes if their only elements are carbon and hydrogen like β -carotene or lycopene, or they can be considered as Xantophylls if they also have oxygen in their structure as for example lutein or zeoxanthin. A primary carotenoid is necessary in the photosynthetic process like β -carotene, lutein or neoxanthin; whereas a secondary carotenoid is not directly involved in the survival of the plant like α -carotene, capsanthin or lycopene [1].

In industrial applications carotenoids have a great impact in the food industry as natural colorants. The double conjugated bounds present in their chemical structure absorb light within the visible range, providing the substances with an intense color ranging from a pale yellow, through a strong orange to a dark red. The additional function related with vitamin A activity and their antioxidant activity generates a great value as high quality colorants [2].

Food colorants have always been object of complains of the food industry consumers, mainly because of the bad fame of the initial synthetic pigments that only had a cosmetic value and were associated with health damage. Nowadays, the food market demands functional foods, and healthy products, while the use of chemical products is considered as negative and the natural additives provide the final product with a healthy value. Furthermore, the introduction of terms as functional, medical and nutraceutical food reinforced the advantages of the natural colorants against the synthetic ones [3].

One of the most abundant and used carotenoid is β -carotene; besides its excellent colorant properties, it has been exhaustively employed as precursor of retinol and retinoic acid which have an important role in human health as vitamin A precursors and as cellular regulatory signal, respectively. Since animals and humans cannot produce carotenoids by themselves, they need to acquire them from their food sources, like vegetables, fruits, fishes and some sea foods [4].

Regardless of the source, carotenoids need a further treatment to be suitable as industrial colorants. Their non-polar nature limits its application to non-aqueous solutions, since they are practically insoluble in water. Different approaches can be taken to minimize this drawback, for example, enhance their dissolution rate by lowering the size of the β -carotene particles, or encapsulating them with hydrophilic materials in order to obtain controlled release systems. Both alternatives involve the use of precipitation processes like solvent-out, salting-out or crystallization; they all lead to products that need further solvent removal involving relatively high temperatures and, consequently, carotenoid destabilization. Light has an activation effect on carotenes alteration and higher temperatures accelerate the degradation processes. What is more, the composition of the medium affects the carotene degradation rate. For example, the half-life of β -carotene during storage in water is 9.6 days while in cyclohexane is only 3.5 days [5].

Because of these drawbacks, the use of supercritical fluids (SCF) is a good alternative in the precipitation processes. The precipitation process can be performed at mild temperature conditions, in an inert atmosphere and particles in the micron and sub-micron range can be obtained [6].

Supercritical fluid based literature has proposed some processes [7,8,9,10] aimed at the production of nanoparticles of organic compounds, that can be used in several industrial fields. These processes try to take advantage of the possibility to modulate the SCF mass transport, solubilisation properties and the intrinsic non toxicity and environmental friendly nature of supercritical carbon dioxide (SC-CO₂), that is the supercritical fluid of election in the applications involving the use of thermolabile and or organic compounds [11].

Reverchon and co-workers have recently proposed an innovative technique for the production of stable aqueous nanodispersion named Supercritical Assisted Injection in Liquid Antisolvent (SAILA) [12]. An expanded liquid solution is formed using SC-CO₂ and an organic solvent in which a solid solute is also

solubilised. Then, the expanded ternary solution is depressurized directly into water (in which a surfactant can be added) where the solute is not soluble and the organic solvent is miscible; therefore, the water based solution works as a liquid antisolvent. Operating in this manner, nanoparticles can be generated. In a previous work the SAILA process has been proposed and successfully demonstrated for polycaprolactone (PCL). Non coalescing nanoparticles down to 64 nm mean diameter were produced; this result is particularly interesting since 100 nm diameter represents a benchmark that is very difficult to overcome when organic compounds are processed. Experimental results on PCL nanoparticles production suggested the formation of nanoparticle guided by almost instantaneous nanomixing plus nucleation and growth.

Therefore, the aim of this work is to extend the application of the SAILA process to the production of β -carotene nanoparticle water suspensions and testing the applicability of this new process to drug and colorant suspensions production. Feasibility of β -carotene nanosuspensions production will be tested and the influence of some SAILA operative conditions on particle size distribution (PSD), such as gas to liquid ratio (GLR) and β -carotene concentration, will be evaluated.

2. Materials & Methods

2.1. Materials

CO₂ (99.9%, SON, Naples, Italy), Polysorbate (Tween 80), Acetone (AC, purity 99.9%), β -carotene (β -C) were all bought from Aldrich Chemical Co. and were used as received.

2.2. Supercritical Assisted Injection into Liquid Antisolvent (SAILA) process layout

A schematic representation of the SAILA process layout is reported in Fig 1. It mainly consists of two feed lines, used to deliver the compressed CO₂ (A) and the solvent, acetone (B) to a mixing vessel (saturator, C). Carbon dioxide is cooled, pumped (P1), preheated and delivered to the saturator (C). The liquid mixture formed by acetone (in which β -C is dissolved), is delivered to the saturator by a membrane pump (P2) and is preheated before the inlet to saturator. The saturator is a high-pressure vessel with an internal volume of 0.15 dm³, heated by thin band heaters (E) and is packed with stainless steel perforated saddles with a high specific surface area (D). It provides a large contacting surface and an adequate residence time (3-5 min, depending on the flow rates) for the liquid-gas mixing. Therefore, an efficient, continuous solubilisation of SC-CO₂ in the liquid β -C acetone solution is obtained. As a result, they form an expanded liquid, whose position in the vapor-liquid equilibrium diagram (VLE) of the system CO₂-acetone depends on the conditions of temperature, pressure and on gas to liquid ratio (GLR, here expressed as weight ratio). The solution at the exit of the saturator is injected into the receiving water plus surfactant phase using an orifice that has a diameter of 100 μ m and a length to diameter ratio of 6.67. The water phase is continuously taken from a reservoir (WR) and pumped with a peristaltic pump (P₃) in the receiving vessel (RV); the suspension is continuously recovered through a regulation valve (V4), that is also used to maintain a constant liquid level in the receiving vessel.

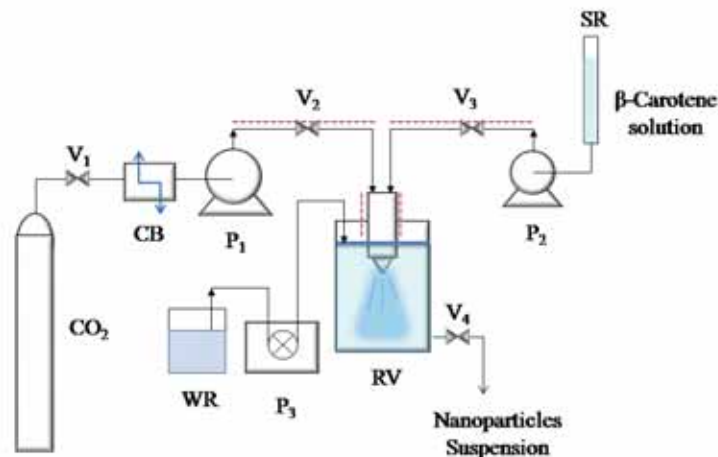


Fig. 1. Schematic representation of the SAILA process layout. CO₂: Carbon Dioxide reservoir; V₁, V₂, V₃, V₄: on-off valves; CB: cooling bath, P₁: CO₂ pump; P₂: liquid pump; P₃: peristaltic pump SR: solvent reservoir; WR: water reservoir; RV receiving vessel

2.3 Nanoparticle morphology, size distributions and solvent residue analysis

Nanoparticle morphology was analysed by FESEM (LEO 1525, Carl Zeiss SMT AG). Samples were prepared by spreading concentrated nanoparticle dispersions over aluminium stubs and drying them at air. Then, the samples were coated with a gold layer, thickness 250 Å (mod.108 Å, Agar Scientific). Particle size (PS) and particle size distribution (PSD) were measured from SEM photomicrographs using the Sigma Scan Pro Software (release 5.0, Aspire Software International Ashburn, VA). Approximately 1000 particles were measured for each particle size distribution calculation. Histograms, representing the particle size distribution, were fitted using Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA).

3. Results and Discussion

The experiments were performed setting the temperature in the saturator at 80°C, CO₂ flow rate at 10 gr/min and varying the acetone solution flow rate. Operating in this way, different gas to liquid ratio (GLR) were produced and, correspondingly, expanded liquid mixtures with different CO₂ molar fractions were obtained. The β-C solution used in the experiments for the study of GLR effect was prepared dissolving β-C in acetone at a concentration of 0.03 mg/mL. The ratio between the solvent (acetone) and the antisolvent (water) flow rates (S/NS) was fixed at 0.25. Small quantities of surfactant were added to water solution to control particles stability. Tween 80, an hydrophilic surfactant, was added to water since it is approved in pharmaceutical formulations and it has been frequently used to control particles coalescence [13]. A consideration is required for the use of a continuous flow of water in the precipitator: this process feature allows to operate at steady state conditions from the point of view of the concentration of the nanoparticles in water, that can influence possible effects of coalescence. Therefore,

continuous operation is obtained instead of a semi-batch process and reproducibility problems are avoided.

In Table 1 data related to the experiments are reported; the decrease of GLR can also produce a lowering of the pressure in the saturator. The representation of the vapour-liquid equilibria (VLEs) of the system acetone-SCCO₂ is reported in Fig 2, adapted from literature [14]. The operating points related to the experiments reported in Table 1 are also reported in this figure. Mean diameter (MD), standard deviation (SD) and polydispersity index (PI) of the produced nanosuspensions were calculate by DLS measurements and are reported in the same table.

Table 1. Mean diameter (MD), standard deviation (SD) and Polidispersity index (PI) of β -C nanoparticles obtained from a 0.03 mg/mL β -C solution in acetone. Saturator temperature was set at 80°C

	GLR	P (bar)	XCO ₂	B-C (mg/mL)	MD (nm) \pm SD	PI
P1	1.58	90	0.68	0.03	37 \pm 5	0.13
P2	1.48	80	0.66	0.03	40 \pm 5	0.12
P3	1.16	75	0.60	0.03	48 \pm 6	0.13
P4	0.81	70	0.52	0.03	56 \pm 7	0.12
P5	0.81	70	0.52	0.1	71 \pm 8	0.15
P6	0.81	70	0.52	0.2	90 \pm 14	0.16

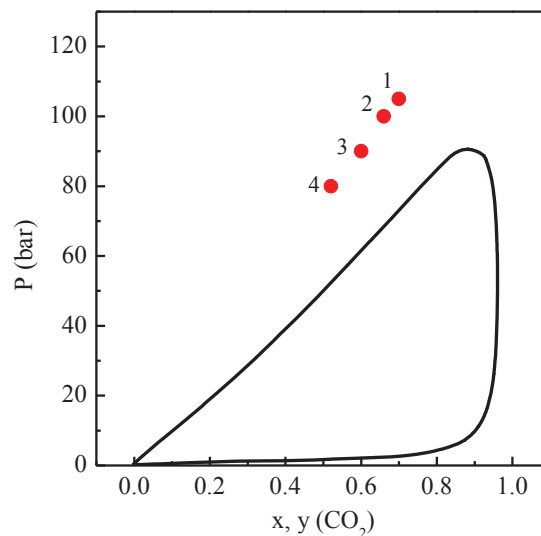


Fig. 2. Representation of the high pressure VLE of the system acetone-SCCO₂ at 80°C, adapted from Sato et al. [14]. The operating points of the experiments reported in Table 1 are also represented.

These results show that SAILA technique was successful in the production of β -C nanoparticle suspension at all GLR tested, moreover the suspensions produced were stable. The macroscopic aspect of the produced suspensions is reported in Fig 3. Suspensions were stable with zeta potential mean value of

about -20 mV. Stability of suspensions was also monitored over time: they maintain stability over months. Examples of corresponding SEM images are reported in Fig 4 and show that nanoparticles were approximately spherical, with a smooth surface and were not aggregated. To obtain this kind of SEM image a nanoparticles dilute dispersion was produced and high contrast images were taken.



Fig. 3. Macroscopic aspect of β -C nanoparticles suspensions produced by SAILA with variation of GLR. Process conditions are reported in Table 1.

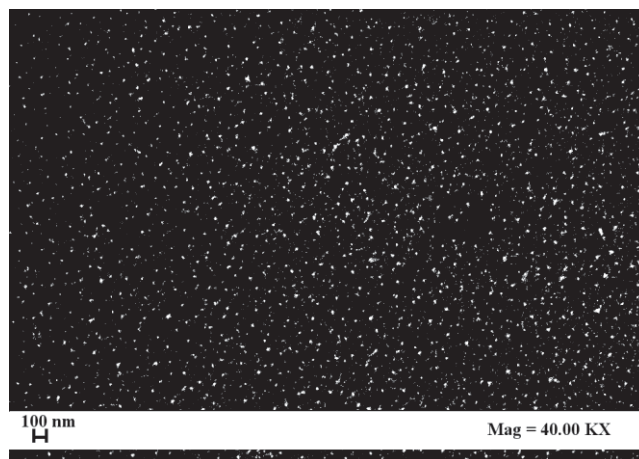


Fig. 4. FESEM image of β -C nanoparticles produced by SAILA (P4). Process conditions are reported in Table 1.

Fig 5 reports a comparison of the volumetric particle size distributions obtained in the experiments performed at the maximum and minimum value of GLR explored. The PSD of the obtained suspensions demonstrated that there is a sensible effect of X_{CO_2} on particle diameters, that is particle size decreases with the increase of GLR and consequently the increase of X_{CO_2} . This result is related to the fact that antisolvent precipitation is obtained when the processed compound is soluble in the solvent and not soluble in the antisolvent. The particle size of the precipitates depends on the efficiency of the micromixing between the two liquids that, in turn, is related to their surface tension [15]. Expanded liquid are characterized by a reduced surface tension that at high gas molar fractions is near to zero [16]. Therefore, the continuous injection of an expanded liquid solution is more effective than the mixing with an ordinary liquid. This characteristic should improve the micromixing with the antisolvent and produce smaller particles increasing the X_{CO_2} , i.e. reducing the surface tension of the expanded liquid. From Figure 5 it is also possible to see that D100 is below 100 nm in all the cases explored.

The smallest β -C nanoparticles produced showed a mean diameter of 56 nm.

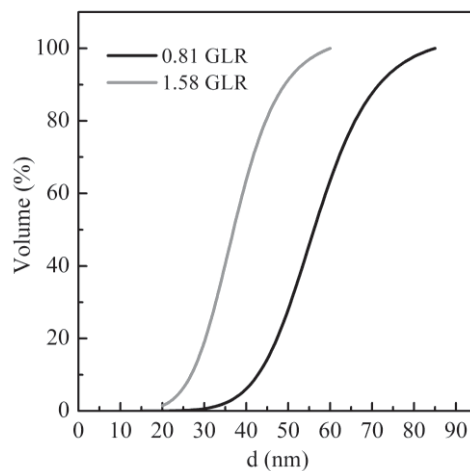


Fig. 5. PSD of β -C nanoparticles suspensions obtained by SAILA at GLR 0.81 and 1.58

The effect of β -C concentration in the acetone solution was also investigated; experiments were performed fixing GLR at 0.81, saturator temperature at 100°C and pressure at 70 bar. The results obtained are summarized in Table 1. Stable suspensions were obtained also with higher concentrations of β -C. A considerable effect on particles MD was observed, as shown in Table 1. This result can be explained considering the nucleation process and its dependence on the concentration of the solute: a higher supersaturation favors particles growth; therefore, increasing the solute concentration, larger particles are obtained. FESEM image of nanoparticles obtained at 0.2 mg/mL concentration is reported in Fig 6. Particles are spherical also in this case and non coalescing with a mean diameter of about 90 nm. Fig 7 reports PSD of nanoparticles obtained at minimum and maximum concentration of β -C.

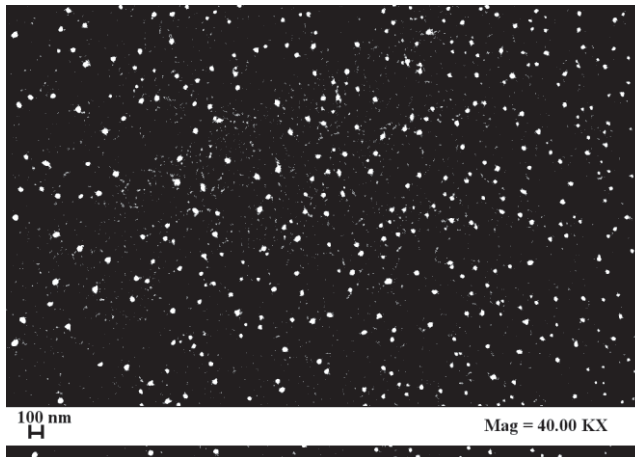


Fig. 6. FESEM image of β -C nanoparticles obtained by SAILA obtained from a solution at 0.2 mg/mL of β -C

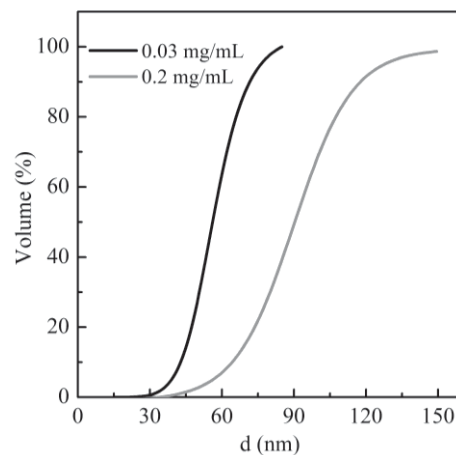


Fig. 7. PSD of β -C nanoparticles suspensions obtained by SAILA operating GLR 0.81.

From Fig 7 and from Fig 6 it is possible to see that in the case of higher concentration larger particles are obtained with D_{100} located at 150 nm, whereas at a concentration of β -C of 0.03 mg/mL PSD is all below 100 nm, with D_{100} at 86 nm.

4. Conclusions

SAILA, the novel supercritical assisted process for the production of nanoparticles of organic compounds, has been successfully demonstrated for β -C. Non coalescing nanoparticles down to 37 nm

mean diameter were produced; this result is particularly interesting since 100 nm diameter represents a benchmark that is very difficult to overcome when organic compounds are processed. Nanoparticle diameters can be modulated varying two main conditions: GLR and β -C concentration in the solvent, with the possibility to produce nanoparticles with a mean diameter of 90 nm and 37 nm and very low PI.

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