Elsevier Editorial System(tm) for Journal of Food Engineering
Manuscript Draft

Manuscript Number:

Title: α-Tocopherol nanosuspensions produced using a supercritical assisted process

Article Type: Research Article

Keywords: α-Tocopherol; Nanodispersion; Supercritical fluids.

Corresponding Author: Dr. Roberta Campardelli, PhD

Corresponding Author's Institution: University of Salerno

First Author: Roberta Campardelli, PhD

Order of Authors: Roberta Campardelli, PhD; Ernesto Reverchon

Abstract: A recently developed supercritical assisted process, named SAILA (Supercritical Assisted Injection in Liquid Antisolvent) has been used for the production of α-tocopherol stable aqueous suspensions. α-tocopherol is a liposoluble vitamin, belonging to the family of Vitamin E, widely used as supplementation and as antioxidant in food, cosmetics and pharmaceutical industries. However, its poor solubility in water, and thus its low bioavailability, make its use problematic. The formulation of this compound in nanosized suspensions is particularly appealing, since it is known that nanoparticles increase the dissolution rate, thus increasing bioavailability. For these reasons, in this work, SAILA has been applied to the production of α-tocopherol nanoparticles suspensions. Process parameters such as kind of solvent-antisolvent, concentration of surfactant, concentration of solute, expanded liquid temperature, pressure and composition have been optimized. Stable α-tocopherol particles suspensions with mean diameters down to 150 nm have been produced and characterized in terms of morphology, particles size distribution and zeta potentials. Particles are spherical and non-coalescing and suspensions stability during storage at 4°C has been verified for 30 days.

Dear Editor,

the manuscript we are submitting entitled "*α-Tocopherol nanosuspensions produced using a supercritical assisted process*" reports the production of stable α-Tocopherol water nanosuspensions produced using a new supercritical fluids (SCFs) assisted technique named SAILA (Supercritical Assisted Injection in Liquid Antisolvent).
Formulation of nanosuspension is a promising approach to overcome bioavailability problems of poor water-soluble compounds. Despite many processes, have been proposed in the literature, the efficient production of particles suspensions with dimensions lower than one micron remains very difficult; furthermore, these processes have some common limitations such as the large use of organic solvents, thermal degradation, large solvent residue, and difficulties in controlling particle size and distribution during processing. This new process has the advantage of producing particles directly in stabilized suspensions ready to be used. In this manuscript, the efficient production of α-Tocopherol nanosuspensions has been demonstrated and optimized, obtaining unimodal sharp nanoparticle distributions stable over time. For this reason this paper would be interesting for the readers of the Journal of Food Engineering.

Best regards,

Ernesto Reverchon

Highlights:

- A supercritical based process is applied for α-Tocopherol nanoparticles production
- α-Tocopherol nanosuspensions with dimensions of 150±30 nm can be obtained.
- Suspensions produced are stable over one month of storage.

α-Tocopherol nanosuspensions produced using a supercritical assisted process

Campardelli R.[1], Reverchon E.[1]*

[1]*Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II,132 Fisciano (SA) 84084, Italy*

A recently developed supercritical assisted process, named SAILA (Supercritical Assisted Injection in Liquid Antisolvent) has been used for the production of α-tocopherol stable aqueous suspensions. α-tocopherol is a liposoluble vitamin, belonging to the family of Vitamin E, widely used as supplementation and as antioxidant in food, cosmetics and pharmaceutical industries. However, its poor solubility in water, and thus its low bioavailability, make its use problematic. The formulation of this compound in nanosized suspensions is particularly appealing, since it is known that nanoparticles increase the dissolution rate, thus increasing bioavailability. For these reasons, in this work, SAILA has been applied to the production of α-tocopherol nanoparticles suspensions. Process parameters such as kind of solvent-antisolvent, concentration of surfactant, concentration of solute, expanded liquid temperature, pressure and composition have been optimized. Stable α-tocopherol particles suspensions with mean diameters down to 150 nm have been produced and characterized in terms of morphology, particles size distribution and zeta potentials. Particles are spherical and non-coalescing and suspensions stability during storage at 4°C has been verified for 30 days.

*Corresponding author.
Ernesto Reverchon Tel.: +39 089964116; Fax: +39 089964057; e-mail address: ereverchon@unisa.it

Keywords: α-Tocopherol; Nanodispersion; Supercritical fluids.

1 Introduction

Functional lipids, such as carotenoids, phytosterols, ω-3 fatty acids and various other compounds, are widely used as active ingredients in food products (Ballabio and Restani 2012). Particularly, natural antioxidants such as such as α-, β-, γ-, δ-tocopherol and tocotrienol are largely used in vitamin supplementation and as antioxidants in food (Cheong et al. 2008; Shahidi 2009). However, the poor water solubility of functional lipids has made their use problematic for food formulations (Tan and Nakajima 2005). For these reasons, it is relevant to find solutions to this problem. Nanoparticles production can represent a good opportunity to improve the dissolution rate of such active ingredients and to increase their bioavailability (Huang et al. 2010).

Nanodispersions formulation is a promising approach to overcome bioavailability problems due to the enlargement of the exposed surface areas that increases the dissolution rate of poor water-soluble compounds (Merisko-Liversidge and Liversidge 2008). Nanodispersions are technically defined as biphasic systems in which solid particles are dispersed in an aqueous vehicle stabilized by surfactants and polymers (Grau et al. 2000). Two approches namely "Bottom up technology" and "Top down technology" are used (Van Eerdenbrugh et al. 2008). Bottom up technology is an assembling method to form nanoparticles by precipitation, microemulsion, melt emulsification methods; whereas, top down technology involves the disintegration of larger particles into nanoparticles, examples of which are high-pressure homogenization and milling methods (Patel and Agrawal 2011). Limitations of these processes are the large use of organic solvents, thermal degradation, large solvent reside, and difficulties in controlling particle size and size distribution during processing. These limitations affect drug particle stability, flow properties, and efficiency of the delivery system. Reduction of particle size of poorly water soluble drugs into the nanoparticle range remains challenging (Hu et al. 2004).

For these reasons, a supercritical fluid based process could be an excellent alternative, to form microparticles and nanoparticles (Reverchon and Della Porta 2003). Processes that use supercritical fluids for particle formation applied to improve the solubility and dissolution of poorly

water soluble drugs are RESS (Rapid Expansion of Supercritical Solution) (Young et al. 2000; Sane and Limtrakul 2009), SAS (Supercritical Antisolvent Precipitation) (Reverchon et al. 2001; Reverchon et al. 2002), SAA (Supercritical Assisted Atomization) (Reverchon and Antonacci 2007; Adami et al. 2011) and SEE (Supercritical Emulsion Extraction) (Campardelli et al. 2013; Shekunov et al. 2006). More specifically, Reverchon and co-workers have recently proposed a supercritical assisted technique for the production of stable aqueous nanodispersions named Supercritical Assisted Injection in Liquid Antisolvent (SAILA) (Campardelli et al. 2012a). In this process, an expanded liquid solution is formed by SC-CO_2 and an organic solvent, in which a solid solute is also solubilised. Then, the 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. This process takes advantage of the fact that the solubilisation of large quantities of a dense gas in a liquid (expanded liquid), largely reduces the surface tension of the solution (Brunner 1994). Indeed, in liquid based processes, the particles size of the precipitates depends on the efficiency of the mixing between the two liquids that is, in turn, related to their surface tension. Therefore, the continuous injection of an expanded liquid solution can be more effective than the mixing with an ordinary liquid (Franck 1984). Moreover SAILA technique is performed as a continuous process; it allows the direct production of particles in stabilized water suspensions in a single step. In previous works, SAILA process has been proposed and successfully demonstrated for polymeric particles production such as polycaprolactone (PCL) (Campardelli et al. 2012a) and polymethylmethacrylate (Campardelli et al.). Non coalescing nanoparticles were produced and the effect of different process conditions on particles size distribution was studied. Some feasibility test on the production of β-carotene nanodispersions with the SAILA process were also performed. (Campardelli et al. 2012b). However a systematic study to demonstrate the efficient production of stable nanodispersions of poor water soluble compounds has not been carried out until now.

For these reasons, in this work, SAILA process has been applied to the production of stabilized α-tocopherol aqueous nanosuspensions. The objective is to find optimized operative conditions to obtain stable nanodispersion of α-tocopherol with sharp particles size distribution. Nanodispersions are also characterized in terms of storage stability.

2 Materials

Carbon dioxide, purity 99.5% was supplied by SON (Naples, Italy). Polysorbate (Tween 80, Aldrich Chemical Co.), Acetone, Ethanol and Isopropanol (purity 99.9%, Aldrich Chemical Co.), distilled water and α-tocopherol (purity 98% Aldrich Chemical Co.) were used as received.

3 Apparatus

SAILA equipment is schematically represented in **Figure 1**. A detailed description of the apparatus is reported elsewhere (Campardelli et al. 2012a). Briefly, the major devices of the apparatus are a saturator, where the expanded liquid is formed at fixed conditions of temperature and pressure. Organic solution (solvent+solute) and SC-CO_2 are delivered to the saturator using two different pumps at a fixed gas to liquid ratio (GLR), expressed on weight basis. Random packings allocated inside the saturator promote the intimate mixing between the two phases that are fed in co-current mode, allowing the formation of the expanded liquid. The expanded liquid mixture obtained is continuously depressurized into an aqueous solution through an injector of a given diameter (80-100 μm). The antisolvent is pumped to the precipitation vessel using a peristaltic pump, at a fixed flow rate. A regulation valve located downstream the precipitation vessel allows to continuously recover the suspension, maintaining the internal water volume constant.

4 Methods

Particles size distribution (PSD), mean diameter (MD), standard deviation (SD) of the produced suspensions were measured by dynamic light scattering (DLS) (Zetasizer, mod. 5000,

Malvern Instruments Ltd). Polydispersity index (PDI) and zeta potential were also measured using the same instrument. Polydispersity index (PDI) measures the dispersion of the particles around the mean diameter, it is the ratio between the standard deviation (SD) and the mean diameter (MD); the smaller is PDI, the sharper is the distribution. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion. A large zeta potential indicates dispersion stability, i.e., the dispersion has reduced tendency to aggregation. Particle morphology was analyzed by FESEM (LEO 1525, Carl Zeiss SMT AG). Samples were prepared by spreading concentrated particle dispersions over Aluminum stubs and drying them at air. Then, the samples were sputter coated with a Gold layer, thickness 250 Å (mod.108 A, Agar Scientific).

5 Results and Discussion

Particles precipitation during SAILA is induced by the injection of the expanded liquid (SC-CO_2 + organic solvent + solute), in the antisolvent phase. Several process parameters can play a role in determining particles size distribution. They can be classified as related to expanded liquid (EL) conditions (pressure, temperature, X_{CO2}, solute concentration), as related to the antisolvent phase (solvent/antisolvent ratio, kind and concentration of surfactants) and their mixing (nozzle diameter, injection pressure). The effect of some of these parameters is discussed in the following sections.

5.1 Expanded Liquid Pressure

In the feasibility test on the processability of α-tocopherol by SAILA, acetone (Ac) was selected as the expanded liquid solvent, because it is a good solvent for the selected solute; furthermore, it is completely miscible with water, that is used as the antisolvent (Campardelli et al. 2012a). An injector diameter of 100 μm was used and the other process conditions were: α-tocopherol concentration in acetone solution 5 mg/mL, saturator temperature 50°C, CO_2 mass flow rate 12 g/min, gas to liquid ratio (GLR) 1.5. Operating in this way, an injection pressure of 70 bar was obtained in the saturator. Water contained 0.2% w/w of Tween 80, used to stabilize the α-

129 tocopherol particles after precipitation. The solvent/antisolvent weight ratio was set at 1/4. At the
130 end of the process, the solvent was removed from produced suspensions by evaporation under
131 reduced pressure. Detailed process conditions are listed in **Table 1** (α-1), where also mean
132 diameters (MD) and polydispersity index (PDI) of the suspensions are reported after solvent
133 elimination.

134 During this experiment α-tocopherol particles suspension was successfully obtained; it was
135 stable and homogeneous with a milky color. Particles with a mean diameter of 1.19 μm ± 0.33 were
136 obtained with a polydispersion index of 0.28 (See experiment in **Table 1,** α-1). A drop of the
137 suspension was put on an aluminum stub an dried at air to analyze particles morphology by SEM.
138 An example of FESEM image of the particles produced in this experiment is reported in **Figure 2**.

139 Micrometric spherical particles with smooth surfaces were obtained. However, process
140 parameters optimization was necessary to reduce particles dimension.

141 For this reason, a second experiment was performed using a 80 μm nozzle diameter. All the
142 other process parameters were not changed, and an operative pressure of 100 bar was established in
143 the saturator (**Table 1** α-2). In the SAILA process a reduction of the diameter of the nozzle, fixing
144 all the other process parameters, causes also an increase of the injection pressure. The use of a
145 smaller injector allowed the production of smaller particles with a MD of 0.22 μm ± 0.06
146 maintaining a PDI of 0.26. From results in **Table 1** and from PSD curves comparison in **Figure 3**,
147 the key role of injection pressure is evident: a large reduction of particles dimension was obtained.
148 A possible explanation is that at higher injection pressures the mixing between solvent and
149 antisolvent is improved thanks to the increase of jet turbulence, as also discussed in a previous work
150 (Campardelli et al. 2014).

151

152 *5.2 Expanded Liquid Solvent*

153 The effect of different liquid solvents was also investigated. Fixed water as the antisolvent,
154 acetone (Ac), ethanol (Et) and isopropanol (iP) were used to produce the α-tocopherol suspensions.

Until now, the SAILA process was performed only using acetone; the behavior of other liquid mixtures was never been explored. The experiments were performed using the 80 μm injector, α-tocopherol concentration in the solvent of 5 mg/mL, saturator temperature 50°C, CO_2 mass flow rate 12 g/min, GLR 1.5 (see **Table 1,** α-2, α-3, α-4 experiments). If we report the composition obtained in these experiments in the corresponding high pressure phase equilibrium diagram, we verify that a single phase is formed in all the tests performed (see **Figure 4**). Indeed, in **Figure 4**, vapor liquid equilibrium curves are plotted for the systems acetone-CO_2, ethanol-CO_2, isopropanol-CO_2 at 50°C. Each curve has a maximum that is known as Mixture Critical Point (MCP); in the region of the diagram located upper the critical pressure and on the right of the MCP, the mixture is in supercritical state; an expanded liquid is formed, instead, in diagram region located on the left of the MCP.

From the results shown in **Table 1** and from PSD curves of **Figure 5**, it is possible to note that α-tocopherol suspensions have been obtained in all cases and the smallest particles have been produced when the mixture acetone-CO_2 was used (α-2): particles with a MD of 0.22 μm ± 0.06 and a PDI of 0.26 were obtained in this case. When the ethanol-CO_2 mixture was used (α-3), particles with larger MD were obtained: about 0.33 μm ± 0.07, but a better control of particles size distribution was found, with a PDI of 0.22. The worst result was, instead, obtained using the system isopropanol-CO_2 (α-4), that gave the largest PSD.

The results observed using different solvents, can be explained firstly considering that, fixing all the process parameters, changing only the solvent, different expanded liquid pressures were established in the saturator; i.e. the higher pressure is obtained working with acetone, and then, in agreement with what has been previously discussed about the role of the injection pressure, the smallest particles have been produced. Furthermore, taking into account the position of process operative points it is possible to note that the smaller particles have been produced using ethanol and acetone, when the operative point is located far away the VLE boundary, i.e. at conditions in which the mixture is located inside the supercritical region. In the case of isopropanol the

miscibility hole is wider and for this reason the operative point of experiment α-4 falls very close the two phase region boundary. Probably at this operative condition a transition regime is established in the saturator. Moreover, considering the properties of solvents used, other considerations are possible. In particular ethanol, the solvent that produced the best result in terms of control of PSD, has the lowest surface tension (δ) compared with acetone and isopropanol (considering the pure solvent property $\delta_{Ac} > \delta_{iP} > \delta_{Et}$) and the highest diffusivity coefficient (D) in water ($D_{Et} > D_{Ac} > D_{iP}$). For these reasons using this solvent a faster and more homogeneous supersaturation can be obtained. This set of experiments points out that the choice of the couple solvent-antisolvent is an important process variable, that may affect not only particles dimension but also the control of PSD.

Considering the results obtained until now, we decided to continue the optimization of the process parameters using ethanol as the expanded liquid solvent. Furthermore, ethanol has also the advantage to be well accepted in pharmaceutical formulations, where it is often used as co-solvent (Strickley 2004).

5.3 Surfactant Concentration in Antisolvent

An important parameter that needs to be optimized and that can control particles dimension and suspensions stability is the concentration of surfactant. For this reason, experiments with different surfactant concentrations in the antisolvent have been performed, using ethanol as expanded liquid solvent, 80 μm injector, α-tocopherol concentration in solvent solution 5 mg/mL, saturator temperature 50°C, CO_2 mass flow rate 12 g/min, GLR 1.5, $X_{CO2} = 0.62$ (see **Table 2)**.

Data reported in **Table 2** shows that it is possible to obtain always a suspension; but, particles mean diameter is larger (0.54 μm ± 0.03) in absence of surfactant and particles size distribution is quite broad. Using a small amount of Tween 80 (0.2% w/w) a reduction of particles mean diameter (0.28 μm ± 0.06) and a better control of particles size distribution has been obtained. Increasing surfactant concentration to 0.5% and 1% w/w an increase of particles mean diameter and

PDI has been progressively obtained. Therefore, there is a clear trend in particle size that shows a minimum value at around 0.2% w/w, as it is possible to see also in **Figure 6**. A possible explanation of these results is the following: in absence of surfactant, coalescence and aggregation phenomena could be responsible of the production of larger particles, this effect is prevented instead using a small amount of surfactant. On the other hand, increasing the concentration of surfactant to 0.5% and 1% w/w, an inversion of the trend is noted that can probably be due to the increase of foam production during injection that induced a worst control of the process. An increase of surfactant concentration can also produce an increase of antisolvent viscosity, that can have a negative effect on mixing (Tsukada et al. 2009). The best performance has been found in correspondence of 0.2% w/w of Tween 80 concentration in the antisolvent phase.

5.4 Expanded Liquid Temperature

The effect of saturator temperature was also investigated. As reported in **Table 3,** experiments were performed at 50°C, 60°C and 80°C, maintaining constant all the other process parameters (80 μm injector, α-tocopherol concentration in ethanol 5 mg/mL, CO_2 mass flow rate 12 g/min, GLR 1.5, Tween 80 0.2% w/w).

Looking at data in **Table 3** and at **Figure 7** it is possible to note that, increasing the saturator temperature, a progressive reduction of particles mean diameter is obtained. In particular, operating at 50°C, particles with mean diameter of 0.28 μm ± 0.06 have been produced; whereas, increasing the temperature to 80°C a reduction of particles mean diameter to 0.23 μm ± 0.07 has been obtained. An increase of temperature also produced an enlargement of PDI.

A possible explanation of this result can be that a temperature increase causes a reduction of expanded liquid surface tension (Vazquez et al. 1995); this fact may favor the disruption of the liquid jet exiting the nozzle, promoting a better mixing. The increase of temperature can also favor the diffusion of the solvent in water and smaller particles are produced. The increase of suspensions PDI can be explained considering that, at higher temperatures, particles coalescence can be

favoured, resulting in larger PSDs. The smallest particles have been obtained at the highest temperature; but, considering that α-tocopherol is a pharmaceutical/nutraceutical product, lower process temperature can be preferred. For this reason, a saturator condition of 60°C can represent a good compromise between the benefit of particles size reduction and the preservation of thermo-sensible compounds (Fernholz 1938).

5.5 Solute Concentration

Experiments at different α-tocopherol concentrations have also been performed to evaluate the effect of solute concentration on PSDs. Operative conditions were fixed considering previous optimizations: 80 μm injector, saturator temperature 60°C, CO_2 mass flow rate 12 g/min, GLR 1.5, X_{CO2} = 0.68, Tween 80 0.2% w/w and α-tocopherol concentration in ethanol solution was varied between 1.5 and 10 mg/mL, as reported in **Table 4**.

An increase in particles MD and PDI can be noted when solute concentration is increased considering both data reported in **Table 4** and in **Figure 8,** where frequency distributions of the experiments performed at different solute concentrations, are compared. In details, considering data reported after ethanol evaporation, in correspondence of the higher α-tocopherol concentration (10 mg/mL) particles with a mean diameter of 0.26 μm ± 0.09 have been obtained; whereas, considering the concentration of 1 mg/mL, smaller particles with MD of about 0.15 μm ± 0.03 have been produced.

The reduction of particles mean diameter at smaller concentrations can be explained considering that a lower quantity of solute is available to contribute to particles growth and particles precipitation in this case is predominantly based on nucleation with reduced growth. The increase of suspensions PDI can be explained considering that the viscosity of the drug solution increases with the increase of solute concentration, which obstacles the diffusion of the solvent from the solution to the antisolvent, leading to a large particles size and relatively broad particle size distributions (Zhu et al. 2010).

5.6 Storage Stability Tests

α-tocopherol suspensions produced using the SAILA process have been stored at 4°C for 30 days, and PSDs and Zeta potential measurements have been performed each day to assay suspensions stability over the time. Results are shown in **Figure 9** for the α-6 experiment. α-tocopherol suspensions are stable during 30 days storage: particles mean diameter and standard deviation remained practically constant during all the tested period of time; also zeta potential of the suspension was large and negative during time with a constant value comprised between -12 and -18 mV.

6 Conclusions

In this work SAILA process has been successfully applied to the production of stable α-tocopherol suspensions. Optimization of the operative conditions allowed us to scale down particles dimensions to about 150 nm, demonstrating that SAILA process can efficiently produce α-tocopherol suspensions ranging in micrometric and nanometric range, depending on process conditions adopted. The smallest particles, about 150 nm in diameter, have been obtained using higher injection pressure, higher saturator temperature and low solute concentration. The process has demonstrated to be able of producing suspensions that are stable during one month of storage. This result is relevant because it confirms that the SAILA process can produce directly stabilized suspensions in a one step process.

Future developments of this work will regard the possibility to control antisolvent phase temperature using a precipitation vessel with an external jacket. Antisolvent phase temperature may have a role in controlling precipitation kinetics, allowing the production of even smaller particles.

Table 1. Process conditions and particles distribution data for SAILA experiments performed at 50°C, GLR 1.5, Tween 80 0.2% w/w with different nozzle diameters and expanded liquid solvents.

	α-1	**α-2**	**α-3**	**α-4**
Solvent	Ac	Ac	Et	iP
Nozzle diameter µm	100	80	80	80
Pressure bar	70	100	86	80
X_{CO2}	0.68	0.68	0.66	0.66
MD µm ± SD	1.19±0.33	0.22±0.06	0.33± 0.07	0.31± 0.10
PDI	0.28	0.26	0.22	0.33

Table 2. Particles distribution data of SAILA experiments performed using ethanol as the expanded liquid solvent, 80 µm injector, saturator pressure and temperature 100 bar and 50°C, X_{CO2}=0.62 and different surfactant (Tween 80) concentrations in the antisolvent phase.

	Surfactant Concentration			
	α-5	α-6	α-7	α-8
Tween 80 % w/w	0	0.2	0.5	1
MD µm ± SD	0.54±0.03	0.28±0.06	0.36± 0.09	0.45±0.03
PDI	0.63	0.22	0.24	0.62

Table 3. Particles distribution data of SAILA experiments performed using ethanol as expanded liquid solvent, 80 µm injector, saturator pressure 100 bar, X_{CO2}=0.68, Tween 80 0.2% w/w, performed at different saturator temperatures.

	EL Temperature		
	α-9	α-10	α-11
Temperature °C	50	60	80
MD µm ± SD	0.28±0.06	0.24±0.07	0.23±0.07
PDI	0.22	0.28	0.33

Table 4. Particles distribution data of SAILA experiments performed using ethanol as expanded liquid solvent, 80 µm injector, saturator pressure and temperature 100 bar and 60°C, X_{CO2}=0.68, Tween 80 0.2% w/w, performed changing α-tocopherol concentration.

	Solute Concentration			
	α-12	α-13	α-14	α-15
α-tocopherol mg/mL	1.5	2.5	5	10
MD µm ± SD	0.15±0.03	0.18±0.05	0.24±0.07	0.26±0.09
PDI	0.26	0.23	0.28	0.36

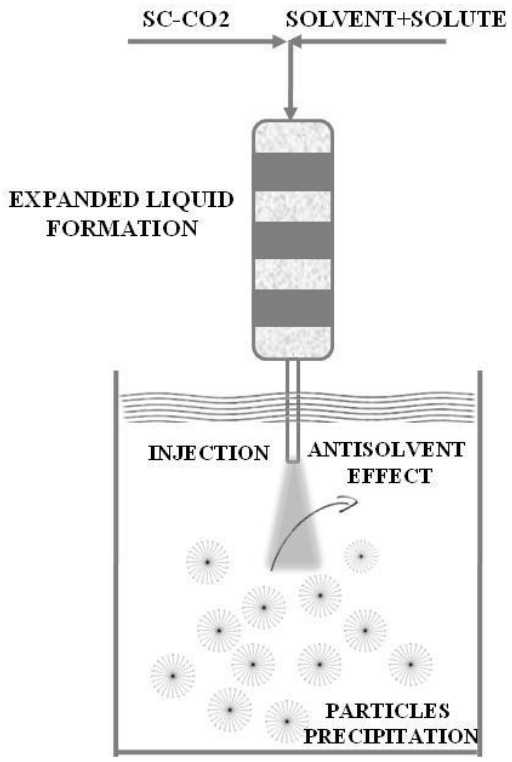

Figure 1. Conceptual representation of SAILA process

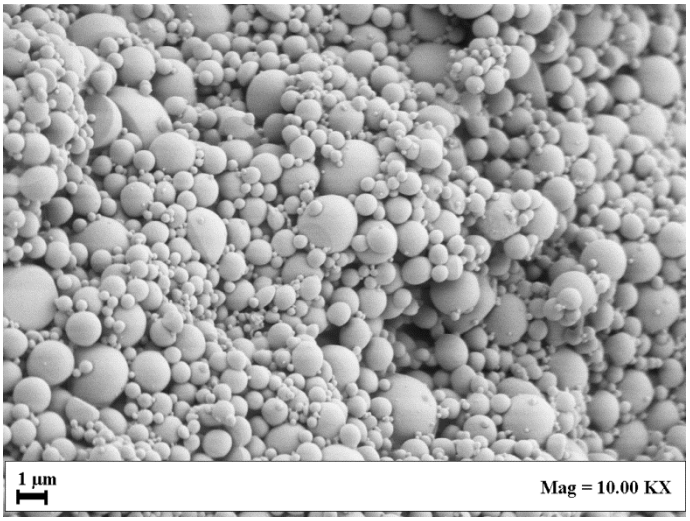

Figure 2. FESEM image of α-tocopherol particles produced in α-1 experiment

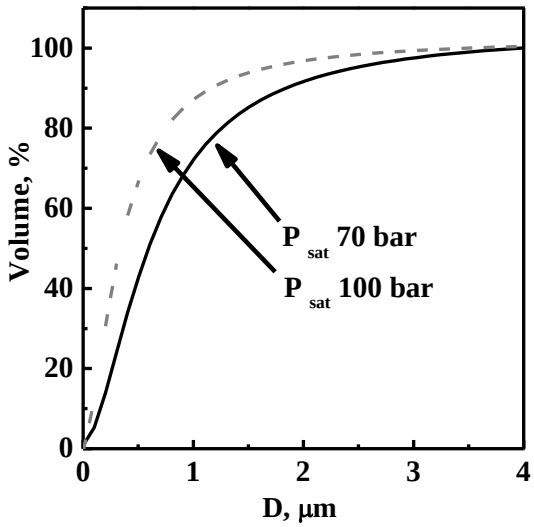

Figure 3. Cumulative PSD of α-tocopherol suspensions produced using different injector diameters:100 μm (with an operative pressure of 70 bar) and 80 μm (with an operative pressure of 100 bar)

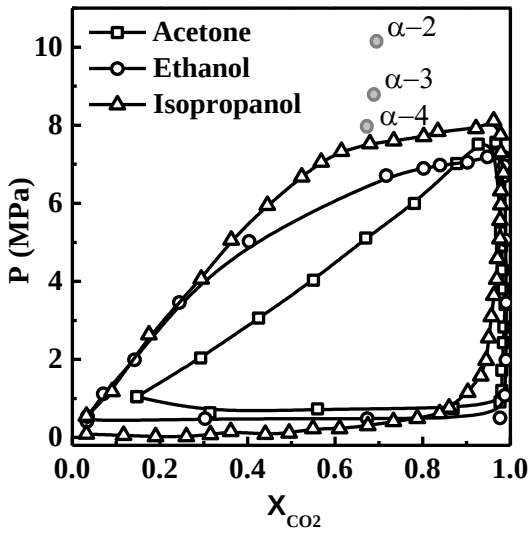

Figure 4. Vapor-liquid equilibrium curves for the systems acetone-CO_2 (□), ethanol-CO_2 (○), isopropanol- CO_2 (Δ) at 50°C adapted from the literature (Stievano and Elvassore 2005; Suzuki et al. 1990). Operative points of experiments listed in Table 1 are also reported (●)

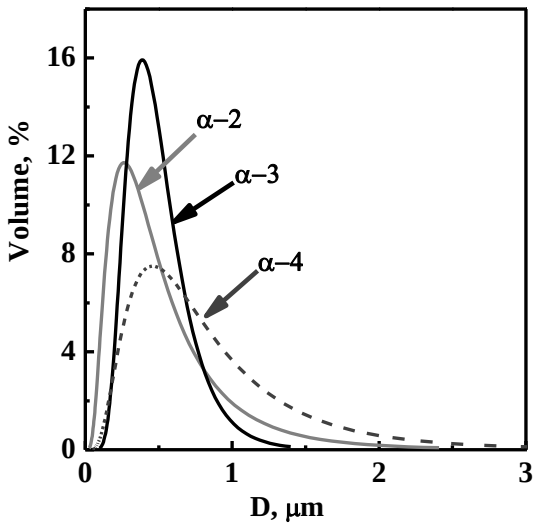

Figure 5. Volumetric PSD of α-tocopherol suspensions produced using different expanded liquid solvents: α-2 Acetone, α-3 Etanol and α-4 isopropanol.

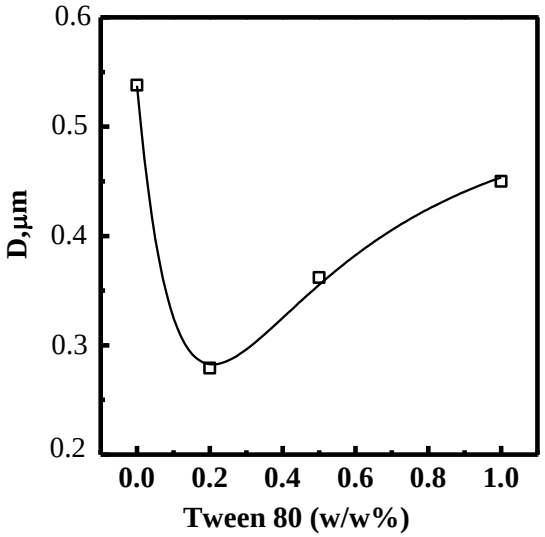

Figure 6. Effect of Tween 80 concentration in the antisolvent on α-tocopherol particles mean diameter

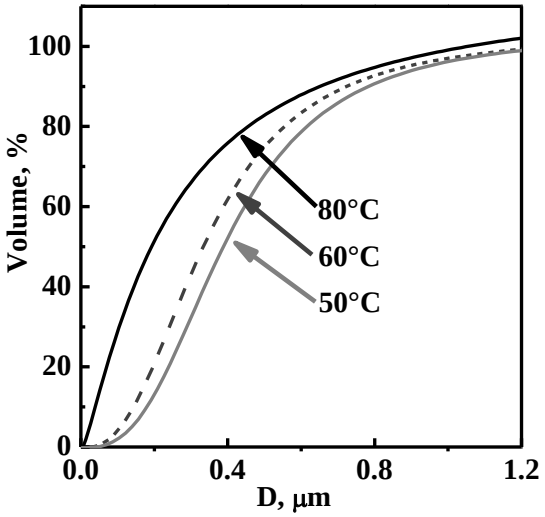

Figure 7. Cumulative PSD of α-tocopherol suspensions produced using different saturator temperatures.

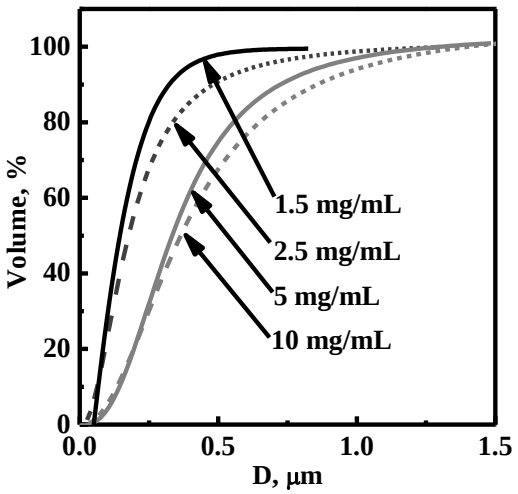

Figure 8. PSD of α-tocopherol suspensions produced using different solute concentrations.

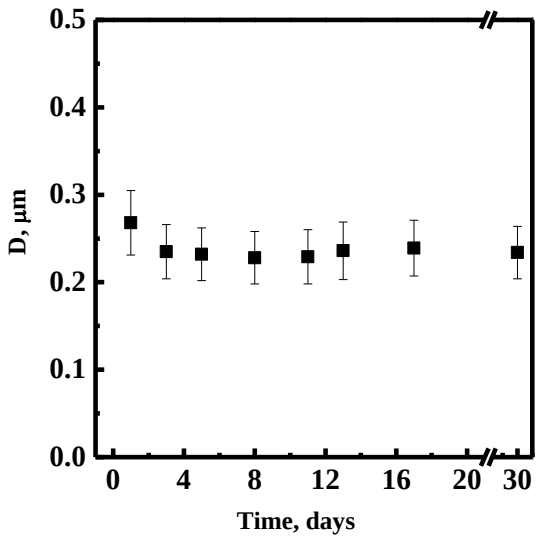

Figure 9. Suspension stability test: α-tocopherol mean diameter during 30 days of storage at 4°C.

References

Adami R, Liparoti S, Reverchon E (2011) A new supercritical assisted atomization configuration, for the micronization of thermolabile compounds. Chemical Engineering Journal 173 (1):55-61. doi:DOI 10.1016/j.cej.2011.07.036

Ballabio C, Restani P (2012) Lipids in functional foods, nutraceuticals and supplements. European Journal of Lipid Science and Technology 114 (4):369-371. doi:10.1002/ejlt.201200111

Brunner G (1994) Gas extraction, Springer Verlag. New York

Campardelli R, Adami R, Della Porta G, Reverchon E (2012a) Nanoparticle precipitation by Supercritical Assisted Injection in a Liquid Antisolvent. Chemical Engineering Journal 192:246-251. doi:DOI 10.1016/j.cej.2012.04.010

Campardelli R, Adami R, Reverchon E (2012b) Preparation of Stable Aqueous Nanodispersions of β-Carotene by Supercritical Assisted Injection in a Liquid Antisolvent. Procedia Engineering 42 (0):1493-1501. doi:http://dx.doi.org/10.1016/j.proeng.2012.07.542

Campardelli R, Della Porta G, Gomez V, Irusta S, Reverchon E, Santamaria J (2013) Encapsulation of titanium dioxide nanoparticles in PLA microspheres using supercritical emulsion extraction to produce bactericidal nanocomposites. J Nanopart Res 15 (10)

Campardelli R, Oleandro E, Adami R, Reverchon E Polymethylmethacrylate (PMMA) Sub-Microparticles produced by Supercritical Assisted Injection in a Liquid Antisolvent. The Journal of Supercritical Fluids (0). doi:http://dx.doi.org/10.1016/j.supflu.2014.05.012

Cheong JN, Tan CP, Man YBC, Misran M (2008) α-Tocopherol nanodispersions: Preparation, characterization and stability evaluation. Journal of Food Engineering 89 (2):204-209. doi:http://dx.doi.org/10.1016/j.jfoodeng.2008.04.018

Fernholz E (1938) On the Constitution of α-Tocopherol. J Am Chem Soc 60 (3):700-705. doi:10.1021/ja01270a057

Franck EU (1984) Physicochemical Properties of Supercritical Solvents (Invited Lecture). Berichte der Bunsengesellschaft für physikalische Chemie 88 (9):820-825. doi:10.1002/bbpc.19840880910

Grau MJ, Kayser O, Müller RH (2000) Nanosuspensions of poorly soluble drugs — reproducibility of small scale production. Int J Pharmaceut 196 (2):155-159. doi:http://dx.doi.org/10.1016/S0378-5173(99)00411-1

Hu J, Johnston KP, Williams RO (2004) Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs. Drug Dev Ind Pharm 30 (3):233-245. doi:doi:10.1081/DDC-120030422

Huang Q, Yu H, Ru Q (2010) Bioavailability and Delivery of Nutraceuticals Using Nanotechnology. J Food Sci 75 (1):R50-R57. doi:10.1111/j.1750-3841.2009.01457.x

Merisko-Liversidge EM, Liversidge GG (2008) Drug Nanoparticles: Formulating Poorly Water-Soluble Compounds. Toxicol Pathol 36 (1):43-48. doi:10.1177/0192623307310946

Patel VR, Agrawal YK (2011) Nanosuspension: An approach to enhance solubility of drugs. Journal of advanced pharmaceutical technology & research 2 (2):81-87

Reverchon E, Antonacci A (2007) Polymer microparticles production by supercritical assisted atomization. The Journal of Supercritical Fluids 39 (3):444-452. doi:http://dx.doi.org/10.1016/j.supflu.2006.03.005

Reverchon E, De Marco I, Della Porta G (2002) Tailoring of nano- and micro-particles of some superconductor precursors by supercritical antisolvent precipitation. J Supercrit Fluid 23 (1):81-87

Reverchon E, Della Porta G (2003) Particle design using supercritical fluids. Chemical Engineering and Technology 26 (8):840-845

Reverchon E, Della Porta G, Pallado P (2001) Supercritical antisolvent precipitation of salbutamol microparticles. Powder Technol 114 (1-3):17-22

Sane A, Limtrakul J (2009) Formation of retinyl palmitate-loaded poly(l-lactide) nanoparticles using rapid expansion of supercritical solutions into liquid solvents (RESOLV). The Journal of Supercritical Fluids 51 (2):230-237. doi:http://dx.doi.org/10.1016/j.supflu.2009.09.003

Shahidi F (2009) Nutraceuticals and functional foods: Whole versus processed foods. Trends Food Sci Tech 20 (9):376-387. doi:http://dx.doi.org/10.1016/j.tifs.2008.08.004

Shekunov BY, Chattopadhyay P, Seitzinger J, Huff R (2006) Nanoparticles of poorly water-soluble drugs prepared by supercritical fluid extraction of emulsions. Pharm Res 23 (1):196-204. doi:10.1007/s11095-005-8635-4

Stievano M, Elvassore N (2005) High-pressure density and vapor–liquid equilibrium for the binary systems carbon dioxide–ethanol, carbon dioxide–acetone and carbon dioxide–dichloromethane. The Journal of Supercritical Fluids 33 (1):7-14. doi:http://dx.doi.org/10.1016/j.supflu.2004.04.003

Strickley R (2004) Solubilizing Excipients in Oral and Injectable Formulations. Pharmaceutical Research 21 (2):201-230. doi:10.1023/B:PHAM.0000016235.32639.23

Suzuki K, Sue H, Itou M, Smith RL, Inomata H, Arai K, Saito S (1990) Isothermal vapor-liquid equilibrium data for binary systems at high pressures: carbon dioxide-methanol, carbon dioxide-ethanol, carbon dioxide-1-propanol, methane-ethanol, methane-1-propanol, ethane-ethanol, and ethane-1-propanol systems. Journal of Chemical & Engineering Data 35 (1):63-66. doi:10.1021/je00059a020

Tan CP, Nakajima M (2005) β-Carotene nanodispersions: preparation, characterization and stability evaluation. Food Chemistry 92 (4):661-671. doi:http://dx.doi.org/10.1016/j.foodchem.2004.08.044

Tsukada Y, Hara K, Bando Y, Huang CC, Kousaka Y, Kawashima Y, Morishita R, Tsujimoto H (2009) Particle size control of poly(dl-lactide-co-glycolide) nanospheres for sterile applications. Int J Pharm 370 (1-2):196-201. doi:10.1016/j.ijpharm.2008.11.019

Van Eerdenbrugh B, Van den Mooter G, Augustijns P (2008) Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharmaceut 364 (1):64-75. doi:http://dx.doi.org/10.1016/j.ijpharm.2008.07.023

Vazquez G, Alvarez E, Navaza JM (1995) Surface Tension of Alcohol Water + Water from 20 to 50 .degree.C. Journal of Chemical & Engineering Data 40 (3):611-614. doi:10.1021/je00019a016

Young TJ, Mawson S, Johnston KP, Henriksen IB, Pace GW, Mishra AK (2000) Rapid Expansion from Supercritical to Aqueous Solution to Produce Submicron Suspensions of Water-Insoluble Drugs. Biotechnology Progress 16 (3):402-407. doi:10.1021/bp000032q

Zhu WZ, Wang JX, Shao L, Zhang HX, Zhang QX, Chen JF (2010) Liquid antisolvent preparation of amorphous cefuroxime axetil nanoparticles in a tube-in-tube microchannel reactor. Int J Pharm 395 (1-2):260-265. doi:10.1016/j.ijpharm.2010.05.018