Preparation, Characterization and Stability Evaluation of α-Tocopherol Nanosuspensions Produced Using A Supercritical Assisted Process

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ABSTRACT

In this work 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 in vitamin supplementation and as antioxidant in food, cosmetics and pharmaceutical industries. However poor solubility, and thus low bioavailability, of this compound has made its use problematic. The formulation of this compound in nanosized suspensions is particularly appealing because it is known that nanoparticles increase the surface areas and dissolution rate, thus increasing the bioavailability. For these reasons in this work SAILA process has been applied for the production of α-tocopherol nanoparticles suspensions. Stable α-tocopherol particles suspensions with mean diameters down to 300 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.

INTRODUCTION

Functional lipids, such as carotenoids, phytosterols, ω-3 fatty acids, natural antioxidants, and various other compounds, are widely used as active ingredients in food products [1]. In particular, natural antioxidants such as α-, β-, γ-, δ-tocopherol and tocotrienol are largely used in vitamin supplementation and as antioxidants in food [2, 3]. However, the poor water solubility of functional lipids has made their use problematic for food formulations. Nanodispersion is a promising approach to overcome bioavailability problems. The increase in bioavailability is due to the increase of the exposed surface areas that increases the dissolution velocity of poor water-soluble compounds [4]. Conventional techniques for the preparation of nanosuspensions, have limitations that include organic solvent use, thermal degradation, large residual solvent content, and difficulties in controlling particle size and size distribution during processing [5].

Reverchon and co-workers have recently proposed a new supercritical assisted technique for the production of stable aqueous nanodispersions named Supercritical Assisted Injection in Liquid Antisolvent (SAILA) [6]. In this process, an expanded liquid solution is formed using SC-CO_2 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. The production of particles takes advantage of the expanded liquid properties, in particular reduced surface tension [7]. Indeed, in processes based on liquid antisolvent, the particles size of the precipitates depends on the efficiency of the mixing between the two liquids that, in turn, is 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 [8]. Moreover the SAILA technique is a continuous process that allows the direct production of particles in stabilized water suspensions in a single step process. In previous works the SAILA process has been proposed and successfully demonstrated for polycaprolactone (PCL) [6]. Non coalescing nanoparticles were produced and the effect of different process conditions on particles size distribution was studied. SAILA process was successfully applied for the production of stable β-carotene nanodispersions [9].

For this reason, the SAILA process will be applied to increase to bioavailability of a liposoluble vitamin, trying to produce stabilized α-tocopherol aqueous nanosuspensions. Objective of this work will be also to find the optimized operative conditions to obtain stable nanodispersion of α-tocopherol with sharp particles size distribution. Nanodispersions will be also characterized in terms of storage stability.

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, α-tocopherol (purity 98% Aldrich Chemical Co.) were used as received.

APPARATUS

SAILA equipment is schematically represented in **Figure 1**. Detailed description of the apparatus is reported elsewhere [6]. Briefly, the principal part of the apparatus is a saturator, where the expanded liquid is formed at desired conditions of temperature and pressure. The organic solution (solvent+solute) and the SC-CO_2 are delivered to the saturator with two different lines in a fixed gas to liquid ratio (GLR), generally expressed as weight ratio; random packings allocated inside the saturator promote the intimate mixing between the

phases, fed in co-current mode, allowing the formation of the expanded liquid. The expanded liquid mixture obtained is continuously depressurized into an aqueous receiving solution through an injector of fixed diameter dimension. The suspension can be recovered using a regulation valve.

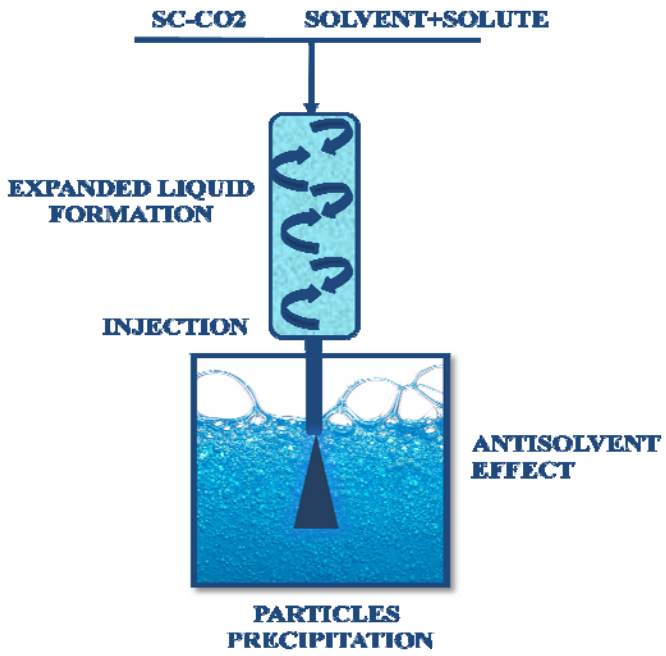

Figure 1. Schematic representation of SAILA process

METHODS

Particles size distributions (PSD) of the produced suspensions were determined by dynamic light scattering (DLS) (Zetasizer, mod. 5000, Malvern Instruments Ltd). Mean diameter (MD), standard deviation (SD) and polydispersity index (PDI) were measured three times for each experiment. 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 Å, Agar Scientific).

RESULTS AND DISCUSSION

In SAILA process, particles precipitation originates from the injection of the expanded liquid, containing the solute, in the antisolvent phase. Several process parameters can affect particles size distribution: expanded liquid (EL) pressure, temperature, X_{CO2}, solute concentration, solvent/antisolvent ratio, kind and concentration of surfactants and nozzle diameter i.e. injection pressure. The effect of a part of these parameters is discussed in the following.

In the first feasibility test on the processability of α-tocopherol, the effect of the expanded liquid solvent was also investigated. Fixing water as antisolvent, acetone (Ac), ethanol (Et) and isopropanol (iP) were used to produce suspensions. In particular, until now, the SAILA process has been performed only using acetone; the behavior with other expanded liquid mixtures has never been explored. The experiments have been performed using a 80 µm

injector, α-tocopherol concentration in solvent solution 5 mg/mL, saturator temperature 50°C, CO_2 mass flow rate 12 gr/min, GLR 1.5 see **Table 1,** α-1, α-2, α-3 experiments. Changing the solvent it is important to ensure the formation of the expanded liquid; with these operative conditions the position of the operative point is represented **Figure 2**, where vapor liquid equilibrium curves are plotted for the systems acetone-CO_2, ethanol-CO_2, isopropanol- CO_2. Each curve has a maximum value 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, instead the expanded liquid region is located on the left of the MCP. Considering α-1, α-2, α-3 experiments the operative points are situated in the EL region in all cases.

	α-1	α-2	α-3
Solvent	Ac	Et	iP
Nozzle diameter µm	80	80	80
Pressure bar	100	86	80
Temperature °C	50	50	50
X_{CO2}	0.68	0.66	0.66
MD nm ± SD	224±58	327± 71	307± 101
PDI	0.26	0.22	0.33
	EL Solvent		

Table 1. Process conditions and particles distribution data of SAILA experiments performed with different expanded liquid solvents.

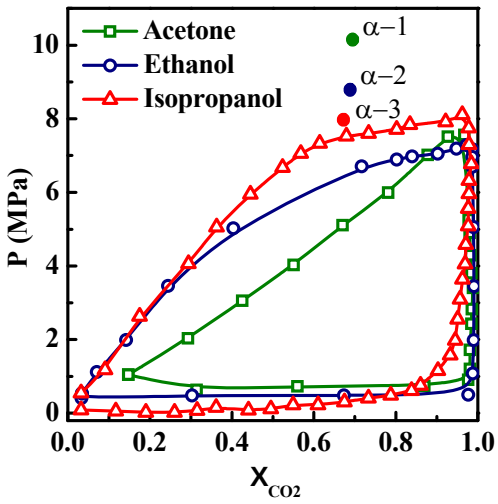

Figure 2.Vapor-liquid equilibrium curves for the systems acetone-CO_2 (□), ethanol-CO_2 (○), isopropanol- CO_2 (Δ) at 50°C adapted from [10, 11]. Operative points of experiments listed in Table 1 are also reported (●)

From results reported in **Table 1** and from PSD frequency curves of **Figure 3**, it is possible to notice that suspensions have been produced in all cases and the smallest particles have been produced when the mixture acetone-CO_2 was used (α-1), particles with a MD of 224 nm±58 and a PDI of 0.26 were obtained in this case. However, when ethanol-CO_2 mixture was used (α-2), particles with larger MD were obtained, about 327 nm±71, 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-3), that gave the largest PSD.

The different behaviors, noticed using various solvents to form the expanded liquid, can be explained considering that fixing all the process parameters, changing only the solvents, different expanded liquid pressures were established in the saturator; indeed, the higher pressure is obtained working with acetone, and then, the smallest particles have been produced using this system; whereas using ethanol and isopropanol lower pressure have been obtained in the saturator, producing, consequently, particles with lager MDs. This set of experiments point 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.

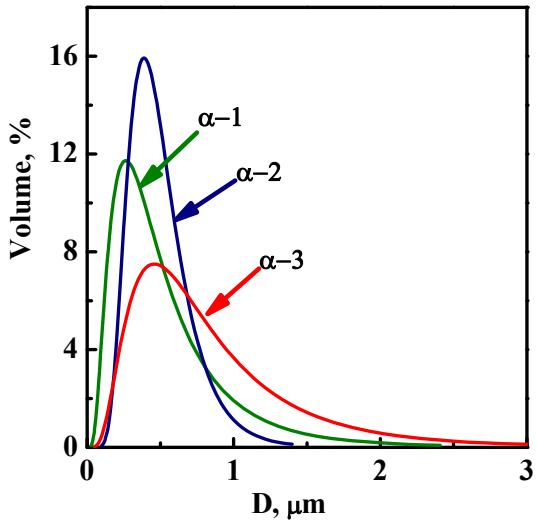

Figure 3. Volumetric PSD of α-tocopherol suspensions produced using different expanded liquid solvents: α-2 Acetone, α-3 Ethanol and α-4 isoPropanol.

Considering results obtained, it was decided to continue the optimization of the operative parameters using ethanol as expanded liquid solvent, because it was the system that have demonstrated to have a better control over PSD. Furthermore, ethanol has also the advantage to be well accepted in pharmaceutical formulations, where it is often used as co-solvent [12].

α-tocopherol suspensions produced with 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 time. Results are shown in **Figure 4** for α-5 experiment. Form this figure it is evident that α-tocopherol particles suspensions are stable during 30 days storage because particles mean diameter and standard deviation remained constant during one month; also zeta potential of all the suspensions analyzed was elevated and negative during time with a constant value comprised between -12 and -18 mV. This result is extremely important because it confirms that the SAILA process has the great advantage of producing directly stabilized suspensions in one step process.

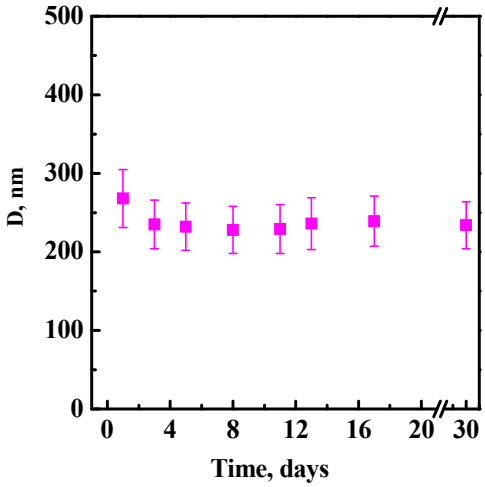

Figure 4. Suspension stability test: α-tocopherol mean diameter during 30 days of storage at 4°C.

CONCLUSIONS

In this work the SAILA process has been applied successfully for the production of stable α-tocopherol suspensions. With this new process it is possible to efficiently produce particles with dimensions tunable with operative conditions, ranging from micrometric to nanometric scale. The smallest particles, about 150 nm, have been obtained using higher injection pressure, higher saturator temperature and low solute concentration. The process has demonstrated to be able of producing directly suspensions that are stable during one month of storage.

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