Supercritical Assisted Atomization under reduced pressure: production of PEG microspheres loaded with lipophilic vitamins

S. Liparoti*, R. Adami, E. Reverchon
Department of Industrial Engineering, University of Salerno
via Ponte don Melillo Fisciano 84084 (SA), Italy

*corresponding author: sliparoti@unisa.it

Abstract

A new arrangement of supercritical assisted atomization (SAA) was proposed to allow the processing of thermolabile compounds. A vacuum system was arranged to the traditional SAA apparatus to reduce the pressure in the precipitation vessel and to allow the evaporation of the solvent at lower temperatures than the traditional ones.
Polyethylene glycol (PEG, Mw=10000) is a very interesting polymer that can be used as carrier for drugs, proteins and vitamins. Indeed, it is biodegradable and freely soluble in water, ethanol and other organic solvents, but its processability is difficult because of the low glass transition and melting temperatures. In this work, SAA operated at reduced precipitation pressure was used to produce microparticles of PEG. The operating conditions used were 40°C and 68 bar in the saturator, 20°C and 0.7 bar in the drying vessel.
Lipophilic vitamin, α-tocopherol, was coprecipitated with PEG. The aim was to improve stability, during shelf storage, and bioavailability of the vitamin. Different percentages of vitamin (5% and 10%) were loaded in PEG and spherical particles, with a mean diameter between 1 and 2 μm, were obtained, with a high encapsulation efficiency. Calorimetric analysis revealed that the microparticles obtained by SAA were crystalline.

Introduction

PEG is widely used in pharmaceutical industry to improve the pharmacokinetics of therapeutic agents [1]. PEG is water soluble, nonionic, non antigenic and non immunogenic. Added to a particle, it becomes an effective protector to inhibit the deactivation of a bioactive compound [2]. It is also used to prepare solid dispersions of poor water soluble drugs. In these solid dispersions the particle size of the drug is reduced, wettability and dispersability are enhanced [3]. Despite its interesting properties, PEG is very difficult to be processed; indeed, it has a very low glass transition temperature (-30°C) and a low melting point (63°C); therefore, the micronization process may induce the partial softens of the particles.
α-Tocopherol acetate (α-TCP) is a lipophilic vitamin. It is an antioxidant and prevents cardiovascular diseases and cancer. However, the use of α-TCP is limited because this compound is thermolabile and suffers the presence of light and oxygen, and losses its stability if dispersed in water because hydrolysis processes take place. To avoid these problems encapsulation of this vitamin or graft to succinate PEG have been proposed [14]. In addition α-TCP does not dissolve in water and surfactants are added in the formulation to enhance bioavailability [4-5].

Emulsion evaporation has been proposed to encapsulate lipophilic vitamins into biodegradable polymers, such as poly(lactic-co-glycolic)acid (PLGA) [6] or inorganic compounds, as silica [7]. Supercritical emulsion extraction has been proposed as an enhancement of emulsion evaporation to reduce the organic solvent residue and to increase the encapsulation efficiency [8]. However PEG can be used only as surfactant in these processes, since it is hydrophilic and lipophilic at the same time [9,10]. Spray Freeze Drying has been proposed to produce particles of PEG for drug delivery device [10]. This process is time consuming and a lyophilization, as post-process, is required to remove the solvent.
To limit the use of organic solvent and reduce the processing time, supercritical fluids based processes have been proposed. For example, rapid expansion from a supercritical solution with a nonsolvent (RESS-N) was used to prepare microcapsules in which PEG, or other biodegradable polymers, were used as carrier [11-12]. Supercritical antisolvent precipitation was also proposed to produce microparticles of PEG and carotene, a lipophilic vitamin [13]. All the techniques, mentioned above, do not allow an efficient micronization of PEG in terms of morphology, dimensions and processing times.
To overcome these limitations SAA at reduced pressure has been proposed to produce microspheres of PEG and α-TCP. This technique allows to operate at conditions that do not induce the degradation of vitamin and the softening of the polymer. The final formulation has the aim of protecting the vitamin during its storage and of enhancing its bioavailability.

Materials

CO_2 (99.9%, SON, Naples, Italy), nitrogen (N_2, 99.9%, SOL, Milan, Italy), acetone (99.5%, Panreac), methanol (99.9%, Carlo Erba), acetonitrile (99.9%, Carlo Erba), ethanol (99.9%, Aldrich Chemical Co.), polyethylene glycol (PEG, Mw:10000, Aldrich Chemical Co.), α-tocopherol acetate semisynthetic (α-TCP, Aldrich Chemical Co.) were used as received.

Method

The new configuration of SAA plant consists of two high-pressure pumps delivering the liquid solution and liquid CO_2 to the saturator. The saturator is a high pressure vessel (I.V. 50 cm^3) loaded with stainless steel perforated saddles which assure a large contact surface between liquid solution and CO_2. The solution obtained in the saturator is sprayed through a thin wall (80 µm diameter) injection nozzle into the precipitator (I.V. 3 dm^3). A controlled flow of N_2 was sent to the precipitator to assist liquid droplets evaporation. A stainless steel filter, located at the bottom of the precipitator, allows the powder collection and the gaseous stream flow out. Downstream the precipitator, a condenser separates the liquid stream from the inert gas and a vacuum pump allows to reduce the pressure in the precipitator.
Morphological characteristic of PEG loaded α-TCP particles were analyzed by Field Emission Scanning Electron Microscope (FESEM). Particle size and particle size distribution were investigated using dynamic laser scattering (DLS) (Zetasizer); the analysis were performed using acetone to disperse the particles.
Thermograms of powder samples were obtained using a differential scanning calorimeter (DSC). The samples (±5 mg) were accurately weighed, crimped in an aluminium pan and heated from to 25 to 100°C at 5°C/min, under a nitrogen purge of 50 mL/min.
Vitamin loading was investigated by dissolving 5 mg of powder in 2.5 mL (5% α-TCP) and 5 mL (10% α-TCP) of methanol, and vitamin concentration were monitored at 292 nm in a

HPLC system, using methanol/acetonitrile (25/75 v/v) as eluent in a Spherisorb ODS-2 column at a flow rate of 1.5 ml/min.
The stability to hydrolysis of the vitamin in water medium was investigated dissolving the powder produced by SAA in water. 1 mL of the resulted solution was eluted in ethanol (50/50 v/v) and the concentration of the vitamin was measured using HPLC analysis as previously discussed.

Results

SAA process is based on the solubilization of SC-CO_2 into the liquid solution. The vapor-liquid equilibrium (VLE) between SC-CO_2 and acetone [17] (Figure 2) gives some indications about the proper conditions that have to be used in the saturator.

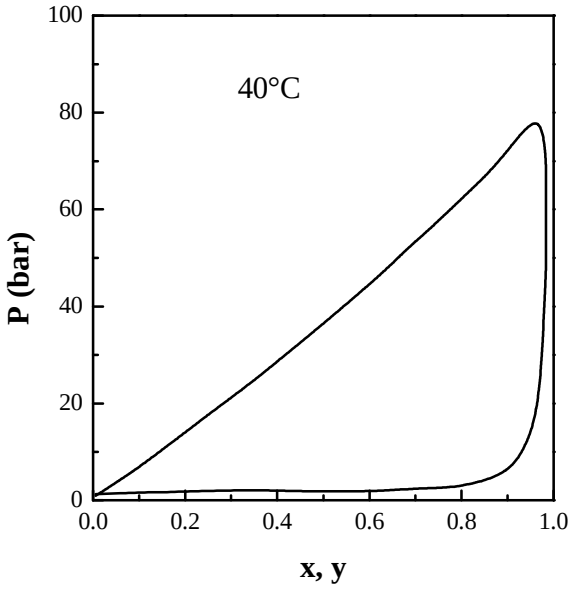

Figure 1 VLE of the system CO_2-acetone, at 40°C, adapted from Sato et al [17].

The complete solubilization of SC-CO_2 in acetone is obtained when the operative conditions at the saturator are located on the left of the two phase region.
All the experiments were performed setting a gas to liquid ratio (GLR) between 3.5 and 4, corresponding to a CO_2 molar fraction of 0.8-0.85. The other conditions used in the saturator were: pressure between 68 and 100 bar; temperature at 40°C, to enhance the solubility of PEG in acetone; concentration of PEG in acetone of about 20 mg/mL. The conditions set in the precipitator were 20°C and 0.7 bar.
The first tests were performed on PEG and white powder was collected on the filter, whereas no materials were found in the saturator at the end of the experiment. An example of PEG microparticles is reported in Figure 1.

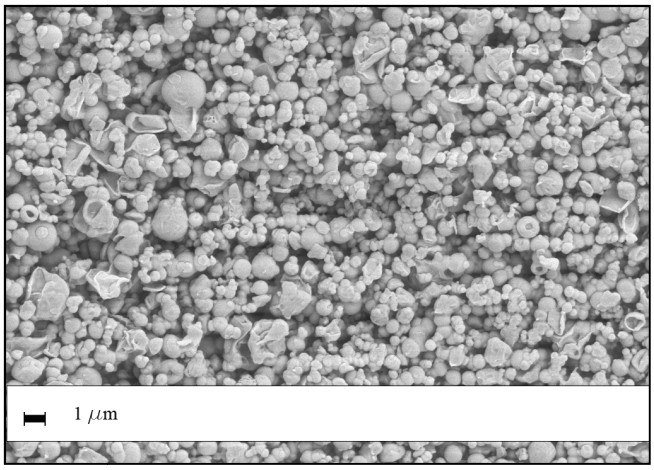

Figure 2 PEG microparticles obtained by SAA.

The particles obtained were not coalescent but the morphology was not uniform. This was probably due to the low glass transition temperature of the polymer that led to an unstable particle structures during the atomization.
Other experiments were performed adding α-TCP in the solution of PEG and acetone that was fed to the saturator.

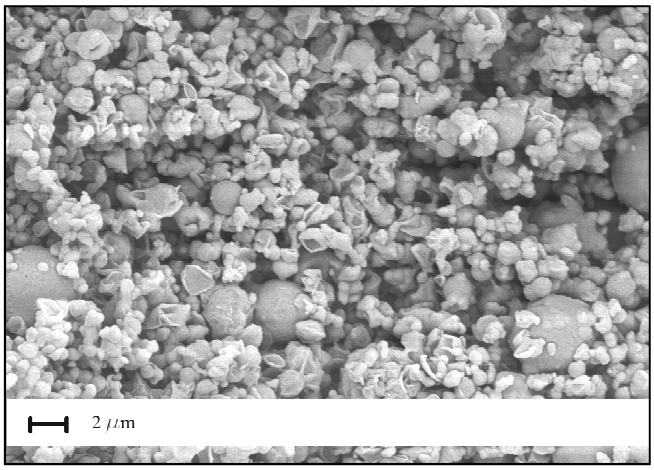

Figure 3 PEG+5% (w/w) of α-TCP. Microparticles obtained by SAA.

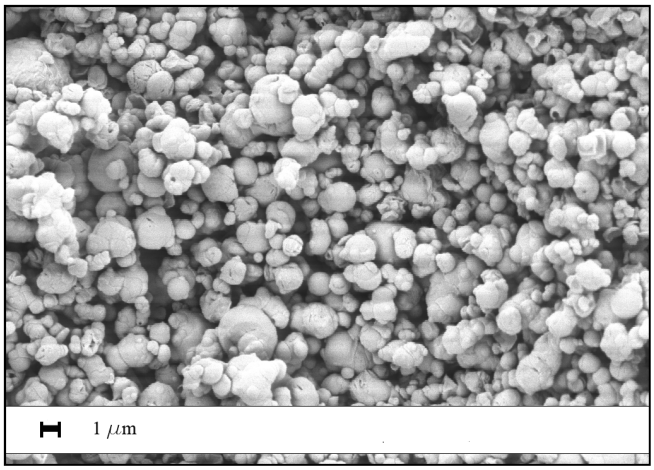

Figure 4 PEG loaded 10% (w) of α-TCP microparticles obtained by SAA.

Figure 3 shows that adding 5% of α-TCP the produced particles had not a stable structure, indeed some particles collapsed. Instead the particles obtained increasing the content of α-TCP to 10% had a more stable structure (see Figure 4) and quasi-spherical particles were obtained.

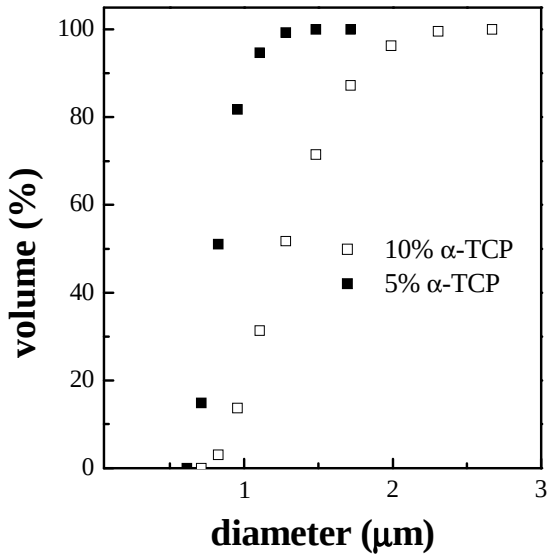

Figure 5 Cumulative volumetric particle size distribution of the particles produced using SAA. Comparison between the particles loaded with different percentage of α-TCP.

Figure 5 shows the particle size distribution related to the particles produced using SAA. The particles have a mean diameter ranged between 0.8 and 1.3 μm. The presence of a larger percentage of α-TCP induced an increasing of particle dimensions.

DSC analysis gave some information about the solid state of the particles produced. Figure 6 shows a comparison between the thermograms related to the raw materials and the thermograms related to the particles produced by SAA process. The thermograms related to the unprocessed PEG shows the double melting behavior of the polymer, due to the presence of imperfect crystals that melt at different temperatures [16]. The thermograms related to the

particles produced by SAA had only the peak at 62°C, corresponding to the more stable crystals structure.

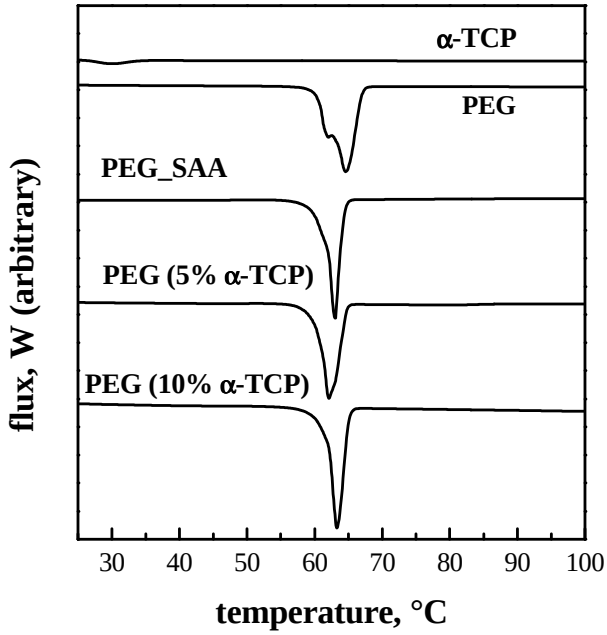

Figure 6 Thermograms: comparison among the analysis performed on the powder produced by SAA-RP and the raw materials, PEG and α-TCP untreated.

The analysis performed using HPLC showed that the α-TCP, that was solubilized in the initial solution, was completely loaded in the polymer matrix. The measured loading was close to theoretical one [15].
Finally the stability to hydrolysis of the vitamin in water medium was investigated using HPLC (Table 1). The vitamin retained at least 67% of its stability when loaded in PEG matrix.

Time, min	Stability %
8	81.56
9	78.26
10	67.54
70	67.48
1 day	67.5

Table 1 Stability to hydrolysis, in water medium, of the vitamin in SAA formulation. The percentage was related to the content of vitamin loaded in polymer matrix.

Conclusion

The SAA process was successfully performed for the micronization of PEG and α-TCP loaded with PEG. The obtained particles had a regular morphology and vitamin preserved its stability when dissolved in water medium.

REFERENCES

[1]. SEAMPLE, S.C., HARASYM, T.O., CLOW, K.A., ANSELL, S.M., KLIMUK, S.K., HOPE, M.J., The Journal of Pharmacology and Experimental Therapeutics, Vol. 312, **2004**, p. 1020

[2]. JUNG, I.I., JOO, H.J., LIM, G.B., RYU, J.H., 9th conference on supercritical fluids and their application, Sorrento, Italy, **2010**, p243.

[3]. DHANARAJU, M.D., THIRUMURUGAN, G., International Journal of PharmTech Research, Vol. 2, **2010**, p. 480.

[4]. YOO, S.H., SONG, Y.B., CHANG, P.S., LEE, H.G., International journal of Biological Macromolecules, Vol. 38, **2008**, p. 25.

[5]. SOMCHUE, W., SERMSRI, W., SHIOWATANA, J., SIRIPINYANOND, A., Food research International, Vol. 42, **2009**, p. 909.

[6]. MATSUMOTO, A., KITAZAWA, T., MURATA, J., HORIKIRI, Y., YAMAHARA, H., Journal of Controlled Release, Vol. 129, **2008**, p. 223.

[7]. HWANG, Y.J., OH, C., OH, S.G., Journal of Controlled Release, Vol. 106, **2005,** p. 339.

[8]. DELLA PORTA, G., CAMPARDELLI, R., FALCO, N., REVERCHON, E., Journal of pharmaceutical science DOI 10.1002/jps.22467, **2011**.

[9]. OULD-OUALI, L., ARIËN, A., ROSENBLATT, J., NATHAN, A., TWADDLE, P., MATALENAS, T., BORGIA, M., ARNOLD, S., LEROY, D., DINGUIZLI, M., ROUXHET, L., BREWSTER, M., PRÉAT, V., Pharmaceutical Research, Vol. 21, **2004**, p.1581.

[10]. BARRON, M.K., YOUNG, T.J., JOHNSTON, K.P., WILLIAMS, R.O., AAPS PharmSciTech, Vol. 4, **2003**, p. 12.

[11]. MATSUYAMA, K., MISHIMA, K., HAYASHI, K.I., ISHIKAWA, H., MATSUYAMA, H., HARADA, T., Journal of Applied Polymer Science, Vol. 89, **2002**, p. 742.

[12]. MISHIMA, K., MATSUYAMA, K., TANABE, D., YAMAUCHI, S., YOUNG, T.J., JOHNSTON, K.P., Materials, Interfaces and Electrochemical Phenomena, Vol. 26, **2000**, p. 857.

[13]. HE, W., SUO, Q., HONG, H., SHAN, A., LI, C., HUANG, Y., LI, Y., ZHU, M., Journal of Material Science, Vol. 42, **2007**, p. 3495.

[14]. WU, S.H.W., HOPKINS, W.K., Pharmaceutical Technology, October, **1999**.

[15]. DELLA PORTA, G., ADAMI, R., DEL GAUDIO, P., PROTA, L., AQUINO, R., REVERCHON, E., Journal Of Pharmaceutical Sciences, Vol. 99, **2010**, p. 4720.

[16]. DELAHAYE, N., DUCLOS, R., SAITER, J.M., International Journal of Pharmaceutics, Vol. 157, **1997**, p. 27.

[17]. SATO, Y., HOSAKA, N., YAMAMOTO, K., INOMATA, H., Fluid Phase Equilibria, Vol.296, **2010**, p.25.