Supercritical fluid extraction of emulsions for the production of vitamin E nanoencapsulates

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INTRODUCTION AND OBJECTIVE

SFEE (*Supercritical Fluid Extraction of Emulsions*) is a new particle formation technology (Chattopadhyay 2006). It uses supercritical CO_2 (sc CO_2) to rapidly extract the organic phase of an emulsion, in which a bioactive compound has been previously dissolved. By eliminating the solvent, the solute precipitates, which generates a suspension of particles of that compound in water. The produced particles have controlled size and morphology, due to the use of the emulsion, which acts as a template, and to the fast kinetics of the sc CO_2 extraction, which avoids particle agglomeration. This method has been mainly used for the entrapment of a compound within a secondary material which protects and stabilizes the core from a variety of physical and chemical factors. Up to now, little work was done, and most of it was related to pharmaceutical compounds encapsulated in PLGA (poly(lactic-co-glycolic acid)). However, this method is a promising technique to encapsulate nutraceuticals for the food industry.

The aim of this work was the application of this technology for the production of nanostructures of vitamin E (vit. E), which is considered one of the most important antioxidants for human nutrition. It has high sensitivity to light, heat and oxygen and it is not soluble in water, what can be avoided by its encapsulation. Furthermore, the encapsulation of a liquid is a very interesting application for the future production of 'liquids into powders'.

MATERIALS AND METHODS

Materials α-tocopherol (≥95%), polycaprolactone (PCL) (MW of 10,000), Tween 80, acetone (≥99,5% (GC)), ethanol (absolute) were all from Sigma Aldrich and used as received. Millipore water was used throughout the study.

Sample preparation The emulsions were formed accordingly to the following procedure: a) addition of the adequate quantity of oil, in which the vit. E (0.51 wt. %) and the coating polymer, PCL (0.63 wt. %) were previously dissolved at 40ºC, b) addition of the adequate quantity of surfactant, c) mechanical shaking during 1 min on the vortex, d) addition of the adequate quantity of water, e) vigorous mechanical shaking for 5 min on the vortex in order to guarantee a homogeneous dispersion, f) control of temperature at 40ºC, by placing the tube within a thermostatic water bath.

Study of the phase behaviour
Twenty compositions in weight were chosen to cover the whole ternary phase diagram, in order to differentiate between emulsion and microemulsion and select the best composition to be then subjected to SFEE.

Formation of the nanoparticles by SFEE
The supercritical extraction apparatus consisted in a 100 ml cylindrical stainless steel vessel in which 50 g of emulsion were placed for each experiment. Liquid cooled CO_2 was delivered using a high pressure membrane pump (Milroyal D, Milton Roy) and was introduced into the extraction vessel at a constant flow rate of $1 g \cdot min^{-1}$. The temperature in the extractor was regulated by a heating jacket and read inside the vessel by a thermocouple type K within ± 1ºC. The pressure in the separator was regulated by a backpressure valve (BPR) and read in a Bourbon manometer within ± 3 bar. A mass flow meter (AlicatScientific, M-10SLPM-D) was used to read the total amount of CO_2 employed. A scheme of the equipment is shown in Fig. 1.

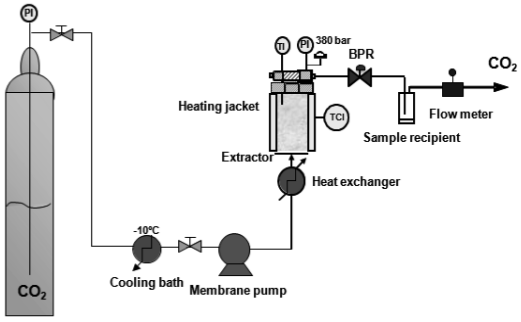

Figure 1: Equipment scheme.

Product characterization To determine the encapsulation efficiency, the suspension of nanoparticles was centrifuged at 15,000 rpm (Digicen 21). The pellet was washed with ethanol and the concentration of vit. E in the supernatant was analysed by UV-vis spectrophotometry (MRC UV1800).

The loading capacity was calculated as the rate between the encapsulated vit. E and the amount of polymer employed.
A sample of the nanoparticles was studied by SEM (JEOL JSM 6335F) and TEM (JEOL JEM 1010) to analyse their morphology.

RESULTS AND DISCUSSION

Determination of the phase behaviour

The phase behaviour of this system was obtained by visual observation of each sample, and plotted as a pseudoternary phase diagram shown in Fig. 2.

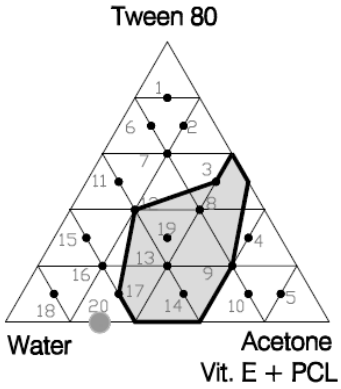

Figure 2: Phase map of the system Tween 80/ acetone + vit. E + PCL / water.

The system shows three behaviours represented with different shading: area without shading or microemulsion; area with a light grey shading or phase separation corresponding to the bicontinuous structure; and finally, sample 20 which corresponds to the emulsion region. This sample, 71.6% of water, 28.3% of acetone and 0.07% of Tween 80 in weight, was then selected to create the nanoparticles.

Formation of the nanoparticles by SFEE

The formation of the nanoparticles of vit. E by SFEE is based on the precipitation of the polymer once the organic solvent is extracted from the emulsion by the supercritical CO_2. The result of this operation is a suspension of the encapsulated nanoparticles in water, which could be recovered by evaporation or by centrifugation.

The operation conditions were selected to get a complete extraction of acetone without extracting the vit. E, according to bibliographic data of the solubility of acetone (Katayama 1975) and vit. E (Chrastil 1982). The temperature and pressure selected were 40ºC and 80 bar, respectively because at these conditions, sc CO_2 and acetone were completely miscible and the solubility of vit. E very low.

Other parameters that can affect acetone extraction are the flow, the operation time and the extractor configuration. With this extractor and with a flow of 1g·min^{-1}, 2 h of operation time were needed to guarantee the complete extraction of the acetone.

Product characterization

The nanoparticles suspension was centrifuged, washed with ethanol, and then analysed by UV-vis spectrophotometry. The encapsulation efficiency was calculated assuming that none of the vit. E was extracted by sc CO_2, as the difference between the initial amount and the one dissolved in the ethanol. Thus, the encapsulation efficiency was around 80%. This result was similar to that obtained by Khayata (2012) using the nanoprecipitation method for the same system. Thus, the loading capacity was 64%.

Regarding to nanoparticles morphology, images obtained by SEM and TEM (See Fig. 3) showed that the particles were spherical and with size around 300 nm. No particle agglomeration was observed.

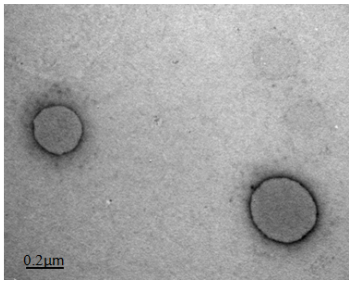

Figure 3: TEM of vit. E loaded nanocapsules.

CONCLUSIONS

The SFEE technology was successfully applied to the production of vit. E nanoencapsulates. High encapsulation efficiency and high loading capacity were obtained. Particle size was of the order of magnitude of the starting emulsion droplet. Morphology was also adequate. Furthermore, despite being a high pressure technology, moderate pressure was used, so the compression costs could be easily overcome by the added value of the product. We are working now on the optimization of the emulsion characteristics regarding to the concentration of the Tween 80 and acetone to reduce the raw materials costs, and to the ratio between vit. E and PCL to increase the coverage ratio. Optimization of the extractor design will also reduce the operation time.

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