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Incorporation of liposoluble vitamins within PVP microparticles using supercritical antisolvent precipitation

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ABSTRACT

In this work, the incorporation of two liposoluble vitamins, α-tocopherol (TOC) and menadione (MEN), within polyvinylpyrrolidone (PVP) microparticles using Supercritical Antisolvent (SAS) coprecipitation is proposed. In order to control microspheres' size and morphology, the effect of SAS main process parameters, such as polymer/vitamin ratio, operating pressure, temperature and overall concentration, was investigated.

In the case of the system PVP/TOC, composite microparticles with mean diameters in the range 1.69–4.08 μm were successfully produced; for the system PVP/MEN, composite microparticles with mean diameters in the range 2.64–5.09 μm were obtained, depending on the operating conditions.

Powders were characterized using UV–vis spectroscopy to calculate the vitamin entrapment efficiency and vitamin dissolution rate, FT-IR to identify possible interactions between the polymer and the vitamin, HPLC to verify the vitamin integrity, and GC to calculate the residual solvent. The analyses revealed that the drug entrapment efficiency was about 53% for TOC and 50% for MEN, and that the vitamin dissolution rate of the coprecipitates was between 3 and 3.5 times faster than the dissolution rate of unprocessed vitamins, respectively.

1. Introduction

Liposoluble vitamins (A, D, E and K) are crucial for human health and can be taken by food or by vitamin supplements. α-tocopherol belongs to Vitamin E group and it is a powerful antioxidant, since it is able to block free radicals, protecting the cell membrane from the oxidation of the lipids [1,2]; moreover, it is used in food industry as antioxidant in emulsions or dairy products to prolong the products' shelf life [3]. Menadione or vitamin K_3 is a synthetic liposoluble vitamin, which shows antihemorrhagic and anticancer activities [4]. Since vitamins are sensitive molecules, their activity and integrity can be easily compromised by light, moisture, oxygen and high temperature, during storage [5]. Moreover, they have a very low solubility in water and, therefore, a reduced bioavailability. In order to preserve their properties and improve their dissolution rate, an effective approach is represented by encapsulation in a polymeric carrier.

Many encapsulation methods for fat soluble vitamins have been proposed in the literature: spray-drying, emulsification/solvent evaporation, melt extrusion, freeze drying, and coacervation [1]. These processes show several limitations: obtainment of large particles with broad particle size distribution (PSD), possible degradation of the active compound and difficulties in the complete elimination of the organic solvents used in the process [6].

Several authors [7–10] proposed tocopherol encapsulation using spray-drying technique; however, they obtained large irregular particles with a wide particle size distribution. Zigoneanu et al. [11] used emulsion evaporation method for the production of α-tocopherol/PLGA nanoparticles; however, they did not report the comparison of the drug dissolution profiles between the unprocessed vitamin and the processed powder to demonstrate the improvement of the dissolution rate. Wang et al. [12] proposed the encapsulation of liposoluble vitamins using complex coacervation technique; they obtained irregular particles with a mean size in the range 50–80 μm and did not report the dissolution behavior of the obtained particles.

In order to overcome the limitations of the conventional techniques, supercritical fluid (SCF) based processes have been extensively proposed in the literature for the micronization and coprecipitation of different kind of compounds [13–17], the extraction of natural matter [18–21], the impregnation of drugs in aerogel [22–24], membranes and scaffolds production [25–27]. Among the micronization techniques, nanoparticles and microparticles of different kind of materials have been successfully obtained by supercritical antisolvent (SAS) precipitation [28–34].

SAS coprecipitation was also attempted in several cases [35–39].

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However, the results were frequently not successful due to the difficulty in forcing the simultaneous precipitation of two solutes (polymer + active compound) to form multicomponent structures. Indeed, especially when nanoparticles are obtained, SAS solid products tend to precipitate separately by homogeneous nucleation and growth; therefore, as a rule, materials that are similar to physical mixtures formed by sub-micro and nanoparticles are obtained. For example, Jin et al. [36], using supercritical antisolvent process, obtained nanoparticles of hydroxypropyl methyl cellulose phthalate + insulin. They showed the in vitro release profiles of insulin/HPMCP nanospheres, but a comparison with the release profiles of unprocessed insulin or with the ones of the physical mixture hydroxypropyl methyl cellulose phthalate + insulin was not shown; therefore, the effective coprecipitation was not demonstrated. Only recently Reverchon and co-workers [5,31,32,40] demonstrated that successful coprecipitates could be obtained, using polyvinylpyrrolidone (PVP) as the coprecipitated polymer. Indeed, PVP was successfully precipitated in form of microparticles by SAS [41] and shows a well established ability to control the crystallization of coprecipitated products, forming spherical microparticles in which the active compound is finely dispersed in the PVP structure. Since literature works shown the difficulties in encapsulating fat soluble vitamins, in this work we tried to apply SAS coprecipitation assisted by PVP to produce microparticles of PVP-α-tocopherol (TOC) and PVP-menadione (MEN) to verify the applicability of this process to two relevant vitamins, extending the range products that can be SAS coprecipitated.

The effects of polymer/vitamin ratio, operating pressure, temperature and overall concentration on particles' morphology, mean size and size distribution will be investigated and discussed. Entrapment efficiency and dissolution tests of coprecipitates will be performed to verify process effectiveness, FT-IR analyses will be used to identify possible interactions between the polymer and the vitamin, and GC separation will be performed to calculate the residual solvent in the obtained powder. The possible precipitation mechanisms will be also discussed.

2. Materials, methods and procedures

2.1. Materials

Polyvinylpyrrolidone (PVP, average molecular weight 10000 g/mol), α-tocoferol (TOC, purity ≥ 96%), Menadione (MEN, purity ≥ 99%) and Dimethylsulfoxide (DMSO, purity 99.5%) were supplied by Sigma–Aldrich (Italy). CO_2 (purity 99%) was purchased from SON (Italy). All materials were used as received.

2.2. SAS apparatus and procedure

The SAS equipment used for the experiments discussed in this work (sketched in Fig. 1) consists of two high pressure pumps to feed the liquid solution and carbon dioxide. A cylindrical vessel with an internal volume of 500 cm^3 is employed as precipitation vessel. The liquid solution is sprayed in the precipitator through a thin wall, 100 μm internal diameter stainless steel nozzle. The temperature control is assured by a PID controller connected with electrically thin bands and the pressure in the vessel is measured using a test gauge manometer and regulated by a micrometering valve. Supercritical CO_2 ($scCO_2$), after preheating, is co-currently delivered to the precipitator through another port located on the top of the vessel. A stainless steel filter (pore diameter of 0.1 μm) located at the bottom of the chamber is used to collect the produced powder and allows the CO_2–organic solvent solution to pass through. A second collection vessel located downstream the precipitator at a lower pressure (18–20 bar) is used to recover the liquid solvent. At the exit of the second vessel, the CO_2 flow rate and the total quantity of antisolvent delivered are respectively measured by a rotameter and a dry test meter.

A SAS experiment starts pressurizing the precipitation vessel with CO_2 until the desired pressure is reached; then, pure solvent is delivered to the vessel for at least 15 min. Once quasi-steady state composition of solvent and antisolvent is reached inside the chamber, the solvent flow is stopped and the liquid solution is delivered through the nozzle, producing the precipitation of the solute. At the end of the solution delivery, the precipitator is washed, flushing only supercritical CO_2, to remove the solution formed by the solvent solubilized in the supercritical antisolvent. When the washing step is completed, CO_2 flow is stopped, the precipitator is gradually depressurized down to atmospheric pressure and the precipitated powder can be collected and characterized.

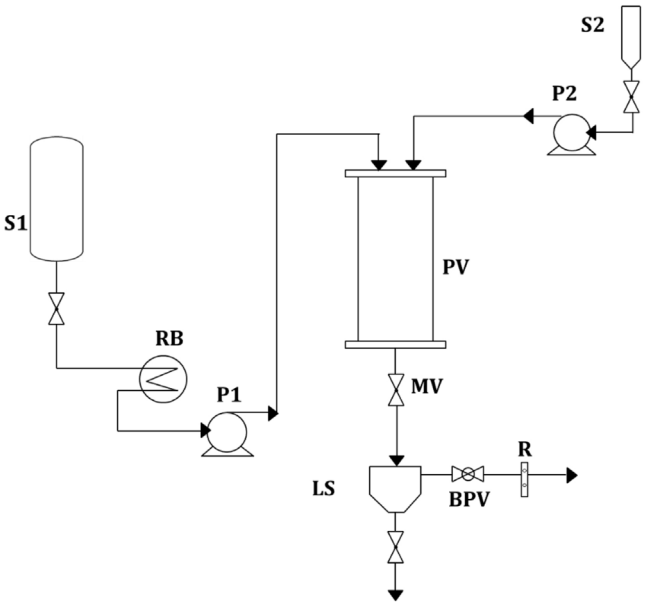

Fig. 1. Schematic representation of SAS apparatus. S1: CO_2 supply; S2: liquid solution supply; RB: refrigerating bath; P1, P2: pumps; PV: precipitation vessel; MV: micrometering valve; LS: liquid separator; BPV: back-pressure valve; R: rotameter.

2.3. Analytical methods

Samples of the precipitated material were observed by a Field Emission Scanning Electron Microscope (FE-SEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). Powder was dispersed on a carbon tab previously stuck to an aluminum stub (Agar Scientific, United Kingdom); then, was coated with gold-palladium (layer thickness 250 Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom).

Particle size distributions (PSDs) of the powders were measured from FE-SEM photomicrographs using the Sigma Scan Pro image analysis software (release 5.0, Aspire Software International Ashburn, VA). Approximately 1000 particles, taken at high enlargements and in various locations inside the precipitator, were analyzed in the elaboration of each particle size distribution. Histograms representing the particle size distributions were fitted using Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA). We were not able to calculate PSDs using the dynamic laser scattering (DLS) technique, since we did not find an effective dispersant in which both PVP and vitamins are not soluble.

Fourier transform infrared (FT-IR) spectra were obtained via M2000 FTIR (MIDAC Co, Costa Mesa, CA), at a resolution of 0.5 cm^{-1}. The scan wavenumber range was 4000–400 cm^{-1}, and 16 scan signals were averaged to reduce the noise. The powder samples were ground and mixed thoroughly with potassium bromide (KBr) as infrared transparent matrix. KBr discs were prepared by compressing the powders in a hydraulic press.

Vitamin dissolution studies were performed using an UV/vis