The Journal of Supercritical Fluids 131 (2018) 124–129

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

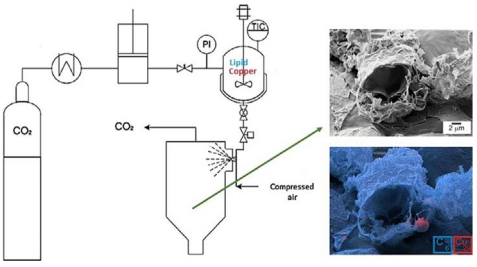
journal homepage: www.elsevier.com/locate/supflu

Production of copper loaded lipid microparticles by PGSS® (particles from gas satured solutions) process

Víctor Martín[a], Vanessa Gonçalves[a,b,c], Soraya Rodríguez-Rojo[a,*], Daniela Nunes[d], Elvira Fortunato[d], Rodrigo Martins[d], María José Cocero[a], Catarina Duarte[b,c]

[a] High Pressure Processes Group, Department of Chemical Engineering and Environmental Technology, School of Engineering, Venue Dr. Mergelina, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain
[b] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Avenida da Republica, 2780-157 Oeiras, Portugal
[c] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[d] i3N/CENIMAT, Department of Materials Science, Faculty of Sciences and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
PGSS®
Copper nanoparticles encapsulation
Lipid microparticles
Dispersion

ABSTRACT

Production of lipid particles loaded with metal nanoparticles by supercritical fluids based processes has been barely studied. In this work, copper nanoparticles were loaded into glyceryl palmitostearate microparticles by PGSS® (Particles from Gas Saturated Solutions). The effect of different variables, temperature (60–80 °C), copper load (0.2–5%w/w) and water addition (0–40%w/w), in particle size and encapsulation efficiency has been studied. The dispersion of metal nanoparticles in the lipid has been determined by SEM-FIB coupled with EDS mapping. In all cases, mean particle size values lower than 70 μm have been obtained, and encapsulation efficiencies around 60% have been achieved. The addition of water has no negative effect in encapsulation efficiency nor in nanoparticles dispersion within the lipid microparticle, being important since nanoparticles are commonly synthetized in aqueous medium.

1. Introduction

Nanoparticles, especially noble metal nanoparticles, have an emergent importance in biomedicine field. Their uses are diverse, for example, in molecular imaging, targeted drug delivery systems, targeted therapies (hyperthermia, gene silencing or radiotherapy), and biosensors. These wide applications are possible thanks to nanoparticle properties such as specific area, superior narrow range of emission, photo stability, broad excitation wavelength, quantum dots and the possibility of being functionalized [1,2].

One of the most interesting metals is copper. This transition metal has biological activity as anti-inflammatory, anti-proliferative, and

* Corresponding author.
E-mail address: sorayarr@iq.uva.es (S. Rodríguez-Rojo).

http://dx.doi.org/10.1016/j.supflu.2017.09.001
Received 25 May 2017; Received in revised form 31 August 2017; Accepted 1 September 2017
Available online 21 September 2017

biocidal agent, and has some radioisotopes useful for nuclear imaging and radiotherapy [3]. On the other hand, copper organometallic complexes can be used to deliver copper ions or radionuclides to diseased tissues or to modify pharmacokinetics. These copper compounds can be managed by organism since copper is an essential microelement in contrary to other transition metals. For example, copper (II) complexes have anti-inflammatory and anti-proliferative properties and thus could be used in chemotherapy. Moreover, copper in metallic form possesses antimicrobial activity, already used in agriculture. It can degrade DNA by means of the generation singlet oxygen [4], therefore, it is studied as anti-cancer and anti-proliferative agent [3,5,6].

In order to apply copper nanoparticles for biomedical applications it is necessary to encapsulate them to protect the metal until it arrives to the desired zone and to avoid the damage in healthy cells owing to its cytotoxicity. Since lipids are well tolerated by human body and have low toxicity, they are adequate carriers for the encapsulation of metal nanoparticles. Besides, they present advantages over other colloidal carriers in terms of active compound stability and protection, being possible to be administered in inhalable, transdermal, intravenous or oral form [7].

Conventional methods for producing lipid microparticles are microemulsions or double emulsions followed by spray drying or spray chilling [8,9]. However, these methods involve the use of organic solvents, severe operation conditions and purification steps. PGSS® (Particles from Gas Saturated Solutions) is a technique with the capacity of avoiding conventional methods mentioned drawbacks [10]. In this process, the lipid is melted with the dissolved or suspended active compound, and the final mixture saturated with supercritical carbon dioxide. Then, this mixture is expanded through a nozzle into an expansion chamber and fine lipid loaded particles are formed [7,11,12]. One of the advantages of PGSS® in relation to other supercritical fluid technologies is that the substance does not need to be soluble in carbon dioxide, like in the case of Rapid expansion of supercritical solutions (RESS). The production of lipid nanoparticles loaded with metal has been barely studied with this process. Up to the authors knowledge only the group of Bertucco worked on the production of lipid microparticles magnetically active with excellent results using triestearin, phosphatidylcholine and magnetite nanoparticles [13]. In contrast, there are studies about processes in which a polymeric matrix is used in spite of lipid. These processes are based on emulsion technology (microemulsions, miniemulsions, double emulsions) [14,15]. Further, this process have been combined with supercritical fluid technology for the elimination of the organic solvent as in the production of poly (lactic-co-glycolic) acid (PLGA) nanoparticle loaded with magnetite formulated by means of supercritical fluid extraction of emulsions [16].

In this work, a study of PGSS® process to obtain copper lipid loaded microparticles was performed. The operation conditions were chosen regarding the nature of the lipid used and the variation of its properties when in contact with supercritical carbon dioxide and its influence on the physical properties of the precipitated particles was investigated. Moreover, the effect of metallic nanoparticle load in the product and encapsulation efficiency was studied. Finally, and since nanoparticles are usually obtained in aqueous dispersion [17], the effect of water in the dispersion of metal in the lipid matrix and particle morphology was analyzed.

2. Materials and methods

2.1. Materials

Precirol® ATO 5 (glyceryl palmitostearate) was kindly supplied by Gattefossé (France). Imwitor® 600 was supplied by Sasol (Germany). Carbon dioxide with 99.95 mol% purity was delivered by Air Liquide (Portugal). Copper nanoparticles were purchased from Alfa Aesar with a particle size of 20–30 nm. All the chemicals have been used without further purification.

2.2. Precipitation of copper loaded lipid particles by particles from gas saturated solutions (PGSS®)

In order to produce the loaded particles, Precirol 5 ATO is placed in a 50 cm^3 high pressure stirred vessel, electrically thermostated at the selected operation temperature. Then, the required amount of copper nanoparticles are added. In the experiments carried out with water, the necessary amount of water and 3 mg of Imwitor® 600 (HLB = 4) are incorporated. Imwitor® is a water/oil emulsifier that is necessary to form a macroemulsion, since Precirol has low hydrophilic lipophilic balance (HLB = 2) [11]. In this case, it resulted in a macroemulsion. Thereafter, the vessel is closed and the mixture stirring (150 rpm) begins. Carbon dioxide is pumped by a high pressure pneumatic piston pump to the vessel until experimental pressure is achieved.

The mixture and the supercritical carbon dioxide are brought into contact during 15 min, since no pressure depression was observed after this period being the ideal mixing time [11]. Then, the stirred mixture is depressurized through a nozzle (250 μm) by means of an automated valve to expansion chamber. In this chamber access, the expanded suspension is mixed with compressed air (0.7 MPa, 25 °C) for improving drying. The particles are collected in an 18 L container. The equipment flow diagram can be seen in Fig. 1.

Some experiments were performed previously to fix the pressure conditions in the pre-expansion chamber. A value of 10 MPa was selected since an increase in the pressure (up to 15 MPa) did not reduce the particle size, varying also the mixing temperature between the studied range, from 60 °C to 80 °C. The variables studied apart from temperature were the copper load, from 0.2 to 5%, and the addition of water from 0 to 40% of the mass of copper and lipid. Random experiments were repeated showing the good reproducibility of the process.

2.3. Particle characterization

Particles have been characterized regarding their size distribution, morphology and metal dispersion in the lipid matrix.

2.3.1. Particle size distribution of lipid loaded microparticles

Particle size distribution was measured by laser diffraction using a Mastersizer 2000 (Malvern Instruments) with red light (max. 4 mW helium–neon, 632.8 nm). This equipment has an accuracy and a reproducibility better than 1%. The particles were dispersed in water with surfactant (Pluronic) to improve the dispersion due to the Precirol 5 ATO low HLB. The results are expressed as particle volume distribution average diameter ($d_{0.5}$) and spam. Average diameter and spam values are an average from three different measurements. Spam is defined as

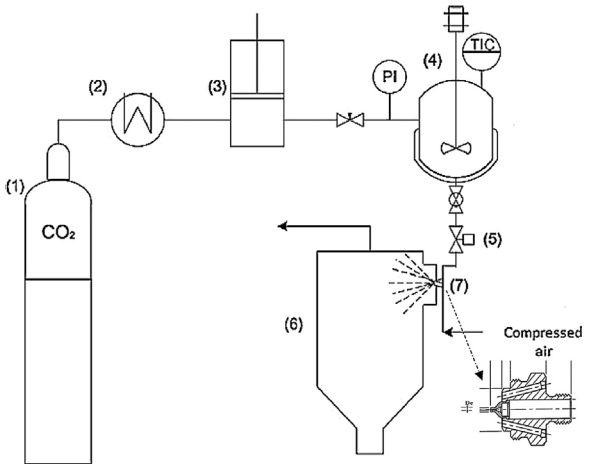


Fig. 1. Experimental setup FAME Separex: (1) carbon dioxide cylinder, (2) cryostate, (3) pneumatic pump, (4) stirred vessel, (5) depressurization valve, (6) cyclone and (7) nozzle d = 250 μm, with external mixture with compressed air.