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Lipid nanoparticles production by supercritical fluid assisted emulsion–diffusion

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ABSTRACT

In this work a supercritical technology is proposed to improve the classical emulsification/diffusion technology for lipid nanoparticles (LNs) production. The process is based on the emulsion diffusion method improved by the addition of a continuous supercritical fluid processing step to eliminate the organic solvent from the nanosuspension obtained.

Different emulsion/diffusion formulations for stearic acid nanoparticles production were tested and, then, processed by supercritical continuous extraction at 80 bar and 45 °C (liquid/gas ratio of 0.1) in a packed column, obtaining an efficient benzyl alcohol elimination. Solvent residues less than 100 ppm were measured. Stearic acid nanoparticles were not extracted or damaged by the supercritical processing step, did not stick on the packing elements and showed mean diameters of 30–50 nm; a value of one order of magnitude smaller than the ones obtained by the conventional emulsion/diffusion technology with a recovery efficiency of 100%. Indeed, the fast and complete elimination of the benzyl alcohol around the nanoparticles reduced the aggregation phenomena responsible of larger lipid particle sizes obtainable by traditional process.

1. Introduction

The production of drug nanocarriers able to overcome therapy failures, such as, poor absorption or specific drug targeting, is one of the challenges of the modern nanomedicine. Nanocarriers can be emulsions, micelles, liposomes or polymer particles [1,2]. Recently, lipid nanoparticles (LNs) have been proposed as nanosystems that may have the advantage of combining the properties of polymer nanoparticles, for convenient drug sustained release [3–5], fat based systems for low toxicity [6,7], and effective targeting [8]. Moreover, the use of LNs for parental, topical and oral administration has been proposed in very promising medical applications [9–11].

LNs suspension is formed as a rule by: a lipid matrix, a stabilizing agent (emulsifiers/surfactants), a continuous medium (water) and can contain residues of the organic solvents used, depending on the manufacture techniques adopted [12]. The term lipid is referred to various kinds of molecules like: triglycerides, glycerides, fatty acids, steroids and waxes. However, triacylglycerides are commonly used as lipid carriers due to their polymorphic behavior that improves the stability of the nanoemulsion and allows a high drug loading by the less ordinate structure of the lipid matrix. The choice of the lipid to be used can be greatly influenced by the drug to be loaded and by the specific target required. Surfactants or surfactant combinations could greatly influence the particle size and the stability of the dispersion, as well as, play an important role in controlling the LNs crystallization process. Indeed, due to nanosize of the particles, the number of lipid molecules that interacts with the surfactant molecules can be large enough to influence the crystallization process and it has been demonstrated that a mixture of nonionic (crystal modulation) and ionic surfactant (stabilization) is recommended to fulfill both criteria of dispersion stability and crystal modulation [13]. In addition, the choice of the emulsifier is largely dependent on the administration route, as well as, the organic solvent used, which should be chosen, as non-toxic, as possible.

Current methods to produce LNs include high-pressure homogenization [14,15], microemulsion technique [16], solvent emulsification/evaporation [14,17] and solvent emulsification/diffusion [18,19]. In the high pressure homogenization technique, lipids are heated above their melting temperature; then, poured into hot surfactant solution, homogenized and cooled to crystallize the lipid droplets and obtain nanoparticles. The

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microemulsion technique consists of the preparation of oil-in-water emulsions at a temperature above the melting point of the lipids with an adequate stirring that ensures the required droplet size; then, the microemulsion is dispersed into a cold aqueous medium, leading to a rapid crystallization of the oil droplets, forming LNs. In the solvent emulsification/evaporation technique, the lipophilic material is dissolved into an organic solvent, an oil-in-water emulsion is produced and, then, evaporated with the production of the LN dispersion. In the solvent diffusion technology two saturated phases are used: an aqueous phase and an organic phase. The drug and the lipids are often dissolved into the organic phase. The mixing of the two phases generates an emulsion in which the resulting droplets are converted into LNs by addition of a large amount of water to induce solvent diffusion toward the continuous medium. Again, solvent and part of water are evaporated under reduced pressure. For all technologies described, the average particle size can be in the range of 100–500 nm. However, high-pressure homogenization is principally limited by the solubility of the drug to be encapsulated in the lipid and by the high shear and/or temperature used; whereas, the other methods often require long processing times for solvents evaporation or extraction and high solvents residues in the produced suspensions can be obtained. The emulsion/diffusion technique is a very promising technique that allows the easy and reproducible LNs preparation, but its application is limited by the problematic removal of the organic solvents used for the oil phase preparation. Indeed, the most used solvent is benzyl alcohol (BA), because it has high solubility in water (i.e., fast diffusion rate) and is non-toxic; however, the main drawback of this solvent, is its boiling point higher than water (T_b = 205 °C) that prevent the elimination by evaporation from a water based suspension.

Supercritical fluid based technologies have been frequently proposed to overcome the traditional processes limitations, and already used for emulsion processing or post-processing. For example, Chattopadhyay et al. [20] proposed a Supercritical technology for emulsion extraction applied to the production of micro and nanoparticle suspensions taking the advantage of the enhanced mass transfer of the supercritical extracting agent. In the process solid particles are formed from droplets thanks to the supercritical extraction of the organic solvent of the oily phase of the emulsion. A similar technology was also successfully described by Della Porta et al. [21,22] in a continuous layout using a high-pressure packed tower to contact the emulsion/supercritical carbon dioxide in counter-current mode, developing a robust and reproducible technology for micro and nanoparticles production. Furthermore, the same apparatus has been also proposed as a possible post-processing of nanoparticle suspensions obtained by nanoprecipitation. In this case nanoparticle suspensions are first produced by conventional nanoprecipitation technique and, then, the organic solvent removed using supercritical fluids [23].

Following this idea, the aim of this work concerns the optimization of lipid nanoparticles production, based on a stearic acid matrix, using the emulsion/diffusion method improved by supercritical fluid post-processing. In the first process step, the solid nanoparticles are produced by a classical solvent emulsion/diffusion method; then, the organic solvent (BA) is continuously extracted in a countercurrent packed column taking advantage of the high solubility of BA in supercritical carbon dioxide (SC-CO_2). Different emulsion formulations, diffusion dilution ratio, as well as, emulsion/diffusion operative temperatures have been tested to obtain lipid nanoparticles. Nanosuspensions have been then, processed by supercritical fluid with the aim of: (1) obtaining a fast elimination of the benzyl alcohol residue and (2) reducing the aggregates due to the presence of solvent and obtaining a more accurate control of the lipid nanoparticle size and distribution. The produced SLNs are characterized by laser scattering for the measurement of their particle size and distribution and by and FE-SEM for the investigation of their morphology.

2. Materials and methods

2.1. Materials

Stearic acid (SA, purity: ≥98.5%, MW: 284.48, Aldrich Chemical Co.) was used for SLN production. Polyoxyethylene (20) sorbitan monooleate (Tween 80®, MW: 1310, Aldrich Chemical Co.) and phosphatidylcholine-enriched fraction (Epikuron 200, purity: 92%, Cargill Inc.) were used as surfactants. Benzyl alcohol (BA, purity: >99.5%, Carlo Erba) was used as organic solvent. Carbon dioxide (purity: 99.9%, SON, Naples, Italy) was used as extracting agent.

2.2. Emulsion preparation & diffusion step

The oil phase utilized in all the experiments is a water-saturated benzyl alcohol solution. In detail, the oily phase was prepared by dissolving 8.75% (w/w) of distilled water in benzyl alcohol at room temperature; the solution was then stirred to reach the thermodynamic equilibrium and the water in excess was removed by decantation. Then, a fixed amount of stearic acid and Epikuron 200 was added. Stearic acid solubility was measured as 15 g/L in the oily phase at room temperature and this concentration was used for all the present study. The water phase was prepared saturating the water with BA. Tween 80 solubility in water saturated phase was measured to be 2.1% (w/w), at ambient temperature. All emulsions were prepared with a high-shear mixer (Silverson mod. L4RT) operating at 7000 rpm/min. The aqueous phase was previously equilibrated and, then, the oil phase added progressively. When all the oil phase was added, mixing was maintained for 4 min. 100 g of emulsion was prepared each time. Water was, then, added to induce the diffusion of benzyl alcohol in water and consequently the formation of stearic acid nanoparticles. Different dilution ratio (emulsion/dilution water) were tested from 1/2 to 1/6.

2.3. Supercritical apparatus

The nanosuspension is continuously fed to the packed column at a constant flow rate by a high pressure piston pump (mod. 305; Gilson, Villiers le Bel, France). The high pressure column (i.d. 1.3 cm) was formed by three stages of stainless steel cylindrical elements of 30 cm height, connected by four way cross-unions and packed with stainless steel packing (1889 m^{-1} specific surface; 0.94 of voidage; ProPak, Scientific Development Company, State College, Pennsylvania). The apparatus was thermally insulated by ceramic cloths and its temperature profile was controlled by six temperature controllers. SC-CO_2 was fed at the bottom of the column by a high-pressure diaphragm pump (model Milroyal B; Milton Roy, Pont Saint-Pierre, France) at a constant flow rate. The supercritical carbon dioxide (SC-CO_2) was pumped from the bottom, to obtain a counter current operation layout. A separator, located downstream the top of the column, was used to recover the extracted oily phase. Lipid nanosuspensions were continuously collected at the bottom of the column by decompression, using a needle valve.

2.4. Analytical procedures

Emulsions and suspensions were observed using an optical microscope (mod. BX 50 Olympus, Tokyo, Japan) equipped with a phase contrast condenser. Samples were prepared by placing a drop of the solution/suspension on a microscope glass slide. Samples were analyzed quickly to avoid any heating due to the microscope light.