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Supercritical assisted process for the efficient production of liposomes containing antibiotics for ocular delivery

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ABSTRACT

Conventional techniques developed for the production of liposomes for ocular delivery show low encapsulation efficiencies (EE).

In this work, a supercritical CO_2 based one-step continuous process, named *Supercritical Assisted Liposome formation* (SuperLip), was used for the production of liposomes to deliver ophthalmic antibiotics, such as ampicillin and ofloxacin. Micrometric and sub-micrometric liposomes with mean diameters in the range from 280 ± 104 nm to 1.76 ± 0.79 μm were successfully produced using drug concentrations in the range from 1% to 6% w/w and water to lipid ratios from 1.7 mg/g to 25 mg/g. Encapsulation efficiencies up to 97% and 99% were obtained for ofloxacin and ampicillin respectively. Storage stability and drug release kinetics of produced liposomes were also studied. Liposomes were stable for at least 3 months, with negligible drug leakage during storage time. At 37 °C ofloxacin and ampicillin were released in a controlled manner within 3 and 4 h respectively.

1. Introduction

The human eye has a complex anatomy and the delivery of drugs to targeted ocular tissues is restricted by various precorneal, dynamic and static ocular barriers [1]. Ocular drug systemic delivery suffers of the difficulty to reach the specific target of ocular tissue. For this reason topical delivery is preferred and can be directly performed by patients [2,3].

Cyclodestrins have been used as ocular drug carriers for hydrophilic molecules, but they suffer of drug leakage. Other side effects are ocular irritation, redness and inflammation [4]. Emulsions for ocular delivery are preferred in water/oil formulation [5,6], because less irritation and better tolerance of the eye has been observed [7]. Mucoadhesive polymers such as chitosan have been introduced to improve pre-corneal residence time [8]. The most used polymer for ocular delivery is Hyaluronic Acid (HA), that was proposed in literature in different nanostructures and solutions [9]. Other conventional drug carriers developed for topical ocular delivery are suspensions and ointments [10]. To overcome the problems related to direct administration of ocular drugs, nanocarriers have been proposed that can assure reduced eye tissues irritation, a better bioavailability and a better biocompatibility with the eye cells. The most widespread examples of nanodrug carriers [11] for ocular delivery are nanomicelles [12], colloids [13], dendrimers [14,15], gels [16,17], microneedles [18,19] and liposomes [20–22]. In this work, liposomes will be studied to understand if it is possible to enhance drug bioavailability through a cell-based vector.

Liposomes are particularly indicated for topical ocular drug delivery due to their biocompatibility. Moreover, lipids are definitely suitable during the interaction with the cell membranes and the biological barriers naturally present in the eye tissues. Indeed, they are artificial vesicles formed by an inner water core, surrounded by an external double lipidic layer. Their similarity with human membrane cells and the amphoteric behaviour of phospholipids give the possibility to encapsulate both hydrophilic and lipophilic compounds. Liposomes showed good efficacy in delivering drugs to posterior and anterior segment of the eye tissues. For this reason, they are considered the most valuable eye drug delivery systems [23–26].

Ampicillin and ofloxacin, antibacterial drug used to stop ocular post-surgery infections, are often delivered using liposomes. [27–31]. However, encapsulation efficiencies (EE) in liposomes produced using conventional techniques, reported in the literature, are low [32,33]. For example, ofloxacin encapsulation efficiency in multilammellar

Abbreviations: MD, mean diameter; SD, standard deviation; PSD, particle size distribution; EE, encapsulation efficiency; PBS, phosphate buffered saline; GLR, gas to liquid ratio; UV–Vis, ultraviolet-visible; PC, phosphatidylcholine; FV, formation vessel; OF, ofloxacin; A, ampicillin

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micrometric liposomes ranging between 53 and 65% was reported by Hosnoy et al. [34] using the thin layer hydration method. Similar results were reported in the case of ampicillin encapsulation into liposomes, using the same production method, with encapsulation efficiencies in the range from 10 to 50% [35]. The reason for the low encapsulation efficiency is not particularly linked to the kind of compound chosen for the liposome entrapment, but it is related to the basic idea for conventional vesicles production. The hydration method generally implies a loss in drug content during water inclusion in the double layer.

Many continuous processes that exploits supercritical carbon dioxide have been recently proposed in literature, such as Continuous Anti-Solvent (CAS) or Supercritical Anti-Solvent (SAS) processes [36,37]. Among these, another novel supercritical assisted process, called SuperLip (Supercritical assisted Liposome formation), has been developed, that allows to obtain stable lipidic vesicles in an efficient way, with improved drug EE. In this process, first water droplets are created; then, lipidic layers are rapidly formed around them. The rapidity of the process is mainly due to the use of Carbon Dioxide and particularly to its high diffusion coefficient in supercritical conditions [38]. High EE for several model compounds, such as fluorescein [39], theophylline [40], bovine serum albumin [41] and olive pomace extract [42] were obtained.

Therefore, the aim of this work is to attempt the encapsulation of ofloxacin and ampicillin into liposomes using SuperLip. Process parameters, such as water flow rate and drug concentration have been studied to obtain high EE, and the correlation between these process parameters, size and drug EE of the vesicles has been proposed. Storage stability and drug release kinetics have also been performed.

2. Apparatus, materials and methods

2.1. Materials

The lipids used for this study have been purchased by Sigma Aldrich as lyophilized powder and have the following composition: soybean L-α phosphatidylcholine from egg yolk (PC, 60% purity) while the rest was composed of a mixture of similar double tailored phospholipids. This raw material was chosen to reduce the R&D cost before trying to commercialize the products once the liposomes production is optimized. Ethanol employed to dissolve phospholipids was purchased from Sigma-Aldrich too (≥99.8% purity). Carbon dioxide was provided by Morlando Group (> 99.4% purity). Distilled water was provided by Carlo Erba, Phosphate Buffer Saline (PBS) was prepared in laboratory. Ampicillin (CAS number 7177-48-2) (A) and ofloxacin (CAS number 82419-36-1) (OF) were obtained by Sigma-Aldrich. All compounds were used as received.

2.2. Apparatus

A block schematization of SuperLip process layout is represented in Fig. 1.

Each block represents a unit process; (1), (2) and (5) indicate feeding elements controlled by pumps. Blocks (3) and (4) work under pressure; particularly, blocks (3) and (4) work at 100 bar while block (7) works at 10 bar. In block (4) the liposomes are formed. Finally, in blocks (9), (6) and (8) separation of products and by-products occurs.

More in details, CO_2 (1) and the ethanolic solution (2), containing the phospholipids, are delivered to the saturator (3), a high pressure static vessel of 0.15 dm^3 internal volume, filled with stainless steel perforated saddles. CO_2 is fed using a Lewa Eco flow pump (mod. LDC-M-2, max, Germany) with a flow rate of 6.5 g/min while the ethanolic solution is pumped using a high pressure precision pump (Model 305, Gilson, France) with a flow rate of 3.5 mL/min. The expanded liquid (EL), formed in the saturator with a Gas to Liquid Ratio of 2.4 on mass base, was delivered to a Formation Vessel (FV) of 0.5 dm^3 internal

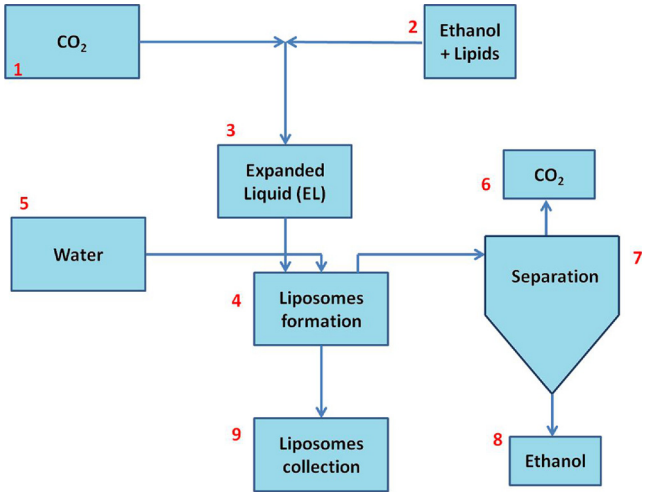

Fig. 1. SuperLip scheme, working at the temperature of 40 °C and a pressure of 100 bar.

volume (4), already half-filled with pure water. During the injection of the EL in the Formation Vessel (4), a water + antibiotic solution (5) is continuously and contemporary pumped using a high pressure pump (Model 305, Gilson, France) and water droplet are formed employing a nozzle with an internal diameter of 80 μm, located on the top of the formation vessel (4). Water flow rate was varied throughout this work to optimize encapsulation efficiency of entrapped compounds. A stainless steel separator (7) is finally employed to perform the separation between CO_2 (6) and ethanol (8) by bringing the pressure down to 10 bar. Depressurization only separates carbon dioxide from ethanol from the top of the formation vessel, while the liposomes have been already collected from the bottom of the chamber, in aqueous suspension. The liposomes produced are collected in an accumulation stainless-steel element and recovered from the bottom (9).

2.3. Methods

Dynamic laser scattering (DLS) technique (mod. Zetasizer Nano S, Worcestershire, UK) was used to obtain particle size distribution (PSD) of the liposomes loaded with ampicillin or ofloxacin. Vesicles suspensions were analyzed without additives or dilution. Each sample was measured three times and the average of the measurements was reported in this work. The same method was also employed to measure surface zeta potential of the produced vesicles. Dynamic laser scattering was also used to perform diameter stability tests. Once a week, for a total period of 3 months, a sample was analyzed by DLS to verify if modifications of mean size and standard deviation were observed during storage.

To measure the encapsulation efficiency, vesicles suspensions were centrifuged at 6500 rpm for 45 min at 4 °C. Then, the concentration of antibiotic in the supernatant (ppm_{sup}) was measured using an UV–vis spectrophotometer (BioSpec-nano, Shimadzu Scientific Instruments, Columbia, USA), at the wave length of 225 nm for ampicillin and 290 nm for ofloxacin assay. The encapsulation efficiency (EE) was calculated as the complement to 100% of the percentage of drug detected in the supernatant, as expressed in the following equation [43]

$$EE = 100 * \left[1 - \left(\frac{ppm_{sup}}{ppm_{loaded}}\right)\right]$$

in which (ppm_{loaded}) is the theoretical ofloxacin/ampicillin amount dissolved in the aqueous solution atomized in the formation vessel and ppm_{sup} is the antiobiotic concentration measured in the supernatant. Each absorbance measurement was repeated three times and the results reported are the mean over 3 different measurements. Encapsulation