UNIVERSITY OF SALERNO

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Ph.D. Course in Industrial Engineering

Curriculum in Chemical Engineering - XXXI Cycle

Optimization of a supercritical assisted process for the production of liposomes for industrial applications

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Abstract

Liposomes are spherical vesicles made of a double lipidic layer that surrounds an inner aqueous core. Several methods for the preparation of liposomes have been developed in the last decades. However, these methods present drawbacks, such as low reproducibility, batch operations, low encapsulation efficiency of hydrophilic compounds, a difficult control of liposome size distribution and high solvent residue, hindering the real industrial potential of these drug delivery systems.

Supercritical fluid (SCF) technologies have been proposed to overcome several limitations of conventional processes for the production of micronized particles carriers, coprecipitates and nanocomposite polymeric structures. Recently, some techniques based on the use of supercritical carbon dioxide have been proposed also for liposome production. However, these methods have still some limitations related to the control of liposome dimension and size distribution and also show very low encapsulation efficiency of hydrophilic drug. The major limitation of these processes, both conventional and supercritical, derives from the hydration step of the lipid layer. Indeed, during this step, only a part of the water used for hydration is actually entrapped into liposomes, resulting in a low overall encapsulation efficiency.

Therefore, the **objective of this Ph.D. thesis** is to develop a novel technology assisted by supercritical carbon dioxide for the production of liposomes of controlled dimensions. The proposed technique was called Supercritical assisted Liposome formation (SuperLip); in this process, first water droplets are produced; then, they are rapidly covered by phospholipids.

In the first year of this Ph.D. work, the effect of several process parameters have been studied such as water flow rate, injector diameter, pressure and Gas to Liquid Ratio of the Expanded Liquid (GLR-EL), i.e. the mass ratio of carbon dioxide and ethanol flow rate. Also, the composition of liposomes was modified changing phospholipid concentration and adding other lipids in the double lipidic layer such as cholesterol. In this way, it is possible to produce vesicles with a good control of particle size distribution (PSD) and high encapsulation efficiency (EE) of hydrophilic and lipophilic (up to 99%) compounds have been obtained. The decrease of water flow rate resulted in the increase of drug encapsulation efficiency; moreover, the use of an injector nozzle with a larger diameter resulted in the production of larger water droplets and larger liposomes. Then, the concentration of lipids did not affect mean size of liposomes or encapsulation efficiency, but it resulted in a delayed drug release due to the formation of several lamellae around water droplets. Cholesterol was also recognized to be responsible of a more compacted double lipidic backbone. The increase of pressure resulted in the formation of smaller water droplets and liposomes.

During the second year, several liposome-based product formulations were developed, according to the operative parameters already optimized during the first year of study. Antibiotics for ocular delivery, proteins and markers for molecule labeling were entrapped into liposomes for pharmaceutical purposes. Then, cosmetic applications were explored, encapsulating antioxidant compounds of hydrophilic and amphiphilic nature. Amphoteric compounds were entrapped either in the inner core, either in the lipidic layer of liposomes to study the differences in the antioxidant inhibition power, depending on vesicles compartment of encapsulation. Dietary supplements were also entrapped for food applications, in order to valorize the by-product that generally are discarded from the agro-alimentary field. A novel textile application was also proposed for the deposition on dye on leather fragments.

Finally, in the **third year of this Ph.D**. project, liposome mechanisms of production involved in SuperLip technique were studied to verify the hypothesis proposed for this process during its development and feasibility validation. *In vitro* studies were also performed with antibiotic loaded liposomes with *E.Coli* and cell citotoxicity studies were started. An economic analysis on SuperLip proposed technique has been performed. SuperLip has a Technology Readiness Level (TRL) of 6/7, since it has been designed in a continuous lab-scale configuration and it is possible to scale it up to industrial level. SuperLip method can produce about 5 liters of liquid liposomes suspensions per day. The idea at the basis of the process has been already validated by product development and samples characterization, as reported in our published works. SuperLip potential applications has always been recognized by external customers, interested in the production of liposomes on demand. A business plan for the commercialization of SuperLip products was attempted to verify if the production of liposomes with this technique could be profitable in the markets. A B2B model has been proposed and an estimation of CAPEX and OPEX was performed to produce a 5-years (2018-2022) prospective for commercialization.