ABSTRACT

The PhD project titled "Development of technological approaches based on supercritical fluids for the production of polymeric micro-nano particulate systems for wound healing" aimed to develop novel formulations for topical administration to wounds using innovative, inexpensive and environmentally-friendly technologies based on the use of supercritical-CO_2 (sc-CO_2): supercritical assisted atomization (SAA) and supercritical assisted extraction (SAE) in tandem with prilling. The specific goal of the project was the designing and development of *"in-situ"* gelling formulations in form of powders or aerogels using polysaccharide-based polymers as carriers for the encapsulated drugs, due to their biocompatibility, biodegradability, low cost and healing improving properties. Optimization of the process parameters were implemented to obtain either submicrometric particulate particles or aerogel beads with desired properties. Size distribution, textural properties, fluid uptake capability and controlled drug release profiles of the optimized formulations have been studied to evaluate the quality of the different wound healing devices.

During the first year, supercritical assisted atomization (SAA) was investigated. SAA was applied for the production of *"in-situ"* gelling dry powders loaded with doxycycline used as antimicrobial drug due to its inhibiting activity against matrix metalloprotease-2 (MMP-2) and metalloprotease-9 (MMP-9) that could enhance the healing process. High mannnuronic content alginate, low methoxyl grade amidated pectin and low molecular weight chitosan, in different combinations, were used as excipients. Process optimization lead to high process yields (up to 89.0%) and the obtained powders showed good technological characteristics since were able to completely gel in three minutes when in contact with simulated wound fluid. Moreover, powders were able to prolong the release of the doxycycline until up to 21 hours after a fast release during the first two hours ("burst effect").

The second year was focused on the development and characterization of aerogel formulations, in form of beads or capsules, obtained by prilling technique in tandem with the supercritical antisolvent extraction (SAE). High mannuronic content alginate was used as carrier for these formulations. Alginate gel beads were produced by prilling using either aqueous or ethanolic calcium chloride solutions as gelling bath thus

producing hydrogels or alcogels in a very narrow size distribution (about 2.4 mm ± 6.0%). Subsequently they were submitted to different supercritical-CO_2 drying processes for the production of aerogel in form of spherical beads. The resulted aerogels showed very high porosities (98.4-99.8%) and surface areas (271.0-537.3%) for the different sc-CO_2 drying processes. Moreover, influence of alginate molecular weight on aerogel properties was studied, resulting to influence the grade of shrinking and the porosity of the aerogels. In addition, the hydrogels and alcogels were also submitted to freeze-drying and oven drying with the purpose to make a comparison in terms of textural properties. Differently, the production of aerogels capsules with controllable shell tickness was designed with the purpose to increase the exudates absorption when beads gel in contact with wounds. For this purpose, core-shell gel microparticles were produced by prilling in co-axial configuration through the development of a new inverse gelation methodology optimized by Artificial Intelligent (AI) tools (Artificial Neural Networks, ANNs, and Neurofuzzy-Logic in combination with genetic algorithms). The obtained core-shell microparticles were formed by a hydrophilic alginate phase as the outer layer and a hydrophobic phase (water-in-oil emulsion) as the inner one of the particles; such core was subsequently removed by supercritical-CO_2 drying producing alginate aerogels with an inner void cavity.

The third year was focused on the development and characterization of drug-loaded aerogel capsules through supercritical antisolvent extraction (SAE) in tandem with prilling. Drug loaded microparticles were produced by the novel inverse gelation previously cited through prilling in co-axial configuration using ketoprofen lysinate, as model drug, and alginate as polymeric excipient. Aerogel capsules with a thin alginate shell layer and a hollow inner cavity, in which the ketoprofen was present, were produced after the supercritical drying of core-shell microparticles. Aerogel capsules showed good textural properties in terms of porosity (up to 93.1%) and surface area being promising formulations for high fluid uptake (about 500% the weight of the aerogel) from the wounds within seconds.

The supercritical drying processes and the characterization of the textural properties of aerogels were carried out during a 7 months period in the University of Santiago de Compostela (Spain).

Hence, such novel technologies, using polymers above mentioned, are promising technologies for the development of a new non-expensive generation of dressing formulations with small particle size *("in-situ"* gelling powders) or large particles in order to be easily handled (aerogels) both with high surface areas that make them able to absorb high amount of exudate from wounds maintaining at the same time the moisture environment at the wound bed acting as non-traumatic dressings.