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PRODUCTION OF PHARMACEUTICAL AND NUTRACEUTICAL FORMULATIONS FOR BIOAVAILABILITY IMPROVEMENT USING SUPERCRITICAL ASSISTED ATOMIZATION

Abstract

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Industrial interest is focused on the development of new pharmaceutical and nutraceutical formulations aimed at the enhancement of bioavailability of poorly water-soluble active compounds. Various factors influence the bioavailability of an active principle in a solid formulation, such as the particle size distribution, the solid state and the morphology. In order to enhance bioavailability, two different approaches were investigated in this thesis: particle size reduction of pure ingredients and production of amorphous formulations consisting of the dispersion of the hydrophobic molecule into a hydrophilic matrix (*carrier*). Supercritical fluid (SCFs) based techniques demonstrated to be a valid alternative to traditional processes, given the SCFs specific properties, such as liquid-like density, very fast mass transfer similar to gas, near-zero surface tension, effective solvent elimination. Among SCFs techniques, Supercritical Assisted Atomization (SAA) was successfully used to produce controlled micro and sub-microparticles of pure compounds, but also composite systems. The enhancement of bioavailability of active ingredients is still poorly investigated in the SAA literature. The ability of the SAA process in crystallinity control and particle size reduction is investigated in order to enhance the bioavailability of new active principles, often not feasible through conventional methods. This Ph.D. work is the most recent advance in the application of this supercritical fluid technology for the production of new and stable pharmaceutical and nutraceutical formulations.

Industrial companies are very interested in the development of these feasible formulation technologies. In fact, this Ph.D. work was supported and granted by Cerbios Pharma, a Swiss pharmaceutical company located in Lugano and specialized in the development and production of chemical and biological active principles. In the frame of the research collaboration between Cerbios Pharma and the Department of Industrial Engineering (DIIN) of the University of Salerno, a part of this thesis concerned the development of pharmaceutical formulations based on active principles suggested by the Company for its world-wide partners, in order to scale up this process for industrialization purposes. Due to secrecy agreements, the proposed active substances were presented using acronyms and no further detailed information was herein reported. Different kinds of active principles were tested on behalf of Cerbios Pharma: BRI (an alpha adrenergic agonists drug), FUL (an anti-estrogen drug), DAS (an anticancer drug), NAP (a non-steroidal anti-inflammatory drug) and GPB (an anticholinergic agent). These drugs were previously unsuccessfully tested using traditional techniques. For each active principle, during this Ph.D. work, a series of SAA experiments was performed in laboratory scale to optimize process conditions, in order to reach the targets requested by the Company. BRI, FUL and NAP were micronized alone, whereas DAS and GPB were dispersed in different carrier matrices producing coprecipitates (Chapter III). In particular, the study performed on NAP compound allowed to validate the SAA industrial plant in Cerbios Pharma and obtain the SwissMedic authorization for *Good Manufacturing Practice* (GMP) production, on January 2017.

To investigate in depth the applicability of the SAA process in the development of new and stable pharmaceutical formulations, *curcumin, luteolin, palmitoylethanolamide* and *nifedipine* were selected as active principles, for their therapeutic and healing properties. All these substances showed low water-solubility and remarkably fast crystallization rates, interfering with the micronization processes; therefore, they were difficult to handle in an industrial perspective. *Polyvinylpyrrolidone, hydroxypropyl-β-cyclodextrin* and *dextran* were investigated as *carrier* since they are non-toxic, water-soluble, biodegradable, biocompatible, *Food & Drug Administration*-

approved and crystal growth inhibitors. The study was focused on the role of the *carrier* in influencing particle size distribution, morphology, solid state, and mostly in inhibiting recrystallization of the active principles and enhancing their bioavailability. In particular, the selected carriers demonstrated to be very efficient in controlling the tendency of active principles to crystallize and agglomerate, by producing SAA stable particles over time. Indeed, well-separated and amorphous microspheres were obtained by SAA process with diameters lower than 1.8 μm and high active principle loading efficiencies, up to 100% in most of the experiments performed. Dissolution tests confirmed improvements of bioavailability up to 12 times, due to the coprecipitation of the pharmaceutical compounds and carrier: the produced composite particles showed active principle dissolution rates much faster compared to the physical mixtures. The active principle/carrier weight ratio and chemical interactions in the coprecipitates revealed to be controlling parameters for dissolution rates (Chapter IV).

The production of composite microspheres loaded with bioactive compounds has been another important challenge handled in this Ph.D. work. In particular, *beta-carotene* and extracts obtained from *propolis* and *saffron petals* (normally considered as industrial wastes) were selected in this framework for their nutraceutical properties. The main outcome of this investigation was not only the improvement of the bioavailability, but also the protection of the antioxidant activity of the bioactive compound. Defined and spherical particles were produced by means of the SAA technique using *polyvinylpyrrolidone* and *hydroxypropyl-β-cyclodextrin* as carriers, with mean diameters influenced by the concentrations under test. The UV-vis analyses confirmed high loading efficiencies (up to 94-100%) and preserved antioxidant activity against light, heat and oxygen. In particular, the study on saffron petals extracts demonstrated that SAA produced particles, using *polyvinylpyrrolidone* as carrier, were able to preserve the antioxidant power over time. After three months, the values of scavenging activity were practically confirmed for SAA coprecipitates and reduced by 35% for crude saffron extracts. As result, the antioxidant-rich particles produced can be used as natural sources of antioxidants and for food supplements, thus proving that it is possible to enlarge the application field of the SAA process to the production of nutraceutical formulations (Chapter V and Chapter VI).

In conclusion, the SAA technique was successfully applied both in pharmaceutical and nutraceutical fields, demonstrating to be an attractive and available process from an industrial point of view.