Stage II. Design of composites containing mesoporous silica modified with inorganic nanoparticles – polyphenolic extract with antibacterial properties

Abstract

In the second stage of the project, all the foreseen activities were carried out and all result indicators were fulfilled with promising results obtaining. The deliverables of this stage are: 6 mesoporous supports modified with inorganic nanoparticles (NPs) with determined features, 18 extracts from wild berries with determined chemical profiles, 8 new formulations polyphenolic extract-mesoporous silica modified with inorganic NPs, chemical and thermal stability data of the encapsulated extracts compared to the free ones, antibacterial activities, biocompatibilities, antioxidant activities, anti-inflammatory activities.

Polyphenolic extracts were obtained using wild bilberry and cranberry leaves and respective fruits as vegetal material. The extracts were prepared by extraction under inert gas pressure, a method that was optimized, which corresponds to fulfil the objective O1 - *optimization of the extraction conditions for obtaining polyphenolic extracts*, but also by conventional or ultrasound-assisted extraction. Extracts were characterized by spectrophotometric methods (including determination of total tannin content and antidiabetic activity by inhibition of α glucosidase activity) and HPLC-PDA. The extraction method under pressure determined a higher content of condensed tannins in the wild bilberry leaf extracts, and the use of ethanol as a solvent determined extracts that contained catechin hydrate, which explained a better antidiabetic activity than that of hydroethanolic or commercial extracts.

During this stage of the project, were developed methods silica surface modification with Ag, Cu, ZnO and TiO_2 NPs to obtain mesoporous matrices with high porosity, which corresponds to the achievement of the objective O2 - *obtaining modified mesoporous matrices with tailored features*. 8 new formulations of polyphenolic extract encapsulated in mesoporous silica modified with inorganic NPs were obtained, which corresponds to objective O3 – *obtaining novel formulations for extracts through encapsulation into a mesoporous matrix with health benefits*. Bilberry leaves extract embedded in silica hallow spheres pure or modified with ZnO (HS-ZnO) are biocompatible, higher cell viabilities been observed for the extract encapsulated in HS-ZnO. The anti-inflammatory potential of these formulations was evaluated against two cytokines, TNF-α and IL-8 on the THP-1 monocyte cell line. Quantification of TNF-α cytokines showed that the extract encapsulated in HS-ZnO present the best anti-inflammatory activity. Formulations of bilberry leves extract encapsulated in silica modified with inorganic NPs showed a synergistic antibacterial activity against *Staphylococcus aureus* bacterial strain due to both the extract and silica support modified with Ag, ZnO or Cu NPs. The biological assessment of the proposed new formulations corresponds to the fulfilment of the objective O5 - *evaluation of cytotoxicity and bactericidal activity of developed formulations containing polyphenols*.

The stability over time of bilberry fruit extracts (BL) free or embedded in mesoporous silica supports obtained in stage 1 was evaluated in this stage through an accelerated degradation study during 28 days, which corresponds to the fulfilment of the objective O4 - *assessment of chemical stability of polyphenols when are encapsulated into mesoporous silica-type matrix*. A sharp decrease in the free radical scavenging activity (RSA) of the free extracts was observed, while in the case of the extract encapsulated in MCM-41 the decrease in the RSA value was 9.6% after 28 days. When BL extract was encapsulated in functionalized silica supports, an enhanced antioxidant activity was observed due to the synergistic effects between the extract and MCM-SH and MCM SH-Fuc supports. The evaluation of the anti-inflammatory activity showed that both the BL ethanolic extract and the BL@MCM SH formulation were strong COX-2 inhibitors, being more selective towards COX-2 than towards COX-1, the BL@MCM-SH formulation showing lower IC_{50} value than the free extract.

The results obtained in this stage of the project have been disseminated in 6 international conferences, 3 ISI papers (one submitted for publication this month) and in a patent application.