Functional Particles Prepared by PGSS®

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ABSTRACT

The design of composite particles as carrier systems of active compounds is a way to achieve more effective therapeutics, to mask unpleasant tastes, to protect ingredients, or even to enable the incorporation of the active compound in an incompatible matrix.
In this study three examples of lipid particles with active compounds, formed by PGSS, are presented: trans-chalcone was used and tested as pharmaceutical example; caffeine was studied for incorporation in cosmetic/dermal products; and mixtures of natural antioxidants were included in lipid particles for food applications. Each of these examples was tested according to the final purpose.

INTRODUCTION

Over the last years several processes have been applied for particle formation using supercritical fluid technology. However, the main focus found in literature has been the process itself and the validation of the techniques. This study aims the evaluation of the particulate systems prepared as a means to demonstrate their applicability and performance in final consumable products. Three examples are presented: one for oral administration of a pharmaceutical product, one for dermal administration for cosmetic/pharmaceutical purposes, and another for incorporation of bioactive compounds in vegetable edible oils. The composite particles were processed by PGSS [1], under experimental conditions evaluated from previous studies of the melting behaviour of the matrices in the presence of carbon dioxide [2-4]. The matrices chosen are commercial formulations of naturally-derived biocompatible lipids classified as GRAS (Generally Recognized As Safe). They have been used to enhance the bioavailability of sensitive drugs, to protect active ingredients from light, moisture and oxidation and as controlled release agents [5-7]. In terms of active compounds, for the oral pharmaceutical example, trans-chalcone, the core structure of a group of compounds with an impressive array of pharmacological activities [8-10], was mixed with two lipid carriers of distinct hydrophobicities (HLB – Hydrophilic-Lipophilic Balance). The particles obtained were analysed by UV spectroscopy, scanning electron microscopy (SEM), laser diffraction and attenuated total reflection infrared (ATR) - FTIR spectroscopic imaging. Dissolution tests on gastro-intestinal simulated fluids were also performed.
Caffeine was used as model compound to be tested for dermal applications. It has known effects in the central nervous system and interesting properties for dermatological application [11-13]. The obtained caffeine-loaded particles, were then incorporated in a hydrogel, and diffusion tests were performed in a Franz-diffusion cell and followed by HPLC-DAD.
Several complex formulations were also prepared with different combinations of natural antioxidants such as tocopherols, ascorbic acid, phytosterols and polyphenolic compounds. The particles were then incorporated in sunflower oil and its stability and properties were evaluated through tests commonly used in industry for characterizing food oils like acidity, Rancimat, peroxide values and frying analysis.

MATERIALS AND METHODS

Materials

Lipid matrices composed of mixtures of mono-, di- and triglycerides and also PEG (polyethylene glycol), were kindly provided by Gattefossé. Trans-chalcone (98%), caffeine (98%) and ascorbic acid (99%) were purchased from Sigma-Aldrich. The other compounds used for vegetable oil incorporation were kindly provided by Sovena, S.A, Portugal. Carbon dioxide (99.5%, industrial grade) was obtained from Air Liquide. All products were used with no further purification.

Particles form gas-saturated solutions (PGSS)

This technique known as PGSS - Particles from Gas Saturated Solutions, was first described by Weidner *et al* [1]. In the apparatus used, described elsewhere [3], carbon dioxide is fed to a high pressure stirred reactor containing the product, at given conditions of pressure and temperature. After a certain stirring equilibration time, the mixtures were depressurized through a valve and a 300 μm nozzle. The solid particles obtained were collected and separated from carbon dioxide in a vessel of about 10L volume.

Chalcone-loaded particles were prepared in 1:1 proportion with two lipid carriers with different HLB. A mixture of both carriers was also tested. Caffeine-loaded particles were prepared with a lipid carrier, and although caffeine is water-soluble, a final content of 140mg/g of particle [3] was achieved. The composition of the several particles formed for incorporation in sunflower oil included ascorbic acid, tocopherols, phytosterols, other phenolic compounds and antifoaming agents. These were based on preliminary tests that considered regulatory issues (like RDD-recommended daily dose and saponification value), complementary effects of the different active compounds, and the effect and price in the final product.

Particle characterization

The morphology of the particles was analysed and imaged by scanning electron microscopy (SEM) after being fixed by mutual conductive adhesive tape on aluminium stubs and covered with gold palladium using a sputter coating. The particle size and size distribution of the prepared microparticles were measured by Laser diffraction spectrometry (Coulter LS 130, Coulter Electronics). The dried powder samples were suspended in deionised water with a surfactant solution and sonicated for 1 minute with an ultra-sound probe (500 W, before measurement.

Chemical composition of the particles

Chalcone loaded-particles were dissolved in dichloromethane and submitted to a UV spectophotometric analysis (Thermo Spectronic Genesys 10UV). Caffeine loaded particles were analysed by HPLC-DAD after a preliminary extraction procedure described elsewhere [3].

Distribution of chalcone in the powders obtained

The distribution of chalcone and lipid carriers in the powders obtained was accessed by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic imaging. Images were collected with a continuous scan FTIR spectrometer (Varian, [14]) coupled with a 64X64 focal plane array (FPA) detector (Santa Barbara, USA). This imaging approach has previously been described elsewhere [15].

Dissolution tests of chalcone-loaded particles

In order to evaluate the difference in performance of the carriers with different HLB used for the chalcone loaded particles, dissolution tests were performed in simulated intestinal and gastric fluids, according to the European Pharmacopeia, prepared without enzymes, to test the pH effect. Saturated solutions of the different particles were left overnight at 37ºC. Samples of these solutions were withdrawn, filtered and analysed by HPLC-DAD after dilution with acetonitrile.

Performance of caffeine-loaded particles in a gel

Particles obtained with lipid carrier + caffeine were incorporated in a gel (o/w emulsion – Simulgel™ 600 PHA, Seppic), in amounts to give 5% wt of caffeine content in the final product. The prepared gels were homogenized for 30 minutes in an ultrasounds bath. To compare with unprocessed caffeine, another gel was equally prepared using the same amount of caffeine as supplied. The gels obtained were tested for caffeine release and permeability using a Franz-cell *in vitro* test. This cell consists of a two compartment glass vessel, separated by a cellulose acetate membrane. The acceptor compartment (below) was filled with a PBS (pH 7,4), which is stirred and thermostatized. In the donor compartment (above) a dose of the gel was added and aliquots of the receptor fluid were collected at given times to follow the diffusion rate of the compound. The amount of caffeine in the aliquots was determined by HPLC-DAD analysis.

Performance of antioxidant-loaded particles in sunflower oil

A sample of the different particles was added to 200 mL of sunflower oil and stirred manually up to 1 min, until homogenization occurred. The incorporation of the active compounds alone was previously tested and was unsuccessful. The oils were characterized according to parameters of the industry, to analyse oxidation products of the oil and thus its degradation degree. Peroxide values were carried out by means of an iodometric assay according to the European Standard EN ISO 3960 and expressed in terms of miliequivalents (meq) of peroxide per kilogram of sample. The acidity tests, measuring the percentage of free fatty acids (% FFA) expressed in oleic acid percentage, were performed according to IUPAC standard methods (2.201, 1987), and consist of a titulation of the free fatty acids with an ethanolic solution of potassium hydroxide. Rancimat measurements, determining the time up to which the oil is resistant to oxidation (induction time), were performed in a Methrom Rancimat (model 679) in triplicate, heated at 120ºC and with an air flow rate of 20 mL/min.

Sunflower oil is also commonly used to fry food and hence, the oxidative stability was also evaluated after simulating a frying process. The procedure of recreating deep-frying was adapted from literature [16]. To simulate the water present in foodstuff 1 g of pre-treat silica (MNKieselgel 60, Machenery-Nagel) is added to 20 g of oil with 10% water. After one minute homogenization in an ultrasound bath, the oil was fried during four hours at 180ºC.

RESULTS

In all experiments performed free flowing powders were obtained, and particles presented porous/laminar morphologies (Figure 1) and average particle sizes (d50, volume%) ranged between 1,7 and 6,7 μm.

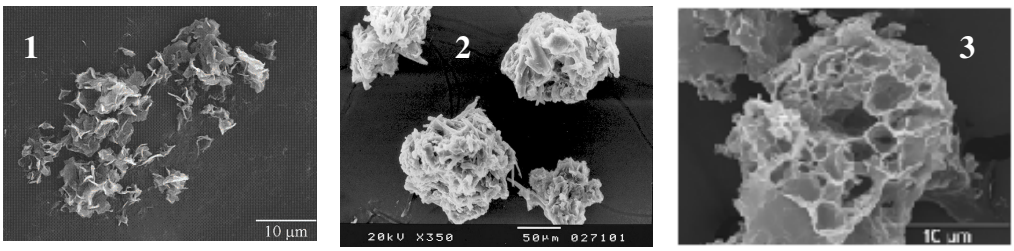

Figure 1: Examples of the SEM pictures obtained for the particles produced with chalcone (1); caffeine (2) and antioxidants (3)

The analysis of the chalcone-loaded particles returned a 50%wt content in chalcone, with a maximum deviation of 3%.

ATR-FTIR images obtained regarding the distribution of chalcone and carriers in the different formulations show there seems to be an influence of HLB value of the carrier in the mixing of the compounds. For formulation with carrier B (HLB=13), images show the most homogeneous mixture, with medium concentration areas common to both substances. For carrier C (HLB=2) on its turn, shows sharp differences in concentration of the compounds, one being the

complementary image of the other, i.e. where there is chalcone there is no carrier and vice-versa. The mixture of the carriers, formulation F, illustrates an intermediate situation, where there still are big contrast areas, but the edges of these areas are smoother indicating a certain degree of mixing.

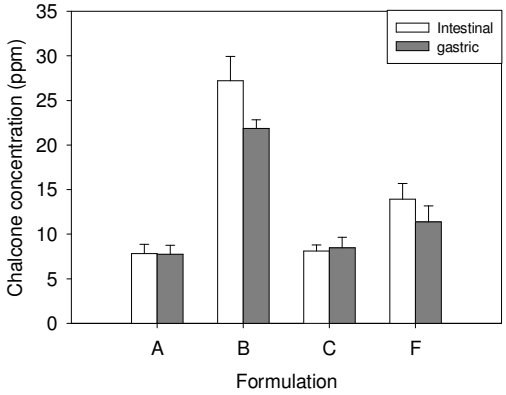

Figure 2: Concentration of trans-chalcone in gastric and intestinal simulated fluids for the formulations A (chalcone alone), B (carrier HLB=13), C (carrier HLB=13), and F (mixture of carriers)

The dissolution tests performed (Figure 2) show the maximum amount of micronized chalcone (no carrier - A) solubilized both in gastric and intestinal simulated fluids was about 8 ppm, as well as for formulation C . Formulation B however, was able to increase the concentration in the gastric and intestinal fluids up to 22 ppm and 27 ppm, respectively. Again the formulation that accounts the mixture of the carriers gave intermediate results, fixing the values of solubilized chalcone at 12 ppm for gastric fluid and 14 ppm intestinal fluid.

The caffeine-loaded particles incorporated in the gel showed a much slower and controlled release (Figure 3), when compared with the gel that had supllied caffeine, validating the possibility of using the particles as controlled release agents.

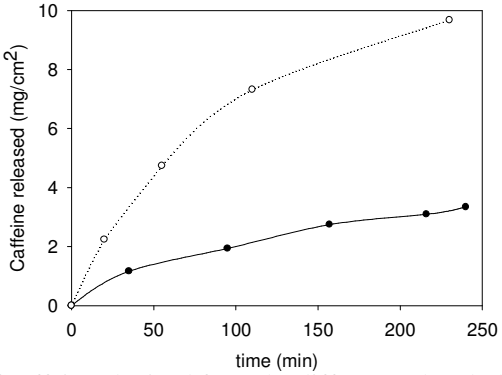

Figure 3: Release profiles of caffeine obtained from the different gels: (dashed line) caffeine alone, (full line) caffeine-loaded particles

From the tests performed in the sunflower oils incorporated with different particle formulations (Figure 4), it can be observed that the induction time measured in Rancimat tests was higher than the original sunflower oil for all formulations, meaning the oils were stable for longer. As for the other tests, the peroxide values and free fatty acid percentage increased with time for the original oil. The incorporation of the particles held back this effect at different levels, depending of the combination of active compounds incorporated within the particles. Formulations E and F were the ones with best overall results, with free fatty acids percentage (%FFA) constant for at least 30 days, which means the oil increased its oxidative resistance.

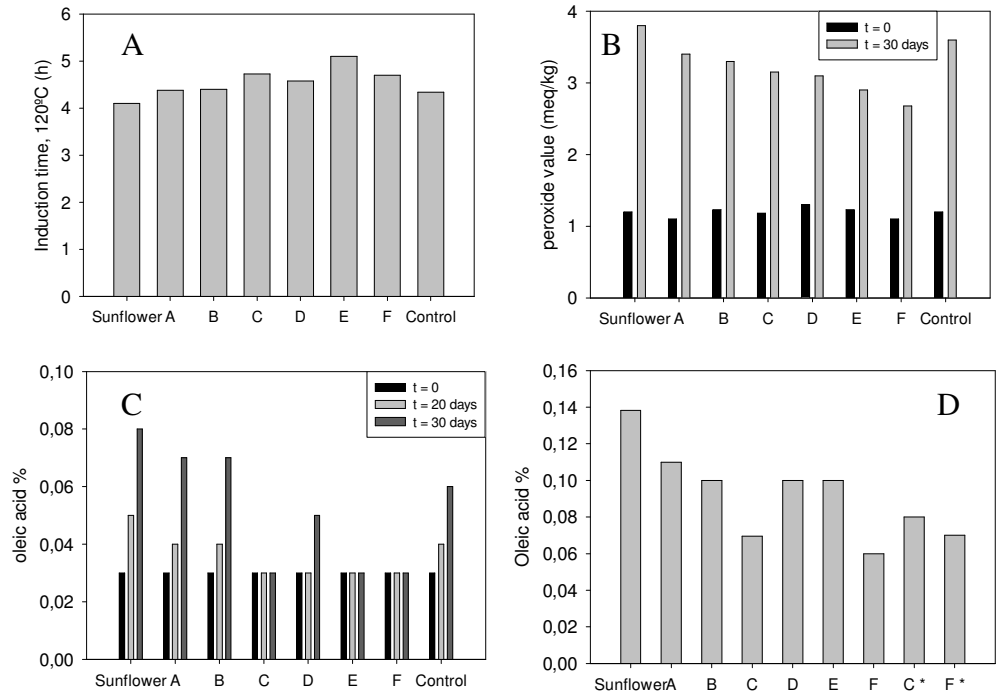

Figure 4: Results of the oxidative stability tests in sunflower oil with several particle formulations incorporated - Rancimat test (induction time - A), peroxide value (B), %FFA (oleic acid %- C and D, where D refers to the results obtained for the frying simulation)

CONCLUSION

Particle formation is a research field with a wide range of applications, transversal to several different industries. This work aimed to demonstrate the viability of application of lipid composite particles produced using supercritical fluids, namely the PGSS method. The tests performed in the particles, according to their final purpose, showed promising results in applying these particulate delivery systems to products in pharmaceutical and food industries.

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