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Casein nanoparticles as carriers for the oral delivery of folic acid

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ABSTRACT

Food grade proteins can be viewed as an adequate material for the preparation of nanoparticles and microparticles. They offer several advantages such as their digestibility, price and a good capability to interact with a wide variety of compounds and nutrients. The aim of this work was to prepare and characterize casein nanoparticles for the oral delivery of folic acid. These nanoparticles were prepared by a coacervation process, stabilized with either lysine or arginine and, finally, dried by spray-drying. For some batches, the effect of a supplementary treatment of nanoparticles (before drying) with high hydrostatic pressure on the properties of the resulting carriers was also evaluated. The resulting nanoparticles displayed a mean size close to 150 nm and a folic acid content of around 25 μg per mg nanoparticle. From the *in vitro* release studies, it was observed that casein nanoparticles acted as gastroresistant devices and, thus, folic acid was only released under simulated intestinal conditions. For the pharmacokinetic study, folic acid was orally administered to laboratory animals as a single dose of 1 mg/kg. Animals treated with folic acid-loaded casein nanoparticles displayed significantly higher serum levels than those observed in animals receiving an aqueous solution of the vitamin. As a consequence the oral bioavailability of folic acid when administered as casein nanoparticles was calculated to be around 52%, a 50% higher than the traditional aqueous solution. Unfortunately, the treatment of casein nanoparticles by high hydrostatic pressure modified neither the release profile of the vitamin nor its oral bioavailability.

1. Introduction

In recent years, nano- and microencapsulation has been a growing interest for pharmaceutical, nutraceutical and food applications. For food applications, these nano- and microencapsulation approaches may be of interest for any of the following reasons: i) protect the compound of interest from its premature degradation (during processing or storage) or undesirable interactions with the environment; ii) mask astringency tastes; iii) facilitate its processability (improving solubility and dispersability); iv) control and/or prolong its release; and/or v) improve its oral bioavailability (Desai & Jin Park, 2005; Quintanilla-Carvajal et al., 2010).

Alimentary proteins, naturally present in food, offer a great potential as a material for the preparation of nanoparticles and microparticles. Overall they are biodegradable, digestible, cheap, offer a nutritional value, and, due to the presence of a number of functional groups, they can interact with a wide variety of compounds in a relatively non-specific way. Another important point is that they are usually considered as GRAS (generally recognized as safe) compounds. In addition, the procedures to transform these proteins into nanoparticles or microparticles are simple and can be performed in an aqueous medium or in environmentally and food grade accepted solvents. Examples of these proteins include legumin and vicilin from peas (*Pisum sativum* L.) (Ezpeleta, Irache, Stainmesse, Gueguen, & Orecchioni, 1996; Irache, Bergougnoux, Ezpeleta, Gueguen, & Orecchioni, 1995), gliadin from wheat (Arangoa, Campanero, Renedo, Ponchel, & Irache, 2001; Ezpeleta et al., 1996), zein from corn (Zhong, Tian, & Zivanovic, 2009) or proteins from soy (*Glycine max* L.) (Teng, Luo, & Wang, 2012).

Another interesting protein for micro- and nanoparticle design is casein. Casein is the major milk protein and possesses many structural and physicochemical properties that facilitate its functionality in drug delivery systems (Semo, Kesselman, Danino, & Livney, 2007). Thus, casein-based devices have been proposed for

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delivering hydrophobic bioactives and drugs including vitamin D2 (Semo et al., 2007), thymol (Pan, Mu, Hu, Yao, & Jiang, 2006), curcumin (Esmaili et al., 2011), and paclitaxel (Shapira, Davidson, Avni, Assaraf, & Livney, 2012).

Folic acid (pteroyl-L-glutamic acid, vitamin B9) is a water-soluble vitamin that is essential in the human diet. The naturally occurring form of folic acid, folate, is typically obtained through consumption of green vegetables or dietary supplements (Brouwer, van Dusseldorp, West, & Steegers-Theunissen, 2001). This vitamin is especially important during the periods of rapid cell division and growth such as pregnancy and infancy when it is often necessary to take specific dosages of folic acid on daily basis (Lamers, 2011). The insufficiency of folic acid has long been known to be related to certain diseases such as neural tube defects in fetus and megaloblastic anaemia (Heseker, 2011). Other ailments associated with low levels of folic acid range from nervous system disturbances to cardiovascular disorders and cancer (Iyer & Tomar, 2009; Kolb & Petrie, 2013). It is assumed that on a population level, nutritional requirements for folate cannot be completely covered by a varied diet, as recommended by national health authorities (Iyer & Tomar, 2009). Thus, fortification with folic acid in one or more of the commonly consumed dietary items (i.e maize flour, milk, bread, etc) has been proposed as the best method to ensure that increased folate intake reduces the risks associated with folate deficiency. However, folic acid and folates are rather unstable molecules. In fact, folic acid is photosensitive and suffers from oxidative degradation, which is enhanced by oxygen, heat and acid pH conditions (Akhtar, Khan, & Ahmad, 1999; Off et al., 2005). This results in a splitting of the molecule into biologically inactive forms (Madziva, Kailasapathy, & Phillips, 2006) and, as suggested from different studies, in a significant decrease in the oral bioavailability of the vitamin (Ohrvik & Witthoft, 2011; O'Leary & Sheehy, 2001).

The aim of this work was to design casein nanoparticles for the oral delivery of folic acid. The encapsulation of this vitamin in these carriers may be of interest to minimize the adverse effects and deteriorative reactions induced during food processing and cooking as well as to improve the oral bioavailability. In order to evaluate the capability of casein nanoparticles to promote the oral bioavailability of folic acid, a pharmacokinetic study in laboratory animals was carried out.

2. Materials and methods

2.1. Materials

Sodium caseinate was obtained from ANVISA (Madrid, Spain). Folic acid, lysine, arginine, pepsin, pancreatin, mannitol and sodium chloride were from Sigma-Aldrich (Germany) whereas ethanol and acetonitrile (HPLC grade) were from Merck (Darmstadt, Germany). AccuDiag™ Folate-Folic acid ELISA Kit was purchased from Diagnostic Automation/Cortez Diagnostics Inc. (USA). All reagents and chemicals used were of analytical grade.

2.2. Preparation of casein nanoparticles

Casein nanoparticles were prepared by simple coacervation procedure followed by a purification step by ultrafiltration and subsequent drying by Spray-drying (Agüeros, M., Esparza, I., González-Ferrero, C., González-Navarro, C.J., Irache, J.M., Romo, A, 2011).

2.2.1. Empty casein nanoparticles (NP)

Briefly, 1 g sodium caseinate and a determined amount of a basic amino acid (either lysine or arginine) were firstly dissolved in 75 mL purified water by magnetic agitation at room temperature. Then, nanoparticles were formed by the addition of 40 mL of a calcium chloride solution in deionized water (0.8% w/v). The suspension was purified by ultrafiltration through a polysulfone membrane cartridge of 50 kDa pore size (Medica SPA, Italy). Finally, 20 mL of an aqueous solution of mannitol (100 mg/mL), was added to the suspension of casein nanoparticles in order to prevent irreversible aggregation of nanoparticles during the drying step, and the suspension was dried in a Büchi Mini Spray Drier B-290 apparatus (Büchi Labortechnik AG, Switzerland) under the following experimental conditions: (i) inlet temperature of 90 °C, (ii) outlet temperature 45–50 °C, (iii) air pressure: 2–5 bar, (iv) pumping rate of 2–6 mL/min, (v) aspirator of 100% and (vi) air flow at 900 L/h.

2.2.2. Folic acid-loaded casein nanoparticles (F-NP)

The preparation of casein nanoparticles loaded with folic acid was similar to that of the empty particles, however some minor adjustments. Thus, 1 g sodium caseinate and 50 mg lysine were dissolved in 75 mL purified water. In parallel, 300 mg folic acid was dissolved in an aqueous solution of lysine (8 mg/mL). Then, 9 mL of the aqueous folic acid solution was added to the caseinate solution and the resulting mixture was incubated at room temperature for 10 min under magnetic stirring. Casein nanoparticles were obtained by the addition of 40 mL of a calcium chloride solution in purified water (0.8% w/v). The suspension was purified and dried as described above.

In order to evaluate the effect of a high pressure treatment on the main properties of these nanoparticles, some batches were subjected to different cycles of high hydrostatic pressure in an ISO-LAB FPG11500 apparatus (Stansted Fluid Power Ltd, UK) prior to the drying step.

For the identification of the different formulations, the following abbreviations were used: FA-NP-C (casein nanoparticles containing folic acid), FA-NP-C-P_x (folic acid-loaded casein nanoparticles stabilized by high pressure) and NP-C (control empty casein nanoparticles).

2.3. Characterization of nanoparticles

2.3.1. Size, zeta potential and morphology

The particle size distribution and zeta potential of the above formulations were measured by photon correlation spectroscopy (PCS) and electrophoretic laser Doppler anemometry, respectively, using a Zetaplus apparatus (Brookhaven Instrument Corporation, USA). The diameter of the nanoparticles was determined after dispersion in distilled water (1:10) and measured at 25 °C with a scattering angle of 90 °C. The zeta potential was measured after dispersion of the dried nanoparticles in 1 mM pH 6 KCl solution.

The morphology and shape of nanoparticles was examined using a field emission scanning electron microscope FE-SEM (ULTRA Plus, Zeiss, The Netherlands). Prior to analysis, particles were washed to remove mannitol. For this purpose, spray-dried nanoparticles were resuspended in distilled water and centrifuged at 27,000× *g* for 10 min. Then, the supernatants were discarded and the obtained pellets were mounted on copper grids. Finally, the pellet was shaded with an amalgam of gold/palladium during fifteen seconds using a sputter coater (K550X Emitech, Ashford, UK).

2.3.2. Yield of the preparative process

In order to quantify the amount of protein transformed into nanoparticles, 10 mg of the nanoparticle formulation was dispersed in water and centrifuged at 17,000× *g* for 20 min. Supernatants were discarded and the pellets were digested with NaOH 0.05 M (casein nanoparticles). Then, the amount of protein was quantified