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Tabletted soy protein cold-set hydrogels as carriers of nutraceutical substances

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ABSTRACT

The swelling of soy protein filamentous hydrogels and tablets thereof and the release of riboflavin from these drug delivery devices were investigated under simulated gastrointestinal conditions in the presence or absence of digestive proteases. Microscopic examination showed riboflavin arranged into crystals dispersed randomly throughout the hydrogel and the tablet powder. Swelling experiments showed a comparable behavior of water uptake for hydrogel and tablet at pH 1.2 as well as tablet at pH 7.5, featuring a low swelling rate. Hydrogel at intestinal pH began to shrink after 1 h, which coincided with a loss its structure. Riboflavin release was faster at pH 7.5 than at pH 1.2 for both devices. Swelling was the principal mechanism of riboflavin release from tablets at pH 7.5, while drug-polymer interactions slowed this release at pH 1.2. In the presence of pepsin at pH 1.2, both devices showed slow zero-order release of riboflavin for 6 h, while both were digested completely in the presence of pancreatin at pH 7.5. These results suggest that these tabletted hydrogels and the hydrogels themselves might both be useful for transporting bioactive molecules through the gastrointestinal tract and delivering them in the small intestine. Considering their non-synthetic nature, they should be of great interest for the development of innovative functional foods.

1. Introduction

Studies of nutraceutical substances such as vitamins, probiotics, bioactive peptides and antioxidants have been growing in number steadily over the past several years and new scientific proof of the physiological benefits of these substances to human health is being discovered every day (Wildman, 2001). However, the consumption of nutraceutical molecules, whether in foods or as dietary supplements, cannot provide the expected benefits if the molecules of interest lose their bioactivity before they are absorbed in the small intestine. It is therefore crucial to protect them against conditions encountered in food processing (temperature, oxygen, light) and in the gastrointestinal tract (pH, presence of enzymes and other nutrients). Although the effectiveness of well-known biomedical and pharmaceutical polymer-based delivery systems for entrapping sensitive nutraceutical molecules and protecting them until they reach the intestines has been shown (Langer & Peppas, 2003), their non-biodegradable nature and requirement for the use of solvents incompatible with food applications has limited their use. The development of natural hydrogels made from food biopolymers and especially proteins shows much more promise (Chen, Remondetto, & Subirade, 2006).

However, food protein hydrogels are generally obtained using heat treatment, which is problematic for heat-sensitive active compounds. Recently, we developed soy protein hydrogels using a cold gelation process already widely practiced with whey protein (Barbut & Foegeding, 1993). Our process involves preheating soy proteins and then adding calcium chloride (Maltais, Remondetto, Gonzalez, & Subirade, 2005). At 10 mM $CaCl_2$, a filamentous structured hydrogel is obtained, while a particulate hydrogel is formed at 20 mM $CaCl_2$. Distinct mechanisms are involved in the formation of these two gel types (Maltais, Remondetto, & Subirade, 2008) and the release of riboflavin from them into gastric and intestinal media also differed somewhat, the more porous and granular particulate type providing faster initial release (Maltais, Remondetto, & Subirade, 2009). Both appear suitable as nutraceutical carriers since they provide slow release in gastric medium (pH 1.2) and faster release in intestinal medium (pH 7.5). However, filamentous networks are easier to work with since they hold better their structure. They are also more likely to be used in the food industry for their smooth texture and their low porosity.

In order to expand the potential range of uses of these delivery devices, we decided to test the release of riboflavin from tablets

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made with freeze-dried cold-set soy protein gel. Tablet manufacturing is widely practiced and produces a highly stable product familiar to consumers and still accounts for more than 80% of all human medical dosage forms (Liu, Sun, Wang, Zhang, & Wang, 2005). We aim to produce a device that provides effective protection of molecules against digestive enzymes and low gastric pH plus prolonged release under intestinal conditions. Since the gel microstructure should be lost during tablet preparation due to aggregation and molecular rearrangements that take place during freeze-drying (Badley et al., 1975; Catsimpoolas, Campbell, & Meyer, 1969; Genovese & Lajolo, 1992) as well as grinding, we expected the behavior of tablets immersed in gastric and intestinal media to differ from that of hydrogel. We therefore compared these two delivery devices using riboflavin, which is a low-molecular-weight ionic molecule that has been shown previously to have no strong interaction with soy protein hydrogels (Maltais et al., 2009).

2. Materials and methods

Anhydrous calcium chloride, riboflavin, pepsin (from porcine stomach; 3200 units/mg of proteins), pancreatin (from porcine pancreas; meets USP specifications) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were reagent grade.

2.1. Soy protein gels

Native soy protein isolate (SPI), prepared at pilot plant scale according to the method of Remondetto, Gonzalez, and Añón (1998), was obtained from the Food Science and Technology Institute, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina. It contained 94% protein on a dry basis as measured by the macro-Kjeldahl method (AOAC, 1984) using N factor 6.25. A 9.5% (w/w) SPI stock solution in double-distilled water was prepared by the method of Maltais et al. (2005) and adjusted to pH 7.0. Protein solution was preheated at 105 °C for 30 min in a water/ethylene glycol bath then cooled and held for 2 h at room temperature (25 °C). After cooling, 0.5 M riboflavin solution was added, followed by double-distilled water to dilute the solutions to 9% protein. This solution was divided into 5-g portions and calcium chloride solution (0.5 M) was then blended into each using a vortex to obtain a final calcium concentration of 10 mM, thus producing filamentous hydrogel. Each gel thus contained 5×10^{-5} mol of riboflavin. They were then stored for 24 h at room temperature in the dark in order to prevent photodecomposition of the riboflavin.

2.2. Soy protein tablets

Riboflavin-loaded gels lyophilized for 24 h were ground into powder. Tablets 13 mm in diameter were prepared by pressing the powder (about 500 mg) in a Carver® Autopellet press (model 3887, Wabash, IN, USA) with a force of 5000 lbs for 1 min. The amount of riboflavin in one tablet was thus about the same as in one gel. Tablets were kept in sealed amber bottles.

2.3. Gastric acid medium

The simulated gastric fluid (United States Pharamcopeia, 2004b) consisted of 2.0 g of sodium chloride, 7.0 mL of 37% hydrochloric acid and 1000 mL of double-distilled water. The final pH was 1.2.

2.4. Intestinal alkaline medium

The simulated intestinal fluid (United States Pharamcopeia, 2004b) consisted of 6.8 g of monobasic potassium phosphate dissolved in 250 mL of double-distilled water and added to 190 mL of 0.2 N sodium hydroxide and 400 mL of double-distilled water. The pH was adjusted to 7.5 using 0.2 N sodium hydroxide and the final volume was brought to 1000 mL with double-distilled water.

2.5. Microscopic analysis

Lyophilized riboflavin-loaded gel powder was re-hydrated with double-distilled water and observed using an Olympus BX50WI optical microscope (Olympus, Melville, NY, USA) fitted with a digital camera (model U-TV1 X, Olympus Optical, Tokyo, Japan). Samples of non-freeze-dried gel were also observed.

2.6. Swelling experiment

The water uptake of hydrogels and tablets was compared at pH 1.2 and 7.5. Gels were unmolded and their surface blotted dry. Both devices were weighed and then immersed in either gastric or intestinal fluid without enzyme. Gels or tablets were periodically removed from the medium, blotted dry and re-weighed. The test was carried out for 24 h. Swelling was calculated as follows:

$$\%\,\text{swelling} = [(W_t - W_0)/W_0] \times 100$$

where W_t is the gel or tablet weight at time t and W_0 is the initial gel or tablet weight.

2.7. Dissolution experiments

Dissolution studies were carried out according to Pharmacopoeia methods (European Pharmacopoeia, 2003; United States Pharmacopeia, 2004a) in 900 mL of simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) with or without enzyme. Pepsin (3.2 g/L) or pancreatin (10.0 g\L) was added to the medium 1 h before adding the soy gel or tablet. A water bath dissolution center (Distek Inc., model 2100C, North Brunswick, NJ, USA) composed of eight vessels immersed in a water bath and interchangeable stirring devices was coupled to a UV-visible spectrophotometer (Agilent Technologies Inc., model 8453, Santa Clara, CA, USA) and an auto-sampling system was used to pump dissolution media through the spectrophotometer flow cells and back to the stirring vessels at predetermined time intervals. The UV-visible ChemStation software (Agilent Technologies Inc.) was used for data acquisition and quantification. Paddle apparatus II with a device developed by Remondetto, Beyssac, and Subirade (2004) to hold the sample at the bottom of the vessel was used for gels and basket apparatus I was used for tablet testing. Agitation speed was set to 100 rpm and the temperature was kept at 37 ± 0.5 °C. Amber vessels were used to prevent photodecomposition of riboflavin. Media were analyzed at 15-min intervals during the first hour and then at 1 h intervals. Sink conditions were maintained throughout the experiments.

2.8. In vitro gastrointestinal assay

To simulate the succession of pH conditions and enzymatic activities occurring during the digestive process, hydrogels and tablets were first subjected to gastric conditions with pepsin (pH 1.2) for 30 min. The SGF was then removed, the vessel washed three times with double-distilled water and intestinal medium with pancreatin (pH 7.5) was added, in which the dissolution was followed for 6 h.