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Development and characterization of iron-pectin beads as a novel system for iron delivery to intestinal cells

F. Ghibaudo[a], E. Gerbino[a], A.A. Hugo[a], M.G. Simões[b], P. Alves[b], B.F.O. Costa[c], V. Campo Dall´ Orto[d,e], A. Gómez-Zavaglia[a,*], P.N. Simões[b]

[a] Center for Research and Development in Food Cryotechnology (CCT-CONICET La Plata, UNLP) RA-1900, Argentina
[b] CIEPQPF, Department of Chemical Engineering, University of Coimbra, Polo II, Pinhal de Marrocos, P-3030-790 Coimbra, Portugal
[c] CFisUC, Physics Department, University of Coimbra, 3004-516 Coimbra, Portugal
[d] Department of Analytical Chemistry and Physical Chemistry, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, 3rd Floor, RA 1113, Argentina
[e] CONICET-Universidad de Buenos Aires, Institute of Chemistry and Drug Metabolism (IQUIMEFA), Junín 956, Buenos Aires, Argentina

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ABSTRACT

Iron deficiency is the most common nutritional deficit worldwide. The goal of this work was to obtain iron-pectin beads by ionic gelation and evaluate their physiological behavior to support their potential application in the food industry. The beads were firstly analyzed by scanning electronic microscopy, and then physical-chemically characterized by performing swelling, thermogravimetric, porosimetry, Mössbauer spectroscopy and X-ray fluorescence analyses, as well as by determining the particle size. Then, physiological assays were carried out by exposing the beads to simulated gastric and intestinal environments, and determining the iron absorption and transepithelial transport into Caco-2/TC7 cells.

Iron-pectin beads were spherical (diameter 1–2 mm), with high density (1.29 g/mL) and porosity (93.28%) at low pressure, indicating their high permeability even when exposed to low pressure. Swelling in simulated intestinal medium (pH 8) was higher than in simulated gastric medium. The source of iron [$FeSO_4$ (control) or iron-pectin beads] did not have any significant effect on the mineral absorption. Regarding transport, the iron added to the apical pole of Caco-2/TC7 monolayers was recovered in the basal compartment, and this was proportional with the exposure time. After 4 h of incubation, the transport of iron arising from the beads was significantly higher than that of the iron from the control ($FeSO_4$). For this reason, iron-pectin beads appear as an interesting system to overcome the low efficiency of iron transport, being a potential strategy to enrich food products with iron, without altering the sensory properties.

1. Introduction

Iron deficiency is the most common nutritional deficit worldwide and represents a public health problem in both industrialized and non-industrialized countries. Iron is an essential trace metal for all organisms. In humans, it plays important biochemical roles, including the binding of oxygen to hemoglobin or as catalytic center of many enzymes (*e.g.*, cytochromes). The iron concentration in the body is regulated at absorption level in the proximal small intestine [1]. The daily requirements of dietary iron are 5–30 mg, depending on the stage of growth, gender and diet. Dietary iron occurs in two forms, as heme iron from meat products, and as non-heme (inorganic) iron present in vegetables and derivatives. The bioavailability of heme iron is 20–30 %, and that of non-heme one is only 1–10 % [2].

To overcome the above mentioned public health problem, supplementation and/or fortification are two strategies generally used for correcting iron deficiency. The first one is targeted to high-risk population. Iron is provided three or four times a day in high doses (usually 60 mg Fe/day), and is not included in food products. This strategy often carries unwanted effects, such as abdominal pain, constipation, diarrhea and vomiting. On the contrary, fortification might be a safer intervention because iron is included in food in lower doses, simulating the physiological environment and avoiding the undesirable side effects [3]. Ferrous sulfate and fumarate sulfate are the most bioavailable iron

Abbreviations: DLS, dynamic light scattering; DMEM, Dulbecco's modified Eagle medium; PBS, phosphate buffered saline; SEM, scanning electronic microscopy; TGA, thermogravimetric analysis; XRF, X-ray fluorescence analysis; ζ, zeta potential

* Corresponding author at: Calle 47 y 116 La Plata, Buenos Aires RA1900, Argentina.
E-mail address: angoza@qui.uc.pt (A. Gómez-Zavaglia).

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compounds. Although they are soluble in water or diluted acid, they often react with other food components to cause off-flavors, color changes or fat oxidation [4]. For this reason, encapsulated ferrous sulfate and fumarate are available in the market for food fortification, preventing iron atoms to alter food sensory properties, and enabling iron to be safely released and absorbed in the small intestine. Therefore, although less soluble forms of iron are less well absorbed, they are often chosen for fortification to avoid undesired sensory changes [4].

An alternative method to prevent this undesirable effect of iron in fortified foods is ionic gelation, through which polymeric beads are obtained using divalent cations. In ionic gelation, cations form insoluble associates with carbohydrate chains, resulting in the so-called "egg-box" complexes [5]. Natural biopolymers, including dietary oligo and polysaccharides, are particularly interesting for this purpose because of their good biocompatibility, non-toxicity and controlled release properties [6]. Pectins are linear chains of partially methyl-esterified (1→4)-linked α-D-galacturonic acid residues. Those of low degree of esterification (< 50%) are useful for ionotropic gelation [7]. As pectins belong to dietary fiber, they are not hydrolyzed in the upper part of the gastro-intestinal tract. For this reason, pectin matrices have been widely used to deliver drugs whose target is the colon [8–11]. Calcium and zinc have been majorly employed as divalent cations for ionotropic gelation, but the use of iron in that function has been very scarcely addressed [12].

Although most dietary iron is absorbed in the duodenum, the colon mucosa also expresses the iron absorption proteins, thus enabling the absorption of 30% of the iron present in the gastrointestinal tract [13]. Non-digestible carbohydrates (*e.g.,* pectins, inulins), resist digestion in the small intestine but are fermented in the colon to short-chain fatty acids, with a variety of health benefits, including the enhancement of iron absorption [14].

Considering the important role of ferrous sulfate salts to enhance iron consumption, the goal of this work was to develop iron-pectin beads by ionotropic gelation as a novel strategy for food fortification. The beads were firstly observed by scanning electronic microscopy, and then physical-chemically characterized by performing swelling, thermogravimetric, porosimetry and X-ray fluorescence analyses. Afterwards, physiological assays were carried out by exposing the beads to simulated gastric and intestinal environments, and by determining the particle size, iron absorption and transepithelial transport in Caco-2/TC7 cells, thus fulfilling a study that supports the safe delivery of iron to the gut.

2. Materials and methods

2.1. Preparation of pectin beads

Pectin from citrus peel (galacturonic acid ≥74.0%, Sigma Aldrich, Buenos Aires, Argentina) was dissolved in 0.060 M acetic acid-sodium acetate (Sigma Aldrich, Buenos Aires, Argentina) at pH 5.0, to obtain a 4% w/v solution. The pectin solution was dripped into a 150 mM $FeSO_4$ solution using a 0.3 mm needle (~10 μL/drop) under continuous agitation for 30 min. The beads were harvested by filtration through a stainless-steel mesh of 0.10 mm and washed three times with distilled water. Then, the beads were frozen at −80 °C and freeze-dried for 48 h on a Heto FD4 equipment (Heto Lab Equipment, Denmark) operating with the condenser at −45 °C at a chamber pressure of 0.04 mbar.

2.2. Scanning electronic microscopy (SEM)

The obtained beads were mounted on metal stubs with double sided adhesive carbon tape, coated with gold using a sputter coater (Polaron Thermo VGScientific, East Grinstead, Sussex, UK) under vacuum and 18 mA at room temperature [15]. The morphology of the beads was observed by SEM, focusing both on the inner and superficial features. Samples were examined using an environmental scanning electron microscope (FEI La B6, Eindhoven, Netherlands) at 14 kV accelerating voltage with an electron detector for low vacuum conditions. For examination of the inner structure, the beads were cut in half with a steel blade.

2.3. In vitro digestion

The freeze-dried beads were digested in simulated gastrointestinal solutions (adapted from [16]). In brief, 10 beads (weighting about 3.5 mg, and representing about 100 μL of the original pectin solution) were suspended for 2 min in 1 mL of saliva [10 mg/mL α-amylase from *Bacillus subtilis* in phosphate buffered saline (PBS) (K_2HPO_4 0.144 g/L; NaCl 9.00 g/L; Na_2HPO_4 0.795 g/L, pH 6.8)] (Sigma Aldrich, St. Louis, MO, USA). Saliva was thoroughly removed, and then 1 mL of simulated gastric digestion (3 mg/mL porcine pepsin, 125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, pH 2.5) (Sigma Aldrich, St. Louis, MO, USA) was added to the sedimented beads. Samples were incubated for 1.5 h at 37 °C under continuous gentle shaking (50 rpm, MaxQ 4000, Thermo Scientific, USA). Afterwards, the gastric juice was thoroughly removed and 1 mL of simulated intestinal juice (1 mg/mL pancreatin, 1.5 mg/mL bile salts, 22 mM NaCl, 3.2 mM KCl, 7.6 mM $NaHCO_3$, pH 8.0) (Sigma Aldrich, St. Louis, MO, USA) was added to the beads and incubated for 3 h at 37 °C, under continuous gentle shaking (50 rpm, MaxQ 4000, Thermo Scientific, USA).

2.4. Swelling characterization of the beads

Freeze-dried beads were weighed on an analytical balance (Adventurer Ohaus, Parsippany, NJ, USA balance sensitivity: 0.1 mg), and then hydrated for 15 min in water and for 60 min in the simulated gastric and intestinal solutions. Afterwards, the beads were dried for 5 min at 20 °C on cellulose paper (Whatman nº 1) and weighed again.

The degree of swelling (given by the ability to absorb water in the interstices of the microspheres) was determined:

$$\frac{Wh-Wd}{Wd} \times 100 \quad (1)$$

where *Wh* is the weight of hydrated beads and *Wd*, the weight of the dehydrated ones.

2.5. Thermogravimetric analysis (TGA)

The thermal stability of pectin and pectin-Fe samples was studied by High-Resolution-Modulated-Thermogravimetry Hi-Res-MTGA (TA Instruments Q500, USA; balance sensitivity: 0.1 μg). The temperature calibration was carried out by measuring the Curie point of nickel standard in open platinum crucibles, under a dry nitrogen purge flow of 100 mL/min, at a heating rate of 2 °C/min, a modulation period of 200 s, and an amplitude temperature of ± 5 °C. Experiments were carried out in the 20–600 °C temperature range.

2.6. Mercury intrusion porosimetry

The porosity and the pore size distribution of the freeze-dried beads were quantified by mercury intrusion porosimetry using the AutoPore IV 9500, from Micromeritics (Atlanta, USA), at a pressure of 0.5 and 30 psi.

2.7. X-ray fluorescence analysis (XRF)

Iron-pectin beads and commercial pectin powder (galacturonic acid ≥74.0%, Sigma Aldrich, Buenos Aires, Argentina) were analyzed by X-ray fluorescence. The measurements were undertaken in air at atmospheric pressure on a Hitachi SEA6000VX bench top high-sensitivity XRF analyzer, with an X-ray tube with a tungsten target, operating at potentials of 15 and 50 kV and a current of 1000 mA, and a 3 mm wide