Inulin as a novel biocompatible coating: Evaluation of surface affinities toward CaHPO₄, α-Fe₂O₃, ZnO, CaHPO₄@ZnO and α-Fe₂O₃@ZnO nanoparticles

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1. Introduction

The development of novel delivery systems for food enrichment and pharmaceutical use is a promising application for nanomaterials...
Micro-nutrient deficiencies of minerals such as iron, zinc and calcium affect a large percent of world population and also impact cognitive and human physical development, reducing life quality and working capabilities [2]. Food fortification constitutes one of the most used strategies to treat nutritional deficiencies. Calcium phosphate (CaHPO_4), hematite (α-Fe_2O_3) and zinc oxide (ZnO) nanoparticles can be easily prepared, are biocompatible, and could be used as food additives for fortification because they are more soluble than their bulk counterparts [3]. The biomedical, pharmaceutical, cosmetic and food applications of calcium phosphate (CaHPO_4), hematite (α-Fe_2O_3) and zinc oxide (ZnO) as bulk materials have been largely explored, which suggest that they may be stable when incorporated into biologically relevant matrices as nanomaterials [3].

Calcium phosphates are important minerals occurring in biological calcification processes and have important roles in the metabolism of animals and plants; they are considered today as important components in food supplements [4]. Zinc oxide is commonly included for fortification of grains, besides of being widely used in the pharmaceutical area, cosmetic and dental prostheses [5]. Hematite is commonly used as a nutritional component for animal feeding, both as a pigment and as a nutrient [6]. However, several challenges have to be confronted in order to incorporate nanomaterials into food without changing their physical or sensorial properties. Solubility of bulk materials is an important issue, but reducing the particle size under the submicrometric sensorial properties. Solubility of bulk materials is an important rate for animal feeding, both as a pigment and as a nutrient [6]. Howev-

terization of nanomaterials for biomedical, pharmaceutical or nutricional applications, as well on the evaluation of their potential toxicological impact [11–13]. Here, we explore the potential of inulin as a novel biocompatible and stabilizing agent for the coating of calcium phosphate (brushite, CaHPO_4·2H_2O), iron oxide (hematite, α-Fe_2O_3), zinc oxide (zincite, ZnO), CaHPO_4·2H_2O@ZnO and α-Fe_2O_3@ZnO nanoparticles, with the later purpose of evaluating their toxicity and evaluate their potential as food fortification additives in dairy products.

2. Materials and methods

FeCl_2·4H_2O (ferrous chloride tetrahydrate, 99%), FeCl_3·6H_2O (ferrous chloride hexahydrate, 98%), HCl (hydrochloric acid, 37%), ammonium hydroxide (NH_4OH, 25–30% of ammonia), ZnCl_2 (zinc chloride, 98%), NaOH (sodium hydroxide, 97%), calcium chloride ([CaCl_2·2H_2O, 99%], dibasic sodium phosphate (NaH_2PO_4·H_2O, 98%) and ethanol (CH_3CH_2OH, 99.93%) were used in the experiments. All chemicals were analytical grade (Sigma–Aldrich, Toluca, Mexico) unless otherwise stated. Inulin (Fruttage SP750, Monterrey, Mexico), a natural polysaccharide with formula C_{6n}H_{10n+2}O_{3n+1}, it was also used. Deionized water was used in the preparation of nanoparticles.

2.1. Synthesis of inorganic nanoparticles

Inorganic nanoparticles of zinc oxide (ZnO), hematite (α-Fe_2O_3) and calcium phosphate (CaHPO_4·2H_2O, brushite) were prepared following previously reported procedures, and used as obtained [5,14,15].

2.1.1. α-Fe_2O_3 nanoparticles

0.270 g (1 mmol) of FeCl_3·6H_2O and 0.198 g (2 mmol) of FeCl_2·4H_2O were dissolved in 10 mL of 2 M hydrochloric acid to form a solution with concentration 1 M for Fe(III) and 2 M for Fe (II). Then, 71 ml of ammonium hydroxide solution (2 M) was added slowly to this solution with vigorous stirring at room temperature along 2 h. The black precipitate was then collected by filtration and rinsed three times with deionized water and ethanol. The washed precipitate was dried at 70 °C overnight. Finally, the dark solid, α-Fe_2O_3 was calcined in air at 500 °C and held at this temperature for 1 h in an oven furnace (Furnace FB1300, Thermolyne, USA) until obtaining red α-Fe_2O_3 nanoparticles (hematite).

2.1.2. ZnO nanoparticles

Zinc oxide nanoparticles were synthesized using ZnCl_2 (zinc chloride 98%), ethanol (CH_3OH) and NaOH (sodium hydroxide 97%). First, a 0.10 M NaOH solution was prepared by dissolving 0.08 g of sodium hydroxide pellets in 20 mL of ethanol and stirred for 20 min at room temperature. Then a 0.10 M ZnCl_2 solution in 20 mL ethanol was slowly added to the previous solution and stirred for 2 h. The obtained precipitate was washed with ethanol, separated by centrifugation and dried at 30 °C overnight to get ZnO nanoparticles, as a fine white powder.

2.1.3. CaHPO_4·2H_2O nanoparticles

CaCl_2·2H_2O (99%), NaH_2PO_4·H_2O (98%) and HCl (37%) were used for the preparation. A 36 mM CaCl_2 solution was prepared by adding 2.407 g (36 mmol) of the calcium (II) precursor. Then the pH of the solution was lowered to pH 5.5–6.0 by adding HCl. This solution was added, drop by drop to a volume of 1 L of Na_2HPO_4 (21.6 mM), monitoring constantly the pH to be kept in the 5.5–6.0 range; at the end of the addition, a final pH of 5.86 was recorded. Then the solution was centrifuged at 8000 rpm for 10 min, the supernatant removed and water added (for each 40 mL of washing solution, 20 mL of water were added). This procedure was repeated twice. The remaining product was recovered and dried at 30 °C overnight to obtain the final white solid.

2.1.4. CaHPO_4@ZnO and α-Fe_2O_3@ZnO nanoparticles

ZnO coating of previously synthesized inorganic nanoparticles was achieved by the slow alkaline hydrolysis of Zn^{2+} in presence of CaHPO_4 or α-Fe_2O_3 nanoparticles as nucleation seeds, a method adapted from that reported by Hong and coworkers [16]. To an aqueous dispersion (20 mL) of 100 mg α-Fe_2O_3 or CaHPO_4 nanoparticles, an aqueous 0.10 M ZnCl_2 solution (20 mL) in ethanol was added; the mixture was heated at 40 °C during 5 min and then, slowly and under magnetic stirring, 20 mL of an aqueous 0.10 M NaOH solution was added over 2 h. After this time, the product was separated by centrifugation, washed twice with absolute ethanol and dried at 30 °C overnight under vacuum to get a fine powder.

2.2. Preparation of inulin-coated nanoparticles

To coat the surface of the inorganic nanoparticles with agave inulin, 100 mg of dried nanoparticles previously prepared were weighed and dispersed in 10 mL of distilled water and sonicated in an ultrasonic bath (Branasonic B5510, Sonics, USA) for 10 min. Simultaneously, a 3% inulin aqueous solution, obtained by dissolv-
ing 0.3 g of inulin in 10 mL of water, was stirred with a magnetic stirrer to be completely dissolved. Then, both solutions were mixed at room temperature and stirred at 500 rpm for 24 h. At the end of this process, to remove the excess of inulin, the material was precipitated by centrifugation, washed once with distilled water and dried overnight in a vacuum oven at 30 °C.

2.3. Characterization

The crystalline phase of the inorganic core of the nanoparticles was determined by X-ray diffraction measurements, using an Empyrean diffractometer system (PANalytical, Netherlands) with Cu Kα radiation (λ = 1.541874 Å), and a X'Celerator detector. The diffraction patterns were collected in the 2θ range of 5–90° (except CaHPO₄, which was measured in the 5–80° range), with a step of 0.017° and 50.2 s measuring time per step. Dynamic light scattering (DLS) measurements were performed for colloidal suspensions of the nanoparticles in deionized water using a Nanotrac Wave (Microtrac, USA). Surface morphology and composition of the inorganic nanoparticles were analyzed using a Vega II-SEM (Tescan, Czech Republic) instrument, equipped with an energy dispersive X-ray spectrometer (EDS) detector. Transmission Electron Microscopy (TEM) studies were carried using a JEM2010 (JEOL, USA) instrument, in samples prepared from a drop of well dispersed supernatant of particle suspensions in absolute ethanol, placed on a carbon-coated 200-mesh copper grid, followed by drying the sample at ambient conditions, before it was attached to the sample holder of the microscope. FTIR spectra were recorded with a Scimitar FTIR 8000 spectrometer (Varian, USA) with a spectral resolution of 4 cm⁻¹, using an ATR (Ge) detector, in the range among 3500–500 cm⁻¹ in transmission mode. For thermal gravimetric analysis (TG-DSC) of the nanoparticles (coated and uncoated), samples were heated in a flow of nitrogen at a constant rate of 20 °C/min in a STA409 system (Netzsch, Germany). Approximately 35 mg of sample, in each case, was placed in an aluminum oxide crucible on a pan of the microbalance and analyzed in a scanning temperature range from 30 to 800 °C.

2.4. Nanoparticle's stability in solution

In order to evaluate the stability in solution of the prepared nanoparticle systems, the dissolution process of 10 mg of the samples dispersed in distilled water at pH 7 was followed along 120 h (5 days). The maximal amount of ions (Fe, Ca, Zn) was determined by digesting 10 mg of the samples in boiling hydrochloric acid for two hours until complete dissolution. The concentration of Zn(II), Fe(III) and Ca(II) released into the solution for the different systems prepared in this work was determined by atomic absorption spectrophotometry (Varian SpectrAA 220FS). Briefly, spaced aliquots of a suspension of nanoparticles in DI water taken along 5 days, were monitored for the concentrations of the specified ions by AAS, using an air/acetylene flame for Zn(II) and Fe (III) or NO₂/acetylene for Ca(II), with a ionization temperature of 1100 °C. All measurements were made by triplicate. The amount of metal ions released was calculated from a calibration curve.

3. Results and discussion

3.1. Synthesis

In order to evaluate the potential usefulness of inulin to prepare water soluble, biocompatible and stable inorganic nanoparticles for food applications, the surface of inorganic CaHPO₄, ZnO, CaHPO₄@ZnO and α-Fe₂O₃@ZnO nanoparticles were treated with inulin of agave and the products were fully characterized by FTIR, XRD, EDS, TGA/DSC and TEM in order to check for their chemical and structural identity. The inorganic nanoparticles were prepared by co-precipitation in water and stirred in 0.5%, 1.0%, 1.5%, 3.0% and 5.0% agave inulin aqueous solutions during 24 h. Inulin coating was only detected for ZnO nanoparticles stirred in 3.0% or higher concentrations, even at longer times of immersion, as confirmed by FTIR spectroscopy. The characteristic vibrational peaks associated with inulin were not detected for hematite or brushite nanoparticles, except when their surfaces were coated first with ZnO (it will be discussed later). Nanoparticles were precipitated and washed in order to remove excess inulin. Based on the recovered inulin from the washing water, only around 4.5–6.0 mg of it was adsorbed on the nanoparticle's surfaces. Based on the weight of the recovered products, yields were in the range from 70% to 90%. Biopolymers may be adsorbed into the surface of nanoparticles by electrostatic, hydrophobic, covalent or hydrogen bonding interactions. Hydrogen bonding results from the interaction of hydroxyl groups (–OH) present along the inulin structure and hydroxylated and protonated surface sites on the nanoparticle, covering its surface as represented in Fig. 1. It has been previously reported how the presence of hydroxyl groups on the nanoparticle surface further enhance the adsorption of biopolymers in comparison with the absence of those in, for example, gold nanoparticles [17]. Furthermore, the use of polysaccharides can reduce the toxicity of a several usually toxic nanomaterial, which is of great importance for biomedical or food applications [18].

3.2. Powder X-ray diffraction

Fig. 2 shows the XRD patterns of the prepared samples. XRD pattern of ZnO corresponds to zincite, with no peaks associated to remaining Zn(OH)₂ in the diffraction pattern (Fig. 2a); the width of the peaks and their intensity suggests that the sample is highly crystalline [19]. However, the product was contaminated with NaCl, as a side product from the reaction of the chemical precursors used for the preparation, as clearly visible by the distinctive XRD pattern characteristic for cubic rock salt. All other XRD peaks correspond to ZnO in the hexagonal wurtzite phase (JCPDS No. 36-1451). Using the Scherrer Eq. 1:

\[ D = \frac{0.94\lambda}{h_{1/2} \cos \theta} \]  

(1)

where \( \lambda \) was 1.541874 Å, \( h_{1/2} \) is the line broadening at the half of the maximum intensity peak after subtracting the instrumental line broadening in radians, and \( \theta \) is the Bragg angle, the average particle size calculated from the first three peaks were found to be among 74–104 nm. The XRD pattern for iron oxide nanoparticles (Fig. 2b) shows the characteristic peaks of the pure rhombohedral phase of α-Fe₂O₃ (hematite. JCPDS: 33-0664) [20]. No diffraction peaks from impurities were found in the sample. The average crystallite sizes calculated from the Scherrer equation, using the two most intense peaks, (1 0 4) and (1 1 0), were found to be 47.1 and 47.4 nm, respectively. Finally, the XRD patterns for the prepared calcium phosphate (Fig. 2c) matched to the standard pattern of pure brushite from JCPDS No. 72-0713 [21]. The average crystallite size, as determined from the Scherrer formula, was of 98 nm.

To confirm the ZnO coating on the α-Fe₂O₃ and CaHPO₄ nanoparticles, the crystalline structure of α-Fe₂O₃@ZnO and CaHPO₄@ZnO systems was determined by XRD. Several characteristic diffraction peaks corresponding to hexagonal wurtzite ZnO (JCPDS No. 36-1451) are present in the diffractogram. No significant changes in the crystalline structure of α-Fe₂O₃, CaHPO₄ or ZnO were found, as the diffraction peaks for hematite, brushite and zincite phases show almost the same 2θ locations as those of the pristine phases (Fig. 3). Enhanced intensity of some peaks is caused by peak overlapping.
3.3. Thermogravimetric analysis (TGA)

The TGA profiles for inulin and inulin-coated nanoparticles are shown in Fig. 4. As expected for inulin, three weight loss zones were identified. Among 25–180 °C, a weight loss related to moisture was found. Then, a second temperature range (180–260 °C) was associated with the decomposition of the polymer, immediately followed by a large loss of mass due to inulin combustion and complete degradation at 250–800 °C. These results are in agreement with those previously reported by Dan and coworkers [22].
For the ZnO nanoparticles (Fig. 4b), only a small (around 3.89%) mass loss was found after heating up to 800 °C, as an indication of material purity [23]. As indicated in the TGA profile, a single decrease in mass over a temperature range from 220 °C to 280 °C can be related to the desorption of chemisorbed water from the surface of ZnO. Further small mass loss at 345 °C and beyond may correspond to the decomposition of residual Zn(OH)₂ in the crystalline lattice. The presence of inulin on the surface of ZnO nanoparticles is supported by the TGA data as a larger mass change (respect to pure ZnO) of 8.1% occurs around 270 °C, characteristic to inulin decomposition. For α-Fe₂O₃ (Fig. 4c) a minimum mass loss of 0.78% was observed in the temperature ranges from 30–140 °C, 180–450 °C and 580–650 °C, corresponding to the evaporation of adsorbed water and further dehydration of remaining [Fe(OH)₃] groups. In the case of the α-Fe₂O₃@inulin nanoparticles the mass loss was very small (2.08%), in comparison to the ZnO@inulin system, which indicates weak inulin binding to the nanoparticle surface. The TGA analysis for the calcium phosphate nanoparticles (Fig. 4d) confirms that they were obtained in the brushite phase. CaHPO₄ nanoparticles showed a 6.3% mass loss around 300 °C, which was due to conversion of calcium phosphate to pyrophosphate. In addition to the loss of adsorbed water, there were a characteristic mass loss at 222 °C which corresponds to CO₂.

Furthermore, the TGA curve for the CaHPO₄@inulin system was almost identical to that of the uncoated CaHPO₄ sample, exhibiting also the transition from brushite to calcium pyrophosphate [24]. The first decomposition step in between 100 °C and 350 °C is related to the loss of two water molecules from the crystal lattice and formation of dehydrated CaHPO₄. The second decomposition step occurs after 350 °C and continues until around 600 °C, and is related to the further loss of water (0.5 water molecules per Ca²⁺ ion) and the formation of Ca₂P₂O₇, which is typical for the high temperature brushite irreversible thermal transformation into calcium pyrophosphate. As not significant mass changes in the TGA profile were observed among uncoated or coated CaHPO₄ samples, it can be conclude that inulin binds poorly to theirs surface.

From the analysis of the TGA curves, it can be suggested that inulin coating of the surface of every nanomaterial can be achieved.
ZnO nanoparticles presents a featureless spectrum in the 3500–2800 cm$^{-1}$ region; in comparison, 3% inulin coated ZnO shows a broad band around 1050 cm$^{-1}$, which may increase the affinity among the sugar and the nanoparticle's surface. Several carboxylated sugars have been explored for nanoparticle coatings [7,25]. Then, in order to prepare inulin coated hematite and brushite nanoparticles, a layer of ZnO has to be first deposited onto the surfaces of those nanoparticles. As showed in Fig. 5, the ZnO coated systems have a stronger interaction with inulin, as indicated by the larger mass losses in the range from 180 to 300 °C, characteristic of the thermal decomposition of the polysaccharide, in contrast with non-significant mass losses observed for the unmodified systems (Fig. 4c and d).

Based on the weight% loss in the range from 200 to 300 °C, which is related to the decomposition of inulin, the polysaccharide adsorbed on the surface of the nanoparticles contributes with 1.5% up to 5% of the total weight of the product. Although it is difficult to estimate the total surface area of the nanoparticles given that they have variable sizes and may present porosity, from the data obtained by TEM and DLS, an approximate surface area was calculated in the range from 11.6 to 79.1 m$^2$/g. Considering those values, the calculated adsorbed amount of inulin per square meter ranges from 1.3 to 7.3 mg/m$^2$, which is not far away of typical values for polymer adsorption on inorganic particles (1 mg/m$^2$).

### 3.4. FTIR spectroscopy

The FTIR spectrum of coated and uncoated nanomaterials are available in the Supplementary Information. The detection of the characteristic inulin chemical functional groups was used to verify if the nanoparticle surface was coated or not with inulin. Pure inulin FTIR spectra shows a broad band around 1050 cm$^{-1}$, two shoulders at 940 and 1130 cm$^{-1}$ and the characteristic O–H stretching vibration around 3300 cm$^{-1}$ [26,27]. The FTIR spectrum of pure ZnO nanoparticles presents a featureless spectrum in the 3500–600 cm$^{-1}$ region; in comparison, 3% inulin coated ZnO shows a FTIR spectra which clearly resembles that of pure inulin, which indicates that ZnO strongly binds inulin to its surface. The CaHPO$_4$ nanoparticles, the FTIR spectra shows three characteristic peaks between 1200 and 600 cm$^{-1}$, which are typical for P–O stretching vibrations and matched well with those reported in the literature [28–30]. No significant change in the FTIR spectra was observed for the inulin coated calcium phosphate nanoparticles, indicating almost no binding of it to their surface. Finally, iron oxide nanoparticles had not bands in the analyzed region, while the inulin coated α-Fe$_2$O$_3$ nanoparticles presented only weak bands related to the chemical groups associated to inulin, in agreement with a poor inulin affinity to its surface. Only the ZnO nanoparticles showed clear spectroscopic evidence of significative surface interactions with unmodified inulin.

When brushite or hematite nanoparticles coated with ZnO were treated with a 3% suspension of inulin during 24 h, a more soluble and stable product was obtained. The FTIR spectra of CaHPO$_4$@ZnO and α-Fe$_2$O$_3$@ZnO nanoparticles treated with inulin shows some of the typical bands associated with the polysaccharide indicating that it is present on the ZnO coated nanoparticles surface, in contrast with what was previously observed for the corresponding uncoated systems. The typical bands associated for inulin C–H stretching modes around 2925 cm$^{-1}$, C–O and C–O–C antisymmetric stretches around 1200–1100 cm$^{-1}$ and the band in 1000 cm$^{-1}$, characteristic for the C–O stretching and H–C–O bending modes are a clear indication of the presence of inulin on the nanoparticles surface.

### 3.5. Transmission electron microscopy (TEM) and energy dispersive spectroscopy (EDS)

The size and distribution of CaHPO$_4$, ZnO, α-Fe$_2$O$_3$ and α-Fe$_2$O$_3$@ZnO, and CaHPO$_4$@ZnO nanoparticles, were determined by TEM. Fig. 6a shows TEM micrographs of brushite nanoparticles with a network of some spherical shaped particles having an average diameter of 76 nm. Fig. 6b shows nearly spherical CaHPO$_4$ nanoparticles coated with 3% inulin with a mean diameter of 57 nm. Fig. 6c shows α-Fe$_2$O$_3$ nanoparticles with a diameter of around 50 nm, with a predominantly spherical morphology, exhibiting good crystallinity. In Fig. 6d α-Fe$_2$O$_3$ nanoparticles coated with 3% inulin, are observed with an average diameter of 71 nm. In Fig. 6e, ZnO nanoparticles with an average diameter of 70 nm are observed; the observed crystallographic planes suggest the high crystallinity of the nanoparticles. Finally, inulin coated ZnO nanoparticles with an average diameter of 92 nm after coating with 3% inulin are shown in Fig. 6f.

Fig. 7 presents typical transmission electron microscope (TEM) images of α-Fe$_2$O$_3$@ZnO and CaHPO$_4$@ZnO nanoparticles.
The composition and nanostructure of the nanoparticles were confirmed by TEM and EDX (energy dispersive X-ray spectroscopy) analysis. The TEM–EDX analysis show that a single nanoparticle consists of two compositions of Fe and Zn (for $\alpha$-Fe$_2$O$_3@$ZnO) and Zn and Ca (for CaHPO$_4@$ZnO), corroborating that the ZnO coating process was successful and that ZnO is on the nanoparticle surface. The $\alpha$-Fe$_2$O$_3@$ZnO and CaHPO$_4@$ZnO nanoparticles were highly crystalline as observed from the SAED analysis (insets, Fig. 7b and d). The complex diffraction pattern containing both diffraction peaks coming from crystal planes for ZnO, $\alpha$-Fe$_2$O$_3$ and CaHPO$_4$, depending of the corresponding system, is also in agreement with the previous results supporting the idea than hematite and brushite phases are coated by ZnO.

The elemental composition of prepared samples was analyzed by EDS (Energy Dispersive Spectroscopy) and the results are available in the supplementary information file. EDS point and area analyses showed for the nanoparticles a ratio 1:1/Ca:P (1.04, Atomic%) is consistent with the CaHPO$_4$ formula; for zinc oxide
nanoparticles a ratio of 1:1/Zn:O (0.86) was found, also in agreement with the Zn stoichiometry; finally, for the iron oxide nanoparticles a 2:3/Fe:O ratio (0.64), in good agreement with the expected α-Fe₂O₃ formula. These ratios are in agreement with the data obtained by XRD and TGA for the expected chemical identity of these nanoparticles. In the case of ZnO coated nanoparticles, the EDS analyses confirms the presence of Zn as part of the surface chemical composition for these systems.

3.6. Particle size distribution (DLS)

Dynamic light scattering (DLS) has been widely used for sizing nanomaterials in liquid suspensions to determine the hydrodynamic size as well as particle size distribution [31]. Scattering of the light beam due to the random motion of nanoparticles dispersed in the suspension change both the direction and intensity of the electromagnetic wave. These changes can be used to estimate the diffusion coefficient of the nanoparticles and from there, their hydrodynamic radii, by using the Stokes–Einstein Eq. (2):

\[
D_f = \frac{k_B T}{6\pi\eta R_{H}}
\]

where \(k_B\) is the Boltzmann constant, \(T\) is the temperature of the suspension, and \(\eta\) is the viscosity of the solvent used to disperse the sample. From the DLS analysis, the brushite nanoparticles have an average size of 38 nm, with a broad distribution from 20 to 80 nm, and some aggregates around 780 and 4970 nm. For the inulin coated brushite nanoparticles, average size was of 96 nm, which is in agreement with an increase of the hydrodynamic radii after chemical modification of the surface with the polysaccharide, with agglomerates in the range of 156 and 979 nm. The increase in average particle size can be related to agglomeration of smaller particles. The tendency to aggregation is maintained even when the nanoparticles are coated with inulin, although the nanomaterial can be easily dispersed using an ultrasonic probe and become more stable. ZnO nanoparticles show an average size of 200 nm in aqueous solution, with particles ranging from 76 to 1150 nm mainly due to agglomeration; after coating with inulin, the average size decreased to 150 nm, with particles having sizes from 100 to 246 nm, indicating that inulin coating avoid particle aggregation, increasing solubility in water. For hematite nanoparticles, a hydrodynamic radii of 221 nm (indicating agglomerate formation) was determined; coating with inulin increases solubility and stability of nanoparticles, reducing agglomeration as indicated by an average size of 85 nm (60–150 nm). For brushite nanoparticles coated with ZnO, the hydrodynamic size was of 55 nm (10–150 nm), while for hematite nanoparticles coated with ZnO, was of 712 nm (460–960 nm). Treating these systems with inulin reduces agglomeration, giving average hydrodynamic radii of 170 nm for CaHPO₄@ZnO/inulin and 200 nm for α-Fe₂O₃@ZnO/inulin, respectively. Changes in sizes are consistent with the deposition of a ZnO coating on the nanoparticles surface. Average sizes obtained from DLS analysis are in agreement with data obtained from XRD and TEM. DLS particle size distribution graphs are available in the supplementary information file.

![Fig. 7. TEM and SAED of (a and b) α-Fe₂O₃@ZnO and (c and d) CaHPO₄@ZnO nanoparticles.](image-url)
3.7. Nanoparticle's stability in solution

The results obtained by following the dissolution of nanoparticles by AAS along 5 days in order to determine their stability in water at pH 7 are available in the Supplementary Information. They clearly shows that inulin acted as a stabilizer for the nanoparticles at pH 7; the uncoated nanoparticles dissolve faster into solution in comparison to inulin coated systems. For example, uncoated ZnO nanoparticle dissolves almost 60% after 5 days in solution, in comparison to barely 10% for the ZnO coated with inulin. For the Fe₂O₃@ZnO system, coating with inulin reduces dissolution from almost 20% to less than 5% for iron, and from nearly 30% to 10% for zinc. In the case of CaHPO₄@ZnO, the uncoated nanoparticles release almost 65% of Ca(II) and 50% of Zn(II) into solution, reducing that amount to only 48% and 10%, respectively, for the inulin coated nanoparticles. These results confirm that coating of the nanoparticles with inulin increase stability in solution, which turns to be useful to control the release of inorganic micronutrients by slowing their dissolution kinetics.

Non-functionalized or homopolymer polysaccharides adsorption onto the surfaces of inorganic particles is rare if not insignificant, as the interactions among them are weak, inhibiting further stabilization. The particular affinity of inulin toward ZnO (nanoparticles or coatings) is appealing. It may be explained by the formation of stronger interactions among a soft-acid σ-acceptor ZnO (a medium bandgap semiconductor) nanoparticle's surface and the inulin polysaccharide chain behaving as a weak, soft-base σ-donor, where the frontier orbital's symmetry and energy have a better match than that corresponding to the interactions among inulin and hematite (a hard-acid, low bandgap semiconductor) or calcium phosphate (a wide bandgap material). To our knowledge, there are no available works in the scientific literature on the study of the interactions among inorganic nanoparticles and polysaccharides. Some recent reviews on polysaccharide-decorated nanoparticles and the interactions of polysaccharides and polymeric nanoparticles have been published, but they focus on functionalized polysaccharides, containing coordinating groups (−COOH, NH₂ or −COOCH₃) [32,33]. The study of the precise nature of the stronger interaction among ZnO and inulin remains to be determined, but it may be of great interest in order to understand the potential interactions of polysaccharides, such as inulin, and inorganic nanoparticles that may influence their environmental or biological fate.

4. Conclusions

A simple chemical method for the preparation of water-soluble inulin coated nanoparticles, containing minerals useful to be considered as additives for food enrichment, was developed. All products were fully characterized by FT-IR, XRD, EDS, TGA/DTA, DLS and electron microscopy (TEM) in order to check for their chemical and structural identity. Although CaHPO₄ and α-Fe₂O₃ nanoparticles had very poor binding interactions toward inulin, coating their surfaces with ZnO allowed the preparation of their correspondent inulin coated system. Nanoparticles with inorganic core average sizes in the range from 50 to 80 nm were obtained and characterized. Inulin (α-D-glucopyranosyl-[β-D-fructofuranosyl](n-1)-D-fructofuranoside) coated nanoparticles were spherical, water soluble and have diameters larger than 80 nm. Inulin biocompatibility, biodegradability and bioactivity as a well-known prebiotic compound, make it an appropriate election to coat inorganic nanoparticles which may become potentially useful as food additives as vehicles for mineral fortification of foods (iron, zinc and calcium). The development of such systems is of strategic significance in order to contribute to the reduction of nutritional deficiencies among vulnerable sectors of the population. ZnO coated nanoparticles stabilized by inulin are water soluble, stable and, as observed from preliminary results currently under performance, they can be easily incorporated into dairy products such as milk and yogurt. However, increased bioavailability may also result in increased toxicological risk. It has been reported that ZnO nanoparticles may block membrane cell transports of sea urchin embryos, affecting their ability to protect from environmental toxins [34]. A careful determination of the toxicity profile of these materials, in order to avoid potential risks and to determine their potential utility as food additives, as well as the determination of the stability and mineral releasing rates of these inorganic nanoparticles at dairy products are currently under investigation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2015.08.057.

References
