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Co-crystallization of zinc sulfate with sucrose: A promissory strategy to render zinc solid dosage forms more palatable

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ABSTRACT

The objective of this work was to develop solid dosage forms to improve zinc nutrition in high-risk populations. Powders containing zinc (17 mg/g) were obtained through co-crystallization in sucrose matrix with high encapsulation efficiency (98%). Co-crystallized powders showed water activity (0.6) and moisture content (2.0%) values characteristic of good stability. Moreover, these products showed an infrared spectrum similar to that of sucrose, indicating that no chemical interactions took place between the matrix components. Co-crystallized powders showed excellent compactibility leading to suitably hard compacts at a low compression force (4.9 kN). Besides, tablets were obtained with optimal values of hardness (4.8 kg_f) and low disintegration times (<5 min) using blends of co-crystallized powder (80% w/w), native corn starch (20% w/w) and magnesium stearate (1 g/100 g). The sensory evaluation of the tablets was performed obtaining a mean overall acceptability rating of 5, which corresponds to the neutral point of the hedonic scale used.

1. Introduction

Zinc (Zn) has an important role for human health, growth and development (Salgueiro et al., 2000; Tapiero and Tew, 2003). This mineral is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients (Chasapis et al., 2012; Salgueiro et al., 2000). Zn has pro-antioxidant properties which could help prevent the illnesses associated with oxidative stress (Goel et al., 2005; Powell, 2000; Zago and Oteiza, 2001). Several authors have reported that Zn inhibits NADPH oxidases, induces the production of metallothionein and competes with metal ions for binding to the cell membrane (e.g. Fe and Cu), thus decreasing the production of OH radicals (Prasad et al., 2004). Foods from animal origin, such as red meat, are considered the major dietary sources of Zn (25–50 mg/kg raw weight). Nevertheless, some populations in developing countries do not have access to micronutrient-rich foods, usually because they are too expensive to buy or are locally unavailable.

Zn deficiency could generate serious physiological disorders including growth retardation, a delay in sexual and skeletal maturation, respiratory infections, diarrhea, loss of appetite and appearance of behavioral change (Shrimpton et al., 2005; WHO, 2002). It is estimated that Zn deficiency is responsible for 4.4% of childhood deaths in Africa, Asia, and Latin America (Fischer Walker et al., 2008; Liberato et al., 2015). The most vulnerable groups to micronutrient deficiencies are pregnant, lactating women and young children, mainly because they have a relatively greater need for vitamins and minerals and they are more susceptible to the harmful consequences of deficiencies.

Nutrition intervention strategies that can be used to reduce the global prevalence of Zn deficiency are fortification, supplementation, and dietary diversification/modification. Several studies showed that the incidence of acute lower respiratory tract infections and malaria may also be reduced by zinc supplementation. Moreover, oral Zn treatment reduced the duration and severity of diarrhea in children from six months to five years old (Liberato et al., 2015). Therefore, the World Health Organization (WHO) and the United Nations Children's Emergency Fund (UNICEF)

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suggested new diarrhea management policies advocating oral Zn to decrease diarrhea deaths in the world's most vulnerable children (WHO and FAO, 2004).

Zn supplementation is most commonly provided as tablets or syrup. The following Zn sources are listed generally regarded as safe (GRAS): Zn gluconate, Zn oxide, Zn stearate, Zn chloride and Zn sulfate. The last is the most commonly used because it has the advantages of low cost and bioavailability (Allen, 1998; Rosado et al., 2012). It has been observed that the Zn from water-soluble salts (e.g. zinc sulfate) is more bioavailable than the Zn from water-insoluble compounds (e.g. zinc oxide) (Allen, 1998). Several authors have reported that some Zn compounds can generate side-effects such as nausea and vomiting (Salgueiro et al., 2002; Solomons et al., 2011). Unpleasant taste has been suggested as a reason for vomiting, but this is more likely due to zinc being a gastric irritant (Liberato et al., 2015). Therefore, the incorporation of these salts in their original state is not always possible and a previous process to face these disadvantages is often necessary.

Co-crystallization is a relatively new method that offers an economic and flexible alternative due to its operational simplicity. This encapsulation technique represents a viable means of enhancing the physical properties of active compounds such as solubility, dispersibility, wettability, anticaking, antidusting, anti-separation, homogeneity, flowability and stability (Bhandari and Hartel, 2002). In the sucrose co-crystallization process, the crystalline structure of this sugar is modified from perfect to irregular agglomerated crystals, to provide a porous matrix in which a second active ingredient can be incorporated. The co-crystallization process involves the concentration of sucrose syrups by evaporation at high temperature until the spontaneous crystallization of sucrose is achieved (Bhandari et al., 1998; Chen et al., 1988). At this point, the rate of crystal formation is so high that nuclei are formed spontaneously (Beristain et al., 1994). This is evidenced by the turbidity observed in the syrup due to the formation of irregular agglomerates. Commonly, the second ingredient is added at the time of spontaneous crystallization followed by cooling. Besides, the addition of the active ingredient into the concentrated syrup can improve the homogeneity of the final product, as it was reported in previous studies (López-Córdoba et al., 2014). The latent heat of crystallization is usually enough to evaporate the moisture, thus the product is substantially dry (i.e., moisture content below 1 wt.%), followed by screening, milling and packaging (Chen et al., 1988).

Co-crystallized products can be used as sugar-based ingredients to mask the bitter taste of active agents. Moreover, they offer direct tableting characteristics which provide significant advantages in the candy and pharmaceutical industries (Awad and Chen, 1993). As direct compression has become the preferred method of tablet manufacturing, demand for compressible sugar-based excipients has increased (Bowe, 1998). Few studies have been published reporting on the use of co-crystallization to drive the encapsulation process (Astolfi-Filho et al., 2005; Awad and Chen, 1993; Beristain et al., 1994, 1996; Bhandari et al., 1998; Bhandari and Hartel, 2002; Chen et al., 1988; Deladino et al., 2007, 2010; López-Córdoba et al., 2014; Maulny et al., 2005; Sardar and Singhal, 2013; Sardar et al., 2013). In addition, the tableting properties of co-crystallized materials containing Zn have not been reported. Recently, we investigated the development of tablets based on co-crystallized sucrose with natural antioxidants of yerba mate (*Ilex paraguariensis*) (López-Córdoba et al., 2015). In the present work, solid dosage forms (powders and tablets) containing Zn were developed based on WHO manufacturing guidelines. Zn-sucrose based excipients were obtained by co-crystallization, characterization of the products was carried out and sensory acceptability was tested. To the best of our knowledge, it is the first time that Zn delivery systems are developed using the co-crystallization technology.

2. Materials and methods

2.1. Preparation of the co-crystallized products

Zn sulfate.$7H_20$ (Parafarm, Argentina) was used as a source of Zn (4.4 mg of Zn sulfate provided 1.1 mg of elemental Zn). The initial moisture content of Zn salt, determined gravimetrically in an oven (SanJor, Argentina) by drying at 105 °C until constant weight, was 37% w/w.

The co-crystallized products were prepared as described by López-Córdoba et al. (2014). Briefly, blends of commercial sucrose (50 g) (Ledesma, Argentina), Zn sulfate (3.5 g) and distilled water (10 mL) were heated on a hot plate at different temperatures (80 and 132 °C). This stage was performed under continuous stirring at 500 rpm, using a vertical agitator (IKA Labortechnik, Staufen, Germany). The temperature and the solid soluble content of the blends were monitored continuously and the supersaturation ratio (S) was calculated as follows:

$$S = \frac{C}{C_o} \quad (1)$$

where C is the concentration of sucrose in the solution and C_o is the saturation concentration of the sugar at the same temperature. Values of $S > 1$ are characteristic of supersaturated solutions while $S = 1$ indicates saturation conditions. The parameter C_o was calculated as described by Hartel et al. (2011).

When the syrup reached the final temperature (80 or 132 °C), the thermal level was kept constant until a slight turbidity was detected, indicating the beginning of the crystallization process. Then, the blends were removed from the heat source and allowed to cool down to room temperature under constant agitation at 700 rpm. The co-crystallized products were dried in a convection oven (SanJor, Argentina) at 40 °C for 15 h and then were ground and sieved through a 500 μm mesh.

Blends of raw sucrose (50 g) and distillated water (10 mL) were crystallized as described above for control purposes. These samples will be referred as “control products”.

2.2. Determination of the Zn content

The Zn content was quantified by atomic absorption spectroscopy using the flame method with a wavelength of 213.9 nm. The analyses were carried out in a Varian spectrometer model EspectrAA 300-plus (Cambridge, United Kingdom). Previously, the samples were digested with concentrated nitric acid.

The entrapment yield (%) was calculated as the ratio between the Zn mass loaded per gram of co-crystallized product and the Zn mass used in the formulation per gram of raw sucrose.

2.3. Characterization of the co-crystallized products

Moisture content (%) was measured gravimetrically by drying the grounded samples in a vacuum oven at 70 °C, until constant weight (AOAC, 1998). Values of water activity (a_w) were determined using AquaLab Serie 3 TE (USA) equipment.

Micrographs of the co-crystallized products were acquired by scanning electron microscopy (SEM) using FEI Quanta 200 equipment (The Netherlands). Zn sulfate distribution on the co-crystallized samples was tested by energy-dispersive X-ray microanalysis (EDX).

The thermal behavior of the powders was evaluated by

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differential scanning calorimetry (DSC) using a Q100 unit (TA Instruments, USA). Samples of 3–5 mg were placed in hermetically sealed aluminum pans and an empty pan was used as reference. Samples were heated from 25 °C to 250 °C at a heating rate of 10 °C/min.

The identification of the main functional groups of the samples was carried out by Fourier transform infrared spectroscopy (FT-IR). The instrument used was a Nicolet 380 (Thermo Scientific, USA) equipped with attenuated total reflectance (ATR) module. The ground samples were placed on the ATR accessory and then were analyzed under transmission mode, taking 64 scans per experiment with a resolution of 4 cm^{-1}. The spectral analysis was performed with Origin software version 8.0 (OriginLab, USA).

X-ray diffraction (XRD) analysis was performed with X'Pert PRO (The Netherlands) equipment at 40 kV with a radiation wavelength of 40 mÅ. Samples were scanned between 2θ values, 5° and 60°.

The flowability of the products was evaluated in terms of the dynamic repose angle and the Hausner (HI) and Carr (CI) indexes (USP 30-NF 25, 2007). The repose angle was determined with a rotating cylindrical chamber, which was tilted gradually until slipping occurred and the angle was measured (Solids handling study bench, CEN, Armfield, United Kingdom).

The Hausner (HI) and Carr (CI) indexes were calculated as follows:

$$HI = \frac{\rho_T}{\rho_B} \tag{2}$$

$$CI = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \tag{3}$$

The loose bulk density (ρ_B) of the powder blends was determined by pouring a known mass delivered freely by gravity into a measuring cylinder. The value of ρ_B parameter was calculated by dividing the mass by the bulk volume. The tapped bulk density (ρ_T) was calculated from the weight of powder and the volume occupied in the cylinder after being hand tapped until a constant value was reached.

Hygroscopicity (HG%) was expressed as the final moisture content achieved after exposing the powder under controlled conditions. Petri dishes filled with co-crystallized products were placed in hermetically sealed glass desiccators containing supersaturated solution of NaCl (75% RH), and stored at 20 °C. Samples were removed at different times and the weight gain was determined until constant value.

2.4. Compactibility curve of the co-crystallized products

Compactibility curves were obtained using the optimized methodology by Gallo et al. (2013). The co-crystallized products were compressed in a hydraulic press (Delfabro, Argentina) at different forces (4.9, 9.8, 14.7 and 19.6 kN) for 5 s. Flat punches of 10 mm diameter were used. The hardness of each compact was determined as the average of 6 measurements using a hardness tester (Scout, Argentina).

2.5. Tablets with zinc

2.5.1. Preparation of the tablets

Tablets based on the co-crystallized products were prepared using the methodology reported in a previous work (López-Córdoba et al., 2015). The co-crystallized products were blended with native corn starch, which is a diluent commonly used in pharmaceutical industries, to reach a mineral concentration around 40% of the recommended dietary allowances (RDA) (WHO and FAO, 2004).

Blends of co-crystallized powders (80% w/w), native corn starch (20% w/w) and magnesium stearate (1 g/100 g of blend) were prepared by shaking the mixture in plastic bags. All ingredients were passed through a 500 μm sieve before being used in the study and also the moisture content and the flowability of the formulations were determined. Then, the powder blends were directly compressed on a single punch-tablet machine (Model SC1, Sanchez, Argentina), regulated to obtain tablets of around 350 mg and constant thickness, using flat-punches with a diameter of 9 mm.

2.5.2. Characterization of the tablets

Zn content of the tablets was determined by atomic absorption spectroscopy following the method mentioned above (Section 2.2). Characterization of the tablets was carried out according to USP 30-NF 25 (2007). For the weight uniformity test, ten tablets were individually weighed and the results were expressed as a mean value of the determinations.

The thicknesses of ten tablets were measured using a Vernier caliper, while the density was calculated as the ratio between the mass and the volume of the tablets. In addition, the tablet hardness was measured with an Erweka hardness tester (Erweka, Germany). *In vitro* disintegration time was evaluated on 6 tablets in 800 mL of distilled water at 37 °C, using a disintegrator tester (Erweka, Germany).

SEM-EDX microanalysis on the inner and the outside surface of the tablets was carried out. Prior to analysis, the tablets were broken diametrically.

2.5.3. In vitro digestion of the Zn tablets using media simulating pH conditions of the gastrointestinal tract

The solutions simulating pH of gastric fluid (SGF, pH 1.2) and intestinal fluid (SIF, pH 6.8) were prepared without enzymes according to the guidelines given by USP 30-NF 25 (2007). Sodium chloride anhydrous and hydrochloric acid 37% (v/v) were employed to formulate the SGF. To prepare the SIF, monobasic potassium phosphate and sodium hydroxide (0.2 N) were used. All reagents were of analytical grade. The analysis was carried out in a dissolution apparatus II (708-DS, Dissolution Apparatus, Agilent Technologies, Santa Clara, USA). Tablets with Zn were weighed and placed into vessels containing 500 mL of SGF at 37 ± 0.5 °C for 180 min, under agitation with a paddle at 50 rpm. Prior to the intestinal digestion step, the pH of the gastric digests was raised to 6.8 by addition of 30 mL of sodium hydroxide solution (1 N). After, 250 mL of the gastric digest were transferred to a vessel containing 250 mL of SIF (pH 6.8) and the blend was incubated for 2 h at similar conditions (37 ± 0.5 °C and 50 rpm). Finally, samples of 10 mL were removed for the determination of the zinc soluble fraction by atomic absorption spectroscopy (Section 2.2). All experiments were conducted in triplicate and mean values were reported.

2.5.4. In vivo disintegration time and sensory evaluation

Tablets based on co-crystallized products were tested and were compared with tablets containing non-cocrystallized ingredients. The time required for complete oral disintegration and the sensory acceptability were tested by 10 untrained judges (6 Males and 4 Females) between the ages of 28 and 35. The volunteers received instructions to not move their tongues during the test. The end point of oral disintegration was considered when a tablet placed on the tongue had disintegrated until no lumps remained. The disintegrated material was held in the mouth for another 30 s, and then spat out. The mouth was rinsed with water between samples and, finally, the acceptability level was recorded on a hedonic numerical scale ranging from 1 to 9 (1: dislike very much; 5: neither

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like nor dislike; 9: like very much).

2.6. Statistical analysis

The statistical analysis was performed using a SYSTAT INC. software (Evanston, USA). The experiments were performed in triplicate and the data were reported as mean ± standard deviation. Assumptions of normality and variance-homogeneity were tested with the Shapiro–Wilk and Levene tests, respectively. Analysis of variance (ANOVA) and Tukey pairwise comparisons were carried out using a level of 95% confidence ($\alpha = 0.05$).

3. Results and discussion

3.1. Co-crystallized products

Co-crystallized products were obtained as described in Section 2.1 from supersaturated syrups of sucrose containing Zn, maintaining the temperature of the blends at 80 °C for approximately 30 min. Under these conditions, the total dissolution of the sucrose was not observed and the supersaturation ratio (S) was 1.1. Moreover, the blends containing zinc showed a higher heating rate (3.1 °C/min) than the control formulations (2.3 °C/min). According to Beristain et al. (1996), sucrose aqueous systems with S values between 1.0 and 1.25 correspond to the metastable zone in which conditions only permit the growth of existing crystals, but not allowing formation of new nuclei. However, the limits of this region can be modified by varying the temperature, the agitation rate and the purity of the raw materials (Beristain et al., 1996; Hartel et al., 2011). On the other hand, the remaining crystals of sucrose probably acted as seeds for the nucleation from the supersaturate solution. At 132 °C, the precursor blend showed coloration changes from white to dark brown and therefore the co-crystallization products were discarded.

The co-crystallization process at 80 °C showed high encapsulation efficiency (98%) and allowed obtaining products with high Zn content (17 mg/g powder). Taking into account the Zn recommended daily allowances (RDA) established by FAO/WHO for an adult (11 mg), these products constitute a useful alternative to supply the mineral requirements. The co-crystallized products showed values of water activity (0.60 ± 0.01) and moisture content (2.0 ± 0.2%) characteristics of good stability (Fu and Labuza, 1993).

Fig. 1 shows the DSC thermographs of raw Zn sulfate, control samples (without Zn) and co-crystallized products. The Zn salt showed several endotherms around 48, 82, 167 and 197 °C. The control samples exhibited an endothermic peak around 192 °C, typical of sucrose melting (Bhandari and Hartel, 2002). For the co-crystallized products no degradation peaks were found below 100 °C. These powders showed a broad endotherm around 201 °C, probably due to the overlapping of the bands located at 192 °C for the control sample and at 197 °C for the raw Zn sulfate. Besides, in the co-crystallized products, the rest of the endotherms present in the thermographs of Zn sulfate were not found (Fig. 1). This fact was ascribed to both the dehydration of the salt during the co-crystallization process and the incorporation of the mineral into the sucrose matrix. Similar observations were reported by Deladino et al. (2007) working with co-crystallized products with calcium lactate and magnesium sulfate. According to Sakata et al. (2005), the stability and behavior of hydrates can vary widely, and hydrate formation and dehydration may occur during processing or storage.

ATR-FTIR spectra of control sample (without Zn) and co-crystallized product are shown in Fig. 2. Both samples showed signals at 3319, 3012, 2970, 2941, 2983, 1128, 1069, 991 and 942 cm^{-1} corresponding to the "fingerprint" of sucrose (Brizuela et al., 2012). This fact suggests that conformational changes of the sugar did not take place during the co-crystallization process. Besides, the analysis of the second-derivative ATR-FTIR spectra of the co-crystallized products (data not shown) allowed the location of the signals around 976 and 1053 cm^{-1} attributed to the vibration modes of the SO_4^{2-} ions (Saha and Podder, 2011). These signals were weak probably due to the low amount of mineral in relation to the sucrose mass.

Fig. 3 shows X-ray diffraction patterns of Zn sulfate, control sample and co-crystallized product. The Zn sulfate salt exhibited characteristic peaks around 2θ (°) = 16.6 and 2θ = 21.1 (Saha and Podder, 2011). The diffractogram of control samples and co-crystallized products showed coincidence with the "fingerprint" of crystalline sucrose, exhibiting peaks at 2θ (°) = 11.7, 12.7, 18.8, 19.6, 24.8, 25.2 and 38.3 (JCPDS, 1999). In both samples amorphous regions were not observed suggesting that the crystalline structure of sucrose was maintained after the co-crystallization process. Similar observations were reported by other authors (Bhandari and Hartel, 2002; Sardar and Singhal, 2013).

The flowability parameters of the Zn sulfate, the control sample

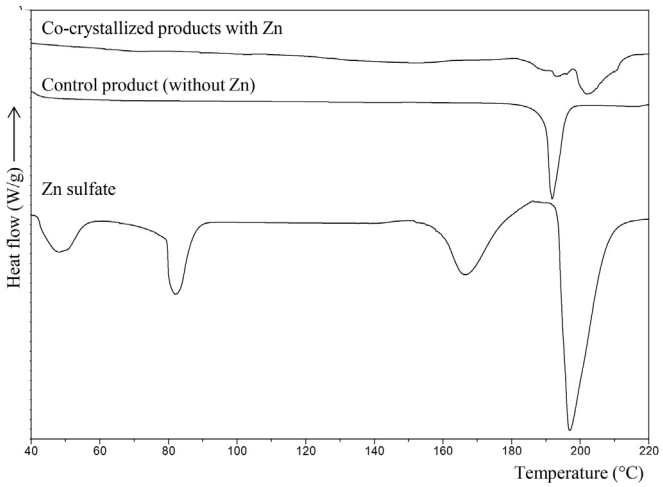

Fig. 1. DSC thermographs of raw Zn sulfate, control samples (without Zn) and co-crystallized products.

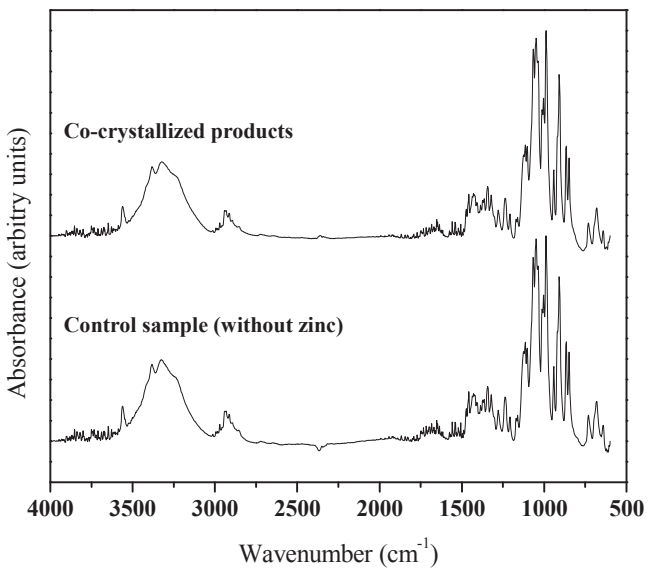

Fig. 2. ATR-FTIR spectra of control sample (without Zn) and co-crystallized products.

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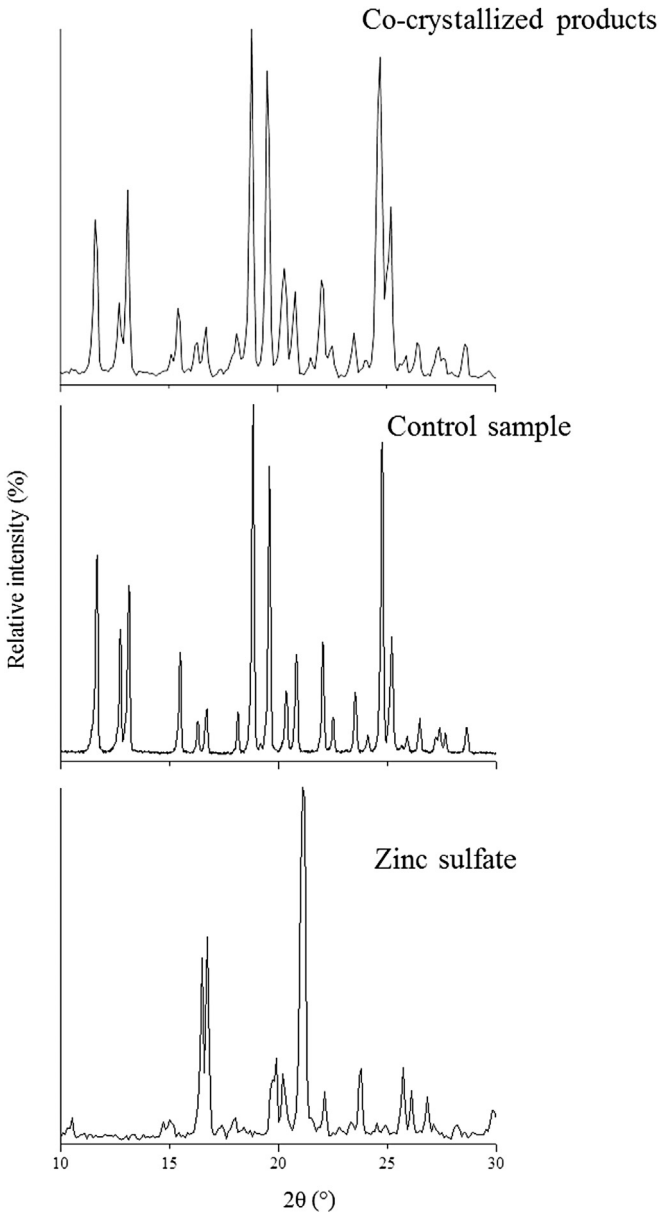

Fig. 3. X-ray diffraction patterns of Zn sulfate, control sample (without Zn) and co-crystallized products.

and the co-crystallized product with Zn are shown in Table 1. The dynamic repose angle ranged between 40 and 50° in all cases. Several authors have reported that materials with repose angle between 40 and 50° can be handled satisfactorily, while products with repose angle higher than 50° correspond to very cohesive materials (Geldart et al., 2006; Santomaso et al., 2003). Hausner (HI) and Carr's (CI) indexes are useful quality parameters to evaluate the flow properties of powders and also their ability to form tablets. Carr indexes correspond to certain flow ratings: up to 10% is excellent, between 10 and 15% is good, 16–20% is poor, between 32 and 37% is very poor and greater than 38% is very, very poor (USP 30-NF 25, 2007). A Hausner ratio <1.25 indicates a powder that is free flowing, whereas a ratio >1.25 indicates poor flowability. Therefore, both indexes indicated that the co-crystallized powder has good flowability and compressibility (USP 30-NF 25, 2007). These indexes were in good agreement with the results of the repose angle measurement (Table 1). Mean hygroscopicity values of 0.080, 0.014 and 2.0% were obtained for Zn sulfate, crystallized sucrose (control) and co-crystallized product, respectively. According to Newman et al. (2008), all these materials can be considered slightly hygroscopic. This behavior represents an important advantage for the physicochemical stability and handling of the co-crystallized materials.

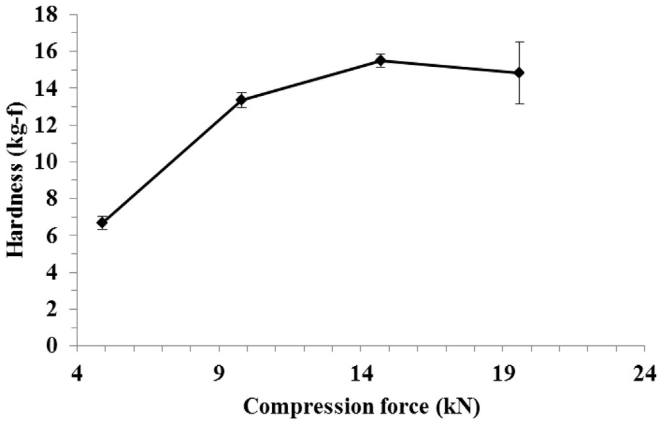

Fig. 4. Compactibility curve of the co-crystallized products.

3.2. Compactibility of the co-crystallized products

Fig. 4 shows the compactibility curve (hardness as a function of compression force) obtained for the co-crystallized products. These materials led to compacts with suitable hardness at low compression force (4.9 kN). At values higher than 14 kN, the hardness of the compacts became almost independent of the applied force. Rizzuto et al. (1984), working with co-crystallized sucrose with dextrin, found that co-crystallized materials tend to the formation of a higher amount of interparticle bridges (interlocking) than in raw sucrose; they are also deformed readily by plastic fracture leading to much harder compacts compared with sucrose (which is a brittle material). For all tested compression forces, compact hardness was above 5 kg_f, indicating that the co-crystallized powder was suitable for direct compression (Ansel et al., 2009).

3.3. Physical properties of the tablets containing Zn

The tablet formulations showed low moisture content (around 2%) and led to tablets with optimal characteristics (Table 2). The tablets showed a Zn content of around 4.5 mg per tablet. This mineral dosage corresponds to 41% of the recommended daily

Table 1
Flowability parameters of raw Zn sulfate, control sample and co-crystallized products.

Samples	Dynamic repose angle (°)	Hausner index	Carr's compressibility index (%)
Raw Zn sulfate	49.1 ± 4.2[a]	1.1 ± 0.05[a]	12.8 ± 3.2[a]
Control product	41.8 ± 4.7[b]	1.0 ± 0.04[b]	7.05 ± 0.5[b]
Co-crystallized product	47.1 ± 5.9[a]	1.2 ± 0.07[a]	12.5 ± 2.9[a]

Different letters in the same column indicate statistical significant differences ($\alpha = 0.05$).

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Table 2
Physical properties of the tablets with Zn.

Physical properties	
Average weight (mg)	344 ± 12
Thickness (mm)	5.0 ± 0.2
Density (g cm^{-3})	1.3 ± 0.2
Hardness (kg_f)	4.8 ± 0.2
In vitro disintegration time (min)	4.6 ± 1.2
In vivo disintegration time (min)	3.7 ± 1.6

intake (RDA) for adults, useful to avoid deficiency and prevent toxicity (WHO and FAO, 2004). Fig. 5 shows SEM micrographs of the co-crystallized products and the tablets with Zn and also the corresponding mapping images of zinc and sulfur obtained by energy-dispersive X-ray (EDX) microanalysis. The co-crystallized products showed a typical structure corresponding to cluster-like agglomerates with irregular cavities between them (Fig. 5a). Besides, EDX microanalysis revealed a homogeneous distribution of both Zn (Fig. 5b) and sulfur (Fig. 5c) on the agglomerate surfaces. With respect to the tablets, SEM images showed a matrix with starch granules distributed homogeneously (Fig. 5d and g). Moreover, uniform distributions of Zn (Fig. 5e and h) and sulfur (Fig. 5f and i) through the tablets were observed by EDX, including the outside surface.

The hardness values of the tablets (Table 2) were considered suitable to withstand the stresses imposed by conventional commercial packaging and distribution (Ando et al., 2007; Lee, 2007; Sugimoto et al., 2006). In addition, *in vitro* and *in vivo* disintegration times of the tablets showed values lower than 5 min. As it is well known, hardness is a critical factor for the disintegration time and the dissolution behavior of the tablets. Frequently, harder tablets take longer to disintegrate than softer tablets (Lee, 2007). In this case, the formulations based on co-crystallized products and excipients (native corn starch and magnesium stearate) allowed obtaining tablets with appropriate hardness which disintegrated faster. This behavior was attributed to both the high solubility of sucrose and Zn salt in water and the effect of native corn starch as disintegrant ingredient (Raymond et al., 2006).

The soluble Zn (%) from the tablets after the *in vitro* digestion was calculated as the ratio between the soluble Zn mass into the intestinal digest and the total Zn content of the tablets (Section 2.5.3). This parameter is frequently used as an indicator of the active compound amount available in the gastrointestinal tract for absorption (so called bioaccessibility) (Cámara et al., 2005; Drago et al., 2005). The tablets based on the co-crystallized products showed a mean soluble Zn value of 85%; this result indicated a good mineral bioaccessibility. The missing zinc amount (15%) could have formed insoluble complexes with starch, as it has been reported in a previous work (López-Córdoba et al., 2015). According to Mundargi et al. (2008) and Vilivalam et al. (2000), the native starch is almost completely broken down, after its oral ingestion, by the pancreatic enzymes that lead to subsequent absorption from the small intestine into the systemic circulation. Higher bioaccessibility (%) could be reached if pancreatic enzymes had been used in the *in vitro* digestion assay. This hypothesis should be addressed in future.

3.4. Sensory evaluation of the Zn tablets

Statistically significant differences were found between the overall acceptability of the tablets based on the co-crystallized products and the tablets based on a physical blend of the ingredients ($p < 0.05$). The tablets containing zinc co-crystallized products showed a mean overall acceptability rating of 5,

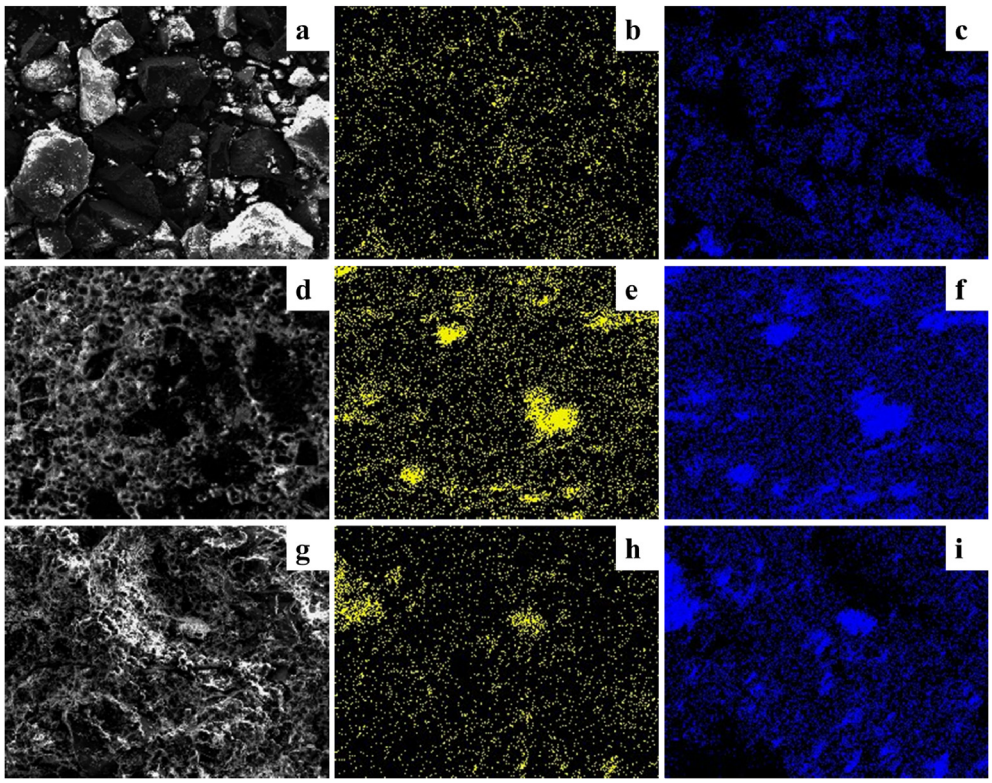

Fig. 5. SEM micrographs (left column) and the corresponding mapping of Zn (center column) and sulfur (right column) obtained by energy-dispersive X-ray microanalysis: (a, b and c) co-crystallized products; (d, e and f) outside surface and (g, h and i) inner surface of the tablets with Zn.

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corresponding to the neutral point of the 9-points hedonic scale (i.e., "neither like nor dislike"). While the tablets containing the non-cocrystallized ingredients showed a mean overall acceptability rating of 3-points (i.e., "dislike very much"). In addition, 22% of judges evaluated the tablets based on Zn co-crystallized products with an acceptability rating between 7 and 9 (i.e., "like very much"), 56% with rating between 4 and 6 (around neutral point) and 22% with rating between 1 and 3 (i.e., "dislike very much"). With respect to the taste, higher degree of bitterness was reported for the tablets based on the physical blend of the ingredients compared to the tablets containing co-crystallized materials. Non-statistically significant differences were found between the appearance and the texture of the samples ($p > 0.05$). In overall, the results suggest that the use of co-crystallized products with Zn in tablet preparations constitutes a potential strategy to render more palatable Zn-dosage forms. Solomons et al. (2011) carried out a sensory study on aqueous solutions of zinc sulfate and NutriSet Zn tablets (Nutriset, Malaunay, France), both containing 30 mg of elemental Zn. The panelists were 10 healthy male volunteers, ranging in age from 18 to 55 years. These authors found that the presentation in tablet form was less emetic and better tolerated than the reagent grade Zn sulfate. They speculated that this behavior would be related to the excipient ingredients in the tablet. Although the pill is predispersed in water before ingestion, it retained some sort of 'coating' effects of the excipient. Such 'shielding' of the Zn might improve its gastric tolerance.

4. Conclusions

Zn solid dosage forms in powder and tablets were developed. The co-crystallization process proved to be a useful technique for the preparation of powders with high Zn content, low water activity and moisture content, good flowability and compactibility. Besides, the co-crystallized products led to tablets with suitable hardness and short oral disintegration times. After an *in vitro* digestion test, the mean soluble amount of Zn from the zinc tablets was found to be 85%; this result indicated a good mineral bioaccessibility. The tablets based on the co-crystallized products with zinc showed higher overall acceptability than tablets based on a physical blend of the ingredients. The developed products constitute a very feasible way to help prevent Zn nutritional deficiency.

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