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Interactions between iron(III) and sucrose, dextran, or starch in complexes

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Abstract

This paper is aimed to study the nature of iron(III)–saccharide interactions in complexes and to look for new iron-containing drugs for supplementing organisms. Iron(III)-saccharide complexes were synthesized by reacting $FeCl_3$ and saccharides (rice starch, sucrose, and dextran) in basic solutions and their characteristics were compared with synthetic iron-oxide. Both crystalline and amorphous iron(III) complexes were obtained and identified by X-ray diffraction (XRD). Amorphous samples were chosen to investigate the interactions between iron(III) and saccharides by continuous-flow dissolution experiments. In acidic solution, the rate of iron release from amorphous iron complexes increased with the following order: iron-rice starch ≈ iron-oxide ≪ iron-sucrose ≈ iron-dextran. The colloidal structure of iron-rice starch was proposed to describe its similar dissolution profile with that of iron-oxide.

Keywords: Iron deficiency; Iron-saccharide; Iron-rice starch; Continuous-flow; Dissolution

1. Introduction

Iron is essential for most living organisms because it is required for many metabolic processes including oxygen transport, drug metabolism, steroid synthesis, DNA synthesis, ATP production and electron transport (Crichton, 1991). However, the concentration of iron within cells must be well controlled due to the cytotoxicity of excess iron. Iron is incorporated into organs and tissues in two major forms: heme and non-heme iron. Most of our dietary intake of iron is in the form of non-heme iron(III) ion. Some components in the diet such as phosphates, phytate, carbonate, and oxalate greatly decrease the bioavailability of iron because precipitates with iron are formed. The use of iron in biological systems is associated with two problems: low solubility of free metal ions and generation of toxic oxidants. Iron absorption can be enhanced by complexing iron with sugars (Gyurcsik & Nagy, 2000). Complexation of iron with saccharides and polyols has been extensively studied because saccharides are able to stabilize iron(III) oxides to maintain relatively high concentration of iron in a soluble form at physiological conditions.

Iron deficiency is one of the most severe nutritional problems worldwide (Scrimshaw, 1991). Iron-saccharide complexes, such as iron-dextran, iron-sucrose, iron-gluconate, and iron-polysaccharide, have been used for the treatment of iron-deficiency anemia (Hudson & Comstock, 2001; Kane, 2003). Oral iron formulations are usually preferred whenever iron replacement therapy is needed. However, some patients who may not be tolerated with oral iron supplements are usually treated with parenteral iron. Iron(II) sulfate has long been accepted for the effective oral treatment of iron deficiency although side effects have been reported. Polysaccharide iron complex (PIC), synthesized by the reaction of $FeCl_3$ and dextrose, is widely used for the oral treatment due to its effectiveness, safe, and free from side effects. Intravenous iron-dextran has been used for the treatment in several groups of patients especially in the US. However, severe side effects, including sarcoma, sterile abscess formulation, tissue necrosis and death have been reported and are associated with the repeated injection of iron-dextran (Kumpf & Holland, 1990). In contrast, clinical use of iron-sucrose in Europe for 50 years has been shown that it is safe for the intravenous treatment of iron-deficiency anemia (Chandler, Harchowal, & Macdougall, 2001; van Wyck et al., 2000; Yee & Besarab, 2002). Hence, it is

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valuable to carefully investigate how iron interacts with saccharides for the design of new iron-containing drugs.

Although the interactions between metals and sugars or polysaccharides have been studied extensively (Gyurcsik & Nagy, 2000), how metals, especially iron(III), interact with polysaccharides is not well understood. The interesting question, whether coordination bonds between iron(III) and the alcoholic hydroxyl group of saccharides are formed, or iron-oxide particles are 'packed' in polysaccharides, still remains unsolved (Sipos, Berkesi, Tombacz, St. Pierre, & Webb, 2003). Sucrose, dextran and rice starch were chosen for study due to their importance in medicine and nutrition. Iron-rice starch, iron-sucrose, iron-dextran complexes and iron-oxide were prepared using the previously reported methods (Bereman & Berg, 1989; Berg, Bowen, Hedges, Bereman, & Vance, 1984). The interactions between iron(III) and saccharides were investigated by kinetic dissolution experiments. Crystallinity of complexes was identified by X-ray diffraction (XRD). Only the amorphous samples were chosen for dissolution experiments using a continuous-flow dissolution system (Chomchoei, Shiowatana, & Pongsakul, 2002; Hinsin, Pdungsap, & Shiowatana, 2002; Shiowatana, McLaren, Chanmekha, & Samphao, 2001; Shiowatana, Tantidanai, Nookabkaew, & Nacapricha, 2001; Tiyapongpattana, Pongsakul, Shiowatana, & Nacapricha, 2004) in acidic solutions of pH 1, which is similar to the pH of gastric juice in the human stomach. Then various analytical tools such as thermal gravimetric analysis (TGA), differential thermal analysis (DTA), electron paramagnetic resonance (EPR), and ^{13}C-cross polarization/magic-angle spinning NMR (^{13}C-CP/MAS NMR) were employed to extract the chemical structures of complexes especially iron-rice starch in order to describe the different characteristic profiles in the dissolution experiments.

2. Experimental

2.1. Materials

Sucrose and dextran (MW 15,000–20,000) were purchased from Fluka (Switzerland). Rice starch was purchased at a local market in Bangkok, Thailand. Iron(III) chloride of purity greater than 98% was obtained from Merck (Germany). Deionized water was used in preparation of reagents.

2.2. Preparation of iron-containing samples

2.2.1. Iron-oxide (*1*)

$FeCl_3$ (3.6 g) was dissolved in deionized water (400 ml). Then 5 M NaOH was added dropwise with stirring to obtain a pH of 9. Red-brown precipitates were observed instantly. The suspension was incubated at 90 °C (incubation temperature) for 2 h with gentle stirring. The precipitates were centrifuged and decanted and then washed with deionized water.

2.2.2. Iron-rice starch complex (*2*), iron-sucrose complex (*3*) and sodium-rice starch

Aqueous starch suspension or sucrose solution (saccharide 5 g in 50 ml of water) was heated at 90 °C for 1 h with continuous agitation. Then 10 ml of 5 M NaOH was added to the solution. The basic saccharide solution was added slowly to the ferric chloride solution (3.6 g of $FeCl_3$ in 400 ml of water). Subsequently, 5 M NaOH was added dropwise to obtain a pH of 9. The suspensions were then incubated for 2 h and the precipitates were centrifuged and washed as in the above procedure.

Sodium-rice starch was prepared with the similar method without adding $FeCl_3$.

2.2.3. Iron-dextran complex (*4*)

Dextran solution (5 g in 50 ml of water) was heated at 90 °C for 1 h with continuous agitation. Then 10 ml of 5 M NaOH was added to the solution. The basic solution was added slowly to the ferric chloride solution. Subsequently, 5 M NaOH was added dropwise to obtain a pH of 9. The suspensions were incubated for 2 h before 150 ml of 2-propanol was added and then the precipitates were centrifuged and washed as in the above procedure.

All iron-containing samples were oven-dried at 50 °C and ground in an agate mortar prior to chemical analysis.

2.3. Characterization

2.3.1. X-ray diffraction

A D8 Advance Bruker analytical X-ray system was operated at the Cu Kα wavelength of 32.297 nm, 40 mA, and 40 keV. The spectra over the range of 20–70° 2θ were recorded at a scan rate of 0.020 2θ/s and a step time of 5 s.

2.3.2. Thermal analysis

Thermal gravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with the TA instruments-SDT-2960 simultaneous analyzer. Samples were placed in an alumina cup and heated from 30 to 450 °C at a heating rate of 5 °C min^{-1} in a nitrogen atmosphere. A purge gas was a flowing dry nitrogen at a rate of 100 ml min^{-1}.

2.3.3. Electron paramagnetic resonance (EPR)

The spectra were recorded for amorphous samples in the X-band region (9.2 GHz, $\lambda = 3.2$ cm) at room temperature. Mn(II) complex was used as the standard for the g-value.

2.3.4. C-cross polarization/magic-angle spinning NMR spectroscopy (^{13}C-CP/MAS NMR)

The ^{13}C-CP/MAS NMR spectra of sodium-rice starch and sample **2** were acquired using the high power cross-polarization sequence at ambient temperature at 125 MHz

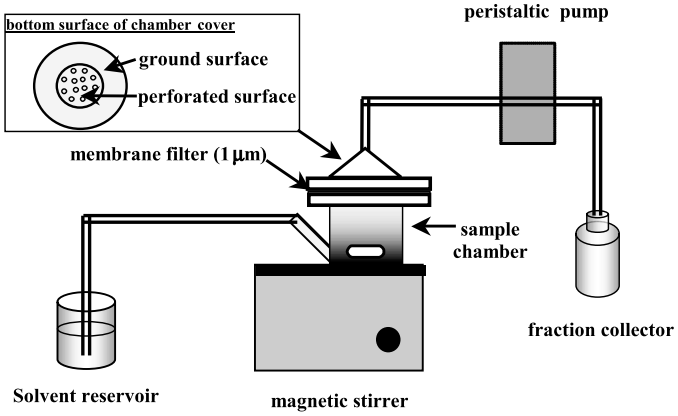

Fig. 1. Diagram of a continuous-flow dissolution system (Shiowatana, Tantidanai et al., 2001b).

on a Bruker DRX-500 spectrometer. The sweep width of spectra was 50,000 Hz and the recycle time was 1 s. The numbers of scans were 5600 and 70,000 for sodium-rice starch and sample **2**, respectively. Chemical shifts were determined by using adamantane as a reference.

2.4. A continuous-flow dissolution setup

A dissolution chamber and its cover were constructed from borosilicate glass to have a capacity of approximately 10 ml. The outlet of the chamber was furnished with a glass microfiber filter membrane to allow dissolved matter to flow through. Leachate was pumped through the chamber using a peristaltic pump at approximately 4 ml min^{-1}, using a tygon tubing of 2 mm inner diameter (Fig 1).

2.5. Kinetic dissolution procedure

A weighed sample, i.e. iron-oxide or iron-saccharide complexes, was transferred to a dissolution chamber. The 0.5 M hydrochloric acid solution was flowed through to dissolve the solid sample. The solutions from the dissolution chamber were collected in small plastic bottles at 3–10 ml volume intervals. The solutions were then diluted with deionized water and subjected to flame atomic absorption spectrometric determination.

3. Results and discussion

3.1. Complex preparation and XRD patterns

The addition of iron(III) to basic saccharide solutions resulted in suspensions of iron complexes. XRD patterns in Fig. 2 clearly showed that the synthetic samples of iron-rice starch and iron-oxide were crystalline which were hematite and goethite (Schwertmann & Cornell, 1991), respectively while iron-dextran and iron-sucrose were amorphous at this preparation condition (pH 11, incubation temperature at 70 °C for 2 days). Crystalline samples were not suitable for the dissolution experiments due to non-reproducible results. Therefore, the condition to prepare the amorphous phase of complexes was optimized. The conditions of iron(III)-rice starch preparation at various pH, incubation time, and incubation temperature were carried out. It was found that at pH 11, incubation temperature at 50, 70 and 90 °C for 2 days, all experiments gave crystalline samples possibly with the hematite phase. At various pH of 5, 7, 9 and 11 and an incubation temperature of 90 °C, crystalline products were obtained for pH 5 and 11 and amorphous products were obtained for pH 7 and 9. Conditions such as pH, incubation temperature, and incubation time must be well controlled in the preparation of iron-containing drugs. Subtle change in the complex preparation can lead to different complex structures and properties.

3.2. Dissolution study of iron(III)-saccharide complexes using a continuous-flow dissolution system

The dissolution or releasing experiments of iron-oxide or ferritin in hydrochloric solution or under reductive conditions have been studied extensively in batch experiments (Hynes & Coinceanainn, 2002). The batch experiment performs dissolution or releasing of metals in an

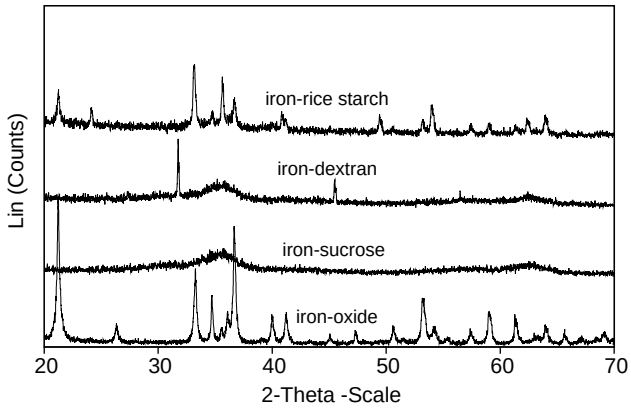

Fig. 2. XRD patterns of samples **1**–**4** (preparation condition: pH 11, incubation temperature 70 °C, 2 days).

equilibrium condition. In this study, continuous-flow dissolution experiments were carried out to gradually dissolve iron(III) complexes with a fresh reagent and iron concentrations in the leachate collected in fractions were determined. Thus, the exhaustive dissolution can be performed. A graphical plot of concentration of iron and dissolution volume provides a dissolution profile which offers the kinetic information about the release of iron from the sample into solution.

The dissolution of ferric ion in an acidic solution was studied using the dissolution profile obtained from the continuous-flow system. The 0.5 M hydrochloric acid was used to dissolve iron-containing samples. The leachate was collected in fractions for the determination of iron concentration by flame atomic absorption spectrometric method. The patterns (narrow or broad) of dissolution profiles was used to indicate the interactions betweens iron(III) and matrices. The dissolution characteristics on the dissolution profiles (Fig. 3) were divided into 2 groups: the first group is iron-dextran and iron-sucrose and the second group is iron-oxide and iron-rice starch complexes. The iron-dextran and iron-sucrose were dissolved in 0.5 M HCl readily after the addition of acid and then the concentration of dissolved iron decreased exponentially. The iron-oxide and iron-starch complex were gradually dissolved after the addition of acid. The dissolution patterns of iron-oxide and iron-rice starch complex were similar. The plots of accumulative concentration of iron in the leachate were

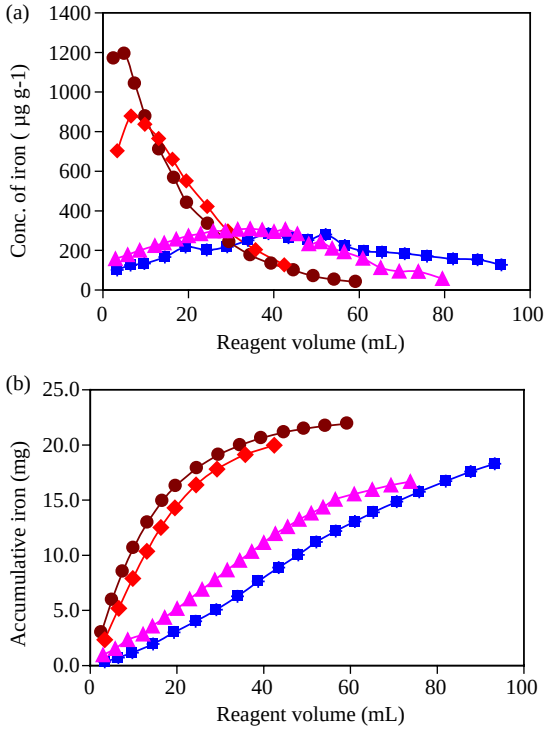

Fig. 3. Dissolution profile (a) and accumulative amount plot (b) obtained from dissolution experiments of iron-oxide (▲), iron-sucrose (♦), iron-dextran (●) and iron-rice starch (■) using 0.5 M hydrochloric acid.

Table 1
Dissolution data of iron from iron-containing samples **1–4**

Iron-containing samples	%Fe dissolved in various concentrations of HCl (M)				
	0.05 M	0.1 M	0.3 M	0.5 M	0.7 M
Iron-oxide (**1**)	n.d.	4.9	24	60	60
Iron-rice starch (**2**)	n.d	4.7	27	44	45
Iron-sucrose (**3**)	14	29	65	78	n.d.
Iron-dextran (**4**)	37	66	83	82	n.d.

n.d. = not determined.

also shown in Fig. 3(b). The approximate rate of iron release from amorphous iron complexes increased with the following order: iron-rice starch ≈ iron-oxide ≪ iron-sucrose ≈ iron-dextran. From Table 1, the % Fe dissolved by various concentrations of HCl increased with the order: iron-rice starch < iron-oxide < iron-sucrose < iron-dextran. The slower release of iron-rice starch complex compared to iron-sucrose and iron-dextran was described as a consequence of the different structures of the iron(III) complexes. However, the rate of release of iron-rice starch complex (**2**) was slightly slower than that of iron-oxide. The argument that the saccharide complexes were overloaded with iron-oxide was ruled out otherwise the rate of release of iron-sucrose should be approximately the same as iron-oxide.

3.3. Structures of iron(III)-rice starch complex

Iron(III)-rice starch was chosen to investigate in more details because of its potential therapeutical use while iron-sucrose and iron-dextran complexes have been well-known in the medical applications (Gyurcsik & Nagy, 2000). The similar characteristics of dissolution patterns of samples **1** and **2** led us to ask a question whether the sample **2** was the iron-oxide adsorbed on the surface of starch. It has been known that the alcoholic hydroxyl group of polysaccharides is deprotonated in alkaline medium and coordinated to iron(III). However, the question about how iron(III) interacts with polysaccharides is still unanswered. Two models have been proposed to explain the structures of iron(III)-polysaccharide complexes(Sipos et al., 2003). The first assumption is that iron(III) is coordinated through the saccharide moieties and forms spatially separated iron(III) centers along the backbone of polysaccharides (site binding model). The second assumption is that iron(III) forms FeOOH precipitate which is covered by the polysaccharide (colloidal model). In our study, the dissolution experiment, TGA, DTA, EPR, and ^{13}C CP/MAS solid state NMR were used to investigate the interactions between iron(III) and saccharides especially rice starch.

TGA and DTA data of saccharides and samples **1–4** under nitrogen atmosphere are summarized in Table 2. This showed that there was some amounts of water (10–15%) which could be located in the matrix or in the coordination sphere of the iron(III) centers. The TGA and DTA of

Table 2
EPR, TGA, and DTA data of saccharides and iron-containing samples

Samples	EPR g-value	TGA	DTA
Iron-oxide (**1**)	1.9417	loss 14% (50–120 °C) loss 5% (30–400 °C)	endo (55 °C) slightly exo (283 °C)
Rice starch	–	loss 10% (50–120 °C) decompose (287 °C)	endo (50 °C) endo (274, 296 °C)
Iron-rice starch (**2**)	1.9547	loss 10% (50–130 °C) loss 34% (130–375 °C)	endo (50 °C) exo (266 °C)
Sucrose	–	decompose (213 °C)	endo (189 °C)(phase tr.) endo (222 °C) endo (283 °C)
Iron-sucrose (**3**)	1.9479	loss 11% (50–120 °C) loss 18% (120–430 °C)	endo (70 °C) endo (186 °C)
Dextran	–	loss 10% (50–120 °C) decompose (230 °C)	endo (50 °C) endo (297 °C)
Iron-dextran (**4**)	1.9417	loss 10% (50–120 °C) loss 25% (220–400 °C)	endo (70 °C) exo (270 °C)

samples **1** and **2** were totally different indicating that sample **2** was not a mixture of saccharide complexes with the excessive iron-oxide sorbed on them. The DTA of sucrose reflected by an endothermic peak centered at 189 °C possibly associated with the phase transition of sucrose. Based on the DTA thermograms of samples **1**–**4**, the thermal effect was exothermic except for iron-sucrose. It was related to the formation of new iron-oxide phases or the loss of coordination water or the redox reaction or metal-catalyzed oxidation (Mitov, Paneva, & Kunev, 2002).

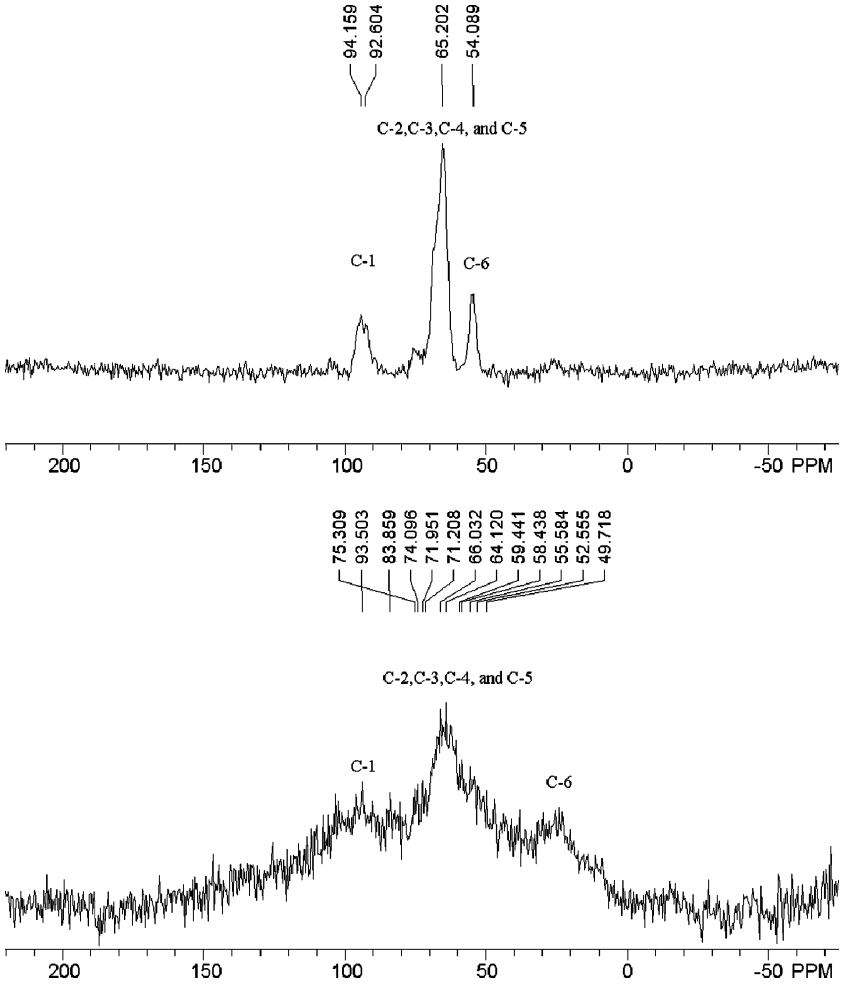

Fig. 4. ^{13}C-CP/MAS NMR spectra of sodium-rice starch and iron-rice starch complex (**2**), respectively.

The X-band EPR spectra of 1–4 in the solid state exhibited one broad band at g ≈1.9. The g-values of **1**–**4** are approximately equal to 1.94 indicating the similar environment of iron(III) centers. It was assigned to a signal arising from strong interactions between the paramagnetic high-spin iron(III) centers in the polynuclear species (Nagy et al., 1986; Wajnberg, El-Jaick, Linhares, & Esquivel, 2001). However, there was no signal of narrow band at g ≈4.3 indicating that there was no isolated high spin iron(III) with a rhombic symmetry in the complexes or the EPR linewidth was too broad. EPR data showed the similar g-values of both complexes indicating that the iron(III) cores of iron-oxide and iron-rice starch were rather similar and starch could stabilize the iron-rice starch complex to release iron slower.

NMR provides the complementary information of saccharide complex structures. The ^{13}C-CP/MAS NMR spectra of sodium-rice starch and iron-rice starch sample **2** were shown in Fig. 4. Based on previous studies (Marchessault, Taylor, Fyfe, & Veregin, 1985), the distinctive peaks of the ^{13}C-CP/MAS NMR spectra at highest and lowest field were assigned to the C-6 and C-1, respectively. Typically, the increase in the local short-range order narrows the ^{13}C resonance dramatically. The broadening resonances may be associated with the long-range order in the local site and probably the interaction between antiferromagnetic iron(III) centers and ^{13}C atoms of glucose units. Clearly the C-6 resonance was shifted to higher field indicating that there were more interactions between iron(III) centers and O-6 units of glucose than other oxygen atoms. The slightly change in the NMR spectrum of iron-rice starch complex was explained with the long-range interactions between iron(III) and starch. This led us to propose that the synthetic iron-rice starch sample **4** should be in the form of iron-oxide stabilized by rice starch. The possible structure of iron-rice starch is that iron-oxide is packed with water inside the helix structure of amylose in which the hydroxyl group of C-6 units of glucose pointing towards the helix center. Our results were in agreement with the structure of iron(III)–chitosan system (Sipos et al., 2003), and polyvinyl alcohol (PVA) and polyacrylic acid (PAA) systems solubilizing iron-containing aggregates (Nesterova, Walton, & Webb, 2000).

4. Conclusions

We have shown the use of the continuous-flow dissolution system with other techniques for shedding light on the interactions between iron(III) and saccharides in complexes. The synthetic iron-rice starch complex was proposed to adopt a colloidal structure although the site-binding model was not ruled out. Furthermore, the continuous-flow dissolution system could be further applied for the therapeutical use to check the releasing of iron from iron-containing drugs and in search of new iron-supplements.

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