Synthesis and Characterization of a Novel Apple Pectin–Fe(III) Complex

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1. INTRODUCTION

Iron deficiency and iron deficiency anemia are the most common nutritional disorders in the world, affecting more people than any other condition.1 Nonetheless, oral administration of iron supplements is typically associated with various gastrointestinal side effects resulting from the release of free iron ions.2,3 Hence, the development of new effective iron supplements exhibiting no or fewer side effects is highly desirable.

A polysaccharide–Fe(III) complex was reported to be an effective oral iron supplement, exhibiting good chemical stability, water solubility, and few side effects.4,5 Furthermore, the complex showed no toxicity under high concentrations.6 However, there are several problems associated with the use of polysaccharide–Fe (III) complexes. For instance, during the iron digestion process, the structure of some polysaccharide–Fe(III) complexes can be destroyed in the condition of the acidic pH of gastric juice. At a low pH, Fe ions are released and dissolved.7 Moreover, some insoluble ferric compounds that cannot be absorbed by the small intestine may form in the intestinal juice.8 The major drawback of such polysaccharide–metal complexes containing Fe3+ cations display antianemic activities.9 Previous work has also demonstrated that polysaccharide–Fe(III) complexes can exert functions of both polysaccharide and Fe.10,11 In this study, we selected the most commonly available commercial AP as a carrier to deliver Fe ions.

The AP–Fe(III) complex exhibiting good bioactivity was synthesized as a potential novel Fe supplement by chelating AP

Pectin is an anionic polysaccharide, primarily composed of α-(1→4)-glycosidic-linked D-galacturonic acid residues and different neutral sugar residues.12 Pectin has recently attracted considerable attention worldwide owing to its various bioactivities and efficacy.13 In the food and beverage industry, pectin has been used as a gelling and thickening agent, fat replacer, and colloidal stabilizer.14,15 Pectin has also shown potential for biomedical applications, including drug delivery, tissue engineering, antioxidant, and wound healing.16,17 However, pectin metal complexes have not been fully investigated and used. Recent studies have reported that pectin exhibits high binding capacity toward metal ions. Pectin ligands can be used to interact with various metals to form new substances, which can be used for the delivery of nutritional supplements.18 In addition, water-soluble pectin–metal complexes containing Fe3+ cations display antianemic activities.19

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with Fe(III). The obtained complex was characterized using Fourier-transform infrared (FTIR) spectroscopy, ultraviolet–visible (UV–vis) spectroscopy, X-ray photoelectron spectroscopy (XPS), and thermogravimetric (TG) analysis. Furthermore, the absorption and antioxidant properties of the AP–Fe(III) complex were investigated via in vitro assays.

2. RESULTS AND DISCUSSION

2.1. Physicochemical Properties of the AP–Fe(III) Complex. The AP–Fe(III) complex was an odorless, reddish brown powder. It was soluble in distilled water but remained insoluble in organic solvents, such as acetone and methanol. The Fe content in the AP–Fe(III) complex was 24.5 ± 1.22%. The content of total carbohydrates was 88.75 ± 1.01% and 69.1 ± 0.98% in AP and the AP–Fe(III) complex, respectively. The galacturonic acid contents of AP and the AP–Fe(III) complex was 66.28 ± 0.86% and 31.83 ± 0.53%, respectively. Compared with AP, the total carbohydrate and galacturonic acid contents decreased in the AP–Fe(III) complex. Furthermore, the molecular weight of the AP–Fe(III) complex was 220.48 kDa and that of AP was 218.15 kDa (Figure 1).

2.2. Analysis of the UV–vis Spectra. UV–vis spectra could be used to evaluate the formation of the AP–Fe(III) complex because the chelation between pectin and Fe3+ led to a change in the absorption intensity. As shown in Figure 2, the UV–vis spectra of AP and the AP–Fe(III) complex did not exhibit absorption peaks at 280 nm, suggesting the absence of proteins. A similar spectral pattern was previously reported by Liu et al. Furthermore, the absorption of the AP–Fe(III) complex in the UV region was evidently higher than that of AP at 0.05 mg/mL. The strong absorption for the AP–Fe(III) complex ranged from 450 to 200 nm due to the electron transition from the ligand to the core Fe atom in the UV region. Hence, the results confirmed the formation of a complex between AP and Fe(III).

2.3. FTIR Analysis. The FTIR spectra of AP and the AP–Fe(III) complex are illustrated in Figure 3. The absorption at high frequency (3475–3412 cm⁻¹) was attributed to the –OH groups. A difference in the shape of the peaks corresponding to the –OH moieties in the spectra of AP and the AP–Fe(III) complex was noted, indicating that the –OH group reacted...
with Fe\textsuperscript{3+} during synthesis. Moreover, the peak at 2929 cm\textsuperscript{-1} was attributed to inter- and intramolecular hydrogen of C–H bonds of the CH\textsubscript{2} and CH\textsubscript{3} groups.\textsuperscript{29} Furthermore, the peaks at 1741 and 1627 cm\textsuperscript{-1} were attributed to the C=O stretching vibration of esterified carboxyl moieties and carboxyl groups,\textsuperscript{30} respectively. For the AP–Fe(III) complex, the intensity of the absorption band at 1741 cm\textsuperscript{-1} significantly decreased, whereas the intensity of the band at 1627 cm\textsuperscript{-1} increased compared with that of AP. This was explained by the conversion of the esterified carboxyl moieties into carboxylate groups under alkaline conditions. Furthermore, the carboxyl groups reacted with Fe\textsuperscript{3+}. The bands at approximately 1600–1500 cm\textsuperscript{-1} assigned to protein amide and aromatic rings were not observed, indicating that lignin and proteins were absent in AP and the AP–Fe(III) complex.\textsuperscript{31} The spectrum of the AP–Fe(III) complex exhibited four new peaks at 849, 688, 640, and 466 cm\textsuperscript{-1}, which were primarily attributed to the structure of the β-FeOOH group in the fabricated complex.\textsuperscript{9,32,33}

**2.4. XPS Analysis.** The elemental analysis of the AP and AP–Fe(III) complex powders was conducted using XPS. As shown in Figure 4A, both C and O atoms were present in AP and the AP–Fe(III) complex. However, Fe was detected during the analysis of the AP–Fe(III) complex. The O 1s and Fe 2p narrow scans are shown in Figure 4B,C, respectively. In the spectrum of pure AP, O 1s exhibited two peaks at 532.74 and 533.39 eV, which were attributed to C=O and C–O/O–H,\textsuperscript{34} respectively. Moreover, after the binding of AP and Fe\textsuperscript{3+}, the binding energy of C=O and C–O shifted from 532.74 and 533.39 eV, to lower energy levels of 532.02 and 532.60 eV, respectively. The peak detected at 535.94 eV corresponded to

**Figure 4.** XPS spectra (A), O spectra (B), and Fe spectra (C) of AP and the AP–Fe(III) complex.
adsorbed water. A new peak observed at 530.39 eV was attributed to Fe−O−Fe and possibly resulted from the interaction between Fe3+ and −COOH.35,36 The Fe 2p peaks were mainly split into two peaks at 709.4 and 722.5 eV, which corresponded to Fe 2p1/2 and Fe 2p3/2 of the AP−Fe(III) complex, respectively. In addition, the AP−Fe (III) complex comprised a β-FeOOH structure, which was obtained by comparison with the standard spectrum. Recent studies on polysaccharide−Fe(III) complexes indicated that the −OH and −COOH groups were bound to Fe3+.24,37,38 Therefore, it was speculated that −OH and −COOH groups in the pectin chains acted as a bridge to connect Fe3+ in the AP−Fe(III).

2.5. Thermal Properties. The derivative thermogravimetry (DTG) and TG curves are shown in Figure 5, AB, respectively. The TG curves of AP and the AP−Fe(III) complex exhibited different shapes. Three regions were observed in the AP curves, which corresponded to different stages of weight loss. This result was consistent with the outcomes of previous studies on pectin.39 Notably, the introduction of Fe resulted in a drastic change in the TG curve of the complex. Moreover, the DTG curves showed that the thermal decomposition rate of the AP−Fe(III) complex was lower than that of AP. The maximum decomposition temperature was determined at 257 and 270 °C for AP and the AP−Fe(III) complex, respectively. It was speculated that the higher decomposition temperature for the AP−Fe(III) complex was caused by the introduction of Fe(III). Moreover, the synthesized complex exhibited good reduction properties and bioavailability.40,41

2.6. Reduction Experiment. Figure 6 shows the absorbance of the AP−Fe(III) complex solution at different pH values and reaction times. The absorbance of the AP−Fe(III) complex solution gradually increased with the increase in the reaction time in the pH range of 3–8. No precipitate was observed for any of the analyzed solutions. This indicated that, in the pH range of 3–8, the AP−Fe(III) complex was not converted into iron hydroxide. Importantly, the complex was soluble and stable in the pH range of 3–8. These findings indicated that Fe3+ in the AP−Fe(III) complex could be reduced to Fe2+ in 6 h. Thus, the AP−Fe(III) complex exhibited good reduction properties and bioavailability.40,41

2.7. In Vitro Iron Release of the AP−Fe(III) Complex and FeSO4. An illustration of the AP−Fe(III) complex in the simulated gastric fluid is demonstrated in Figure 7A. The AP−Fe(III) complex formed a gel in the simulated gastric fluid. The gelling capacity of the complex appeared to increase the viscosity of the gastric fluid (Figure 7B). The gelling capacity is an important property of AP,25 and the introduction of Fe(III) retains the gelling property of AP. When K3[Fe(CN)6]·6H2O and KSCN were separately added into the simulated gastric fluid of the AP−Fe(III) complex, no characteristic color appeared. This result indicated that no free Fe ions were released from the AP−Fe(III) complex after digestion in simulated gastric fluid for 2 h. FeSO4 is widely accepted as a...
reference in studies of iron supplement.1 However, the iron release from FeSO₄ was established at 100% after digestion in simulated gastric fluid for 0.5 h. These results confirmed that the structure of the AP–Fe(III) complex was not destroyed under acidic conditions of the gastric fluid. This may be due to the presence of the iron core surrounded by AP ligands, which...

Figure 7. (A) Images from the polarized microscope of the gel in the simulated gastric fluid. (B) Viscosity curves of the AP–Fe(III) complex in gastric fluid. (C) Iron release from the AP–Fe(III) complex and FeSO₄ in gastric fluid (pH = 1.2) and intestinal fluid (pH = 6.8).

Figure 8. Scavenging ability of AP and the AP–Fe(III) complex for the DPPH radical (A) and ABTS radical (B).
stabilized the metal in the complex. As shown in Figure 7C, the iron ion release reached 96.5% after digestion for 4 h in the simulated intestinal fluid. Moreover, no insoluble ferric/ferrous compounds formed in the intestinal medium. Iron was released from the AP–Fe(III) complex and could maintain better solubility and higher bioavailability, which implied that the complex could be well absorbed. In addition, the structure of the AP–Fe(III) complex protected the iron ion when passing through the stomach, where the release of Fe ions causes stomach irritation and leads to the decline of activity. Hence, more active iron was delivered to and concentrated in the intestinal fluid. These results clearly demonstrate the potential of the AP–Fe(III) complex as a new iron Fe supplement in the treatment of iron deficiency anemia.

2.8. Antioxidant Activity. The free DPPH radical is a stable radical and can exhibit a maximum absorbance at 517 nm. When free DPPH radicals meet a proton donor, the free radicals are scavenged and a decrease in the absorbance is observed.43 The scavenging ability of free DPPH radicals can be used to evaluate the antioxidant activity of polysaccharides and polysaccharide–iron(III) complexes.33 As shown in Figure 8A, the scavenging activities of AP and the AP–Fe(III) complex dose-dependently increased with concentrations from 0.1 to 3.0 mg/mL. However, the detected scavenging activities were lower than those of ascorbic acid. At a concentration of 3.0 mg/mL, the highest scavenging rates of AP and the AP–Fe(III) complex were found to be 67.65 and 59.97%, respectively. The IC_{50} values of AP and the AP–Fe(III) complex were determined at 1.36 and 2.06 mg/mL.

To further investigate the antioxidant activities of AP and the AP–Fe(III) complex, the ABTS radical scavenging activities were assessed. The scavenging effects of ascorbic acid, AP, and the AP–Fe(III) complex toward the ABTS radical are shown in Figure 8B. Evidently, similar to the DPPH radical assay, the ABTS antioxidant activities increased in a dose-dependent manner. The obtained results demonstrated that the scavenging effect of AP rapidly increased with increasing concentrations. The AP–Fe(III) complex exhibited a relatively slow scavenging rate. The highest scavenging rates for AP and the AP–Fe(III) complex were 94.71 and 75.28%, respectively, at a concentration of 3.0 mg/mL. The IC_{50} values of AP and the AP–Fe(III) complex were found to be 0.66 and 1.33 mg/mL, respectively. Notably, the IC_{50} value of the complex was more than twice higher than that of AP. In addition, the IC_{50} values were lower than those reported in a previous study, i.e., 13.52 mg/mL for a Crassostrea gigas polysaccharide and 11.92 mg/mL for an Astragalus membranaceus polysaccharide–iron(III) complex.44,45 It is also noteworthy that, for AP, the introduction of iron weakened its antioxidant activity.

The outcomes obtained in this study were generally consistent with those of previous reports,44,45 which showed that the DPPH and ABTS scavenging activities of polysaccharides were higher than those of polysaccharide–iron(III) complexes. The difference in the antioxidant activities can be explained by the presence of numerous active hydroxyl and carboxyl groups in the structure of AP, particularly in galacturonic acid, which can act as electron donors to the free radicals.46 In contrast, the high molecular weight of the AP–Fe(III) complex and the presence of steric hindrance meant that the complex was not easily accessible for donation of electrons or hydrogen atoms, which could stabilize the reactive radicals. Having antioxidant capacity is usually a desirable feature for food and nutritional supplements. These results showed that the AP–Fe(III) complex demonstrated more health benefits in comparison to the iron supplements with no antioxidant capacity.33

3. CONCLUSIONS

In this study, a novel AP–Fe(III) complex was successfully synthesized and characterized. The chelated Fe complex and its core exhibited a polymerized \( \beta '-\text{FeOOH} \) structure. The introduction of Fe endowed the complex with enhanced thermal stability and improved its water solubility. The results of the reduction experiments demonstrated that the complex displayed good reduction properties and bioavailability in the pH range of 3–8. The Fe release of the AP–Fe(III) complex and FeSO\(_4\) was investigated using an in vitro assay, which indicated that the complex possessed good gastric tolerance and Fe was only released in the intestinal fluid. No Fe ions were detected in the simulated gastric fluid. Hence, AP was considered an appropriate ligand for chelating iron to promote digestion and absorption. Furthermore, the antioxidant activity of the AP–Fe(III) complex was evaluated based on the IC_{50} against the free DPPH and ABTS radicals. The outcomes of this analysis suggested that the AP–Fe(III) complex retained certain antioxidant activities, which were weaker than those of AP. Overall, the AP–Fe(III) complex can be used as a novel iron supplementation with the advantage to relieve stomach irritation caused by free iron ions.

4. EXPERIMENTAL SECTION

4.1. Materials and Reagents. Apple pomace used in this work was provided by Shanxi Huiyuan Fruit Juice Co., Ltd., Datong, China. 2,2-Diphenyl-1-picyrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The dextran standards were obtained from the American Polymer Standards Corporation. All other chemical reagents used in the experiments were of analytical grade.

4.2. Extraction and Purification of AP. Dried apple pomace powder was washed with distilled water (solid to liquid ratio of 1:15) for 15 min under constant mechanical stirring at 600 rpm and then centrifuged to remove the supernatant. Pectin was extracted using deionized water (solid to liquid ratio of 1:20). The pH value was adjusted to 1.5–2.0 using 2 M HCl, and the mixture was then heated to 90–95 °C in a water bath and maintained for 60 min under constant mechanical stirring at 600 rpm. After extraction, the soluble portion was recovered via filtration. The filtrate was collected for alcohol precipitation and cooled to room temperature. Subsequently, 1.5 times the volume of 95% ethanol was added to the filtrate. After 4 h, the precipitate was washed three times with 5% (v/v) HCl in 95% ethanol and anhydrous ethanol. Thereafter, the precipitate was freeze-dried. The degree of esterification was determined at 61.74% using a titrimetric method.

4.3. Synthesis of the AP–Fe(III) Complex. AP and trisodium citrate were added to distilled water at a mass ratio of 3:1. Then, 2 M FeCl\(_3\)-H\(_2\)O was added dropwise to the solution, which was continuously stirred until the appearance of a reddish brown precipitate. The pH of the mixture was adjusted to 8–9 using 2 M NaOH and 2 M HCl. The solution
was heated to 80 °C and stirred for 1.5 h. The reaction mixture was centrifuged at 6000 rpm for 10 min, concentrated, and dialyzed in distilled water. Finally, the supernatant was precipitated using 95% ethanol and then freeze-dried to obtain the AP–Fe(III) complex.

4.4. Physicochemical Properties. The Fe content was determined according to the 1,10-phenanthroline method previously described by Wang et al. with minor modifications. Briefly, 20 mg of the complex was dissolved in 100 mL of 1 M HCl. Then, 1 mL of sample was mixed with 2 mL of 0.2% ascorbic acid, 2 mL of sodium acetate trihydrate buffer solution (pH = 4.5), and 1 mL of 10% 1,10-phenanthroline solution. After 20 min, the UV–vis absorbance of the complex was recorded at 510 nm (PerkinElmer, U.S.A.). A ferrous sulfate solution was used to obtain a calibration curve (y = 0.1511x – 0.0123; R² = 0.9995), and the iron content of the complex was calculated.

The total carbohydrate and galacturonic acid contents were determined using a phenol sulfuric acid and colorimetric carbazole method, respectively.

4.5. Determination of the Molecular Weight. The molecular weight of the sample was determined using high performance size-exclusion chromatography on an Empower 3 system equipped with a Waters AQ 450 column (4.6 × 150 mm, 2.5 μm) and a refractive index detector. The sample (4.0 mg/mL) was centrifuged at 6000 rpm for 15 min and then filtered through a 0.22 μm filter. Then, 10 μL of the supernatant was injected during each run. The column and detector temperatures were maintained at 35 °C. A 0.05 M solution of NaNO₃ was used as the mobile phase at a flow rate of 0.5 mL/min. The calibration curve was calculated using dextran standards with known molecular weights.

4.6. UV–Vis Spectra. For UV–vis analysis, 0.05 mg/mL aqueous solutions of AP and AP–Fe(III) were prepared. The spectra were recorded using a UV–vis spectrophotometer (PerkinElmer, U.S.A.) in the range of 200–700 nm with distilled water (as the blank) in a quartz cuvette. The experiment was performed in triplicate.

4.7. FTIR Analysis. FTIR spectra of AP and the AP–Fe(III) complex were measured using the Tensor 27 spectrophotometer (Bruker, Germany). The samples were mixed with KBr and compressed into pellets for FTIR analysis at a frequency of 4000–400 cm⁻¹.

4.8. XPS Analysis. XPS characterizations were conducted using an Escalab Xi⁺ electron spectrometer (Thermo Fisher Scientific, U.S.A.). The high-resolution XPS spectra of O 1s and Fe 2p were analyzed, employing Advantage software.

4.9. Thermal Analysis. Thermal analysis was performed at temperatures of 25–600 °C at a heating rate of 10 °C/min using the Mettler Toledo TGA-1 analyzer. Nitrogen was used as the carrier gas at a flow rate of 50 mL/min.

4.10. Reduction Experiment. The pH of the solutions was adjusted to 3–8 using 0.2 M aqueous solutions of NaOH and HCl. Five milligrams of the AP–Fe(III) complex was dissolved in 10 mL solutions of different pH values. One milliliter of the solution was mixed with 2 mL of 0.2% ascorbic acid and 1 mL of 10% 1,10-phenanthroline solution. The absorbance was recorded at 510 nm with an interval of 0.5 h. The operation was performed in triplicate.

4.11. In Vitro Fe Release of AP–Fe(III) and FeSO₄. The release of Fe from the AP–Fe(III) complex was measured according to the 1,10-phenanthroline method. The AP–Fe(III) complex and FeSO₄ were dissolved in distilled water to get a final concentration of 2 mg/mL. A simulated gastric fluid containing 2 g of NaCl, 3.2 g of pepsin (15,000 units), and 7 mL of HCl at a pH of 1.2 was used. Then, 5 mL of 2 mg/mL solution of the AP–Fe(III) complex or a 2 mg/mL solution of FeSO₄ was mixed with 5 mL of simulated gastric fluid and incubated for 2 h. The solution was then added to the simulated intestinal fluid (phosphate buffer media, pH = 6.8) and incubated for an additional 4 h. During the entire process, the fluids were maintained at 37 °C and 100 rpm. Next, 5 mL of the samples was withdrawn for the analysis of Fe release. The withdrawn volume was immediately replaced with an equivalent volume of fresh fluids. K₃[Fe(CN)₆]·6H₂O and KSCN were used to identify free Fe²⁺ and Fe³⁺ in the simulated gastric fluid of the AP–Fe(III) complex. The release of Fe ions from the AP–Fe(III) complex was measured at 510 nm using the 1,10-phenanthroline method. Polarized microscopy (NOVEL, Nanjing, China) was used to observe the freshly prepared gel in the simulated gastric fluid at 50x magnification. The AP–Fe(III) complex in the simulated gastric fluid was placed on a glass slide without a cover glass at room temperature. Viscosity was measured using a rotational rheometer (MCR 102, Anton Paar GmbH, Austria). Parallel plates with a diameter of 40 mm and a gap size of 1 mm were used. A temperature of 37 °C was maintained, and the shear rate was 0.1–100 s⁻¹. The viscosity curve was recorded.

4.12. DPPH Radical Scavenging Assay. The DPPH radical scavenging activity was evaluated according to a previously reported method. All samples were dissolved in distilled water, and then 1 mL of sample solutions was mixed with 4 mL of 0.1 mmol/L DPPH in methanol. The mixture was shaken for 30 min at room temperature.

The absorbance of the mixture was then determined at 517 nm. Ascorbic was used as the positive control. The following formula was used to calculate the DPPH radical scavenging activity:

\[
\text{DPPH radical scavenging activity} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\%
\]

4.13. ABTS Radical Scavenging Assay. The ABTS radical scavenging activity was measured according to a previously reported method. For this, 7 mmol/L ABTS solution was incubated with a 2.45 mmol/L solution of potassium persulfate in the dark for 12–16 h. The prepared ABTS solution was diluted 50–60 times with phosphate buffer (pH = 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 0.4 mL of sample was added to 3 mL of ABTS solution. The mixture was incubated for 6 min at 25 °C in the dark, and the absorbance of the mixture was measured at 734 nm. Ascorbic acid was used as the positive control. The ABTS radical scavenging activity was calculated according to the following formula:

\[
\text{ABTS radical scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%
\]
Complete contact information is available at:

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Notes

The authors declare no competing financial interest.

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