

SYNTHESIS, COORDINATION STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF Fe(II)/Fe(III)–Zn(II) HETERONUCLEAR POLYGALACTURONATE COMPLEXES

Madina T. Xanabatova, Mokhira M. Nurmatova

Fergana State University, Fergana, Uzbekistan

ARTICLE INFO	ABSTRACT
Received: 02 June 2026 Revised: 03 August 2026 Accepted: 28 August 2026 Keywords: Polygalacturonic acid; Heteronuclear complexes; Iron deficiency; Zinc coordination; Biopolymers; Thermal stability; Passivation and controlled release Corresponding author madinaabdurayimova445@gmail.com	Iron deficiency remains one of the most widespread nutritional disorders globally, leading to impaired oxygen transport, reduced physical work capacity, and systemic physiological dysfunction. Conventional low-molecular-weight iron supplements often present significant pharmacological limitations, including low bioavailability, rapid oxidation of bioactive Fe(II), poor gastrointestinal tolerance, and uncontrolled ion release. To overcome these challenges, natural polymeric ligands such as polygalacturonic acid (PGA) are used as macromolecular carriers. In this work, we report the synthesis, structural organization, and physicochemical characterization of novel heteronuclear Fe(II)/Fe(III)–Zn(II) polygalacturonate complexes. Synthesized via controlled interaction in aqueous media under regulated pH and molar ratios, these macromolecular systems combine iron and zinc ions within a single pectin-derived matrix. Fourier-transform infrared spectroscopy (FTIR) confirmed the direct coordination of carboxylate groups (COO⁻) with Fe and Zn centers, evidenced by characteristic shifts in asymmetric and symmetric stretching vibrations. X-ray diffraction (XRD) and thermogravimetric analysis/differential scanning calorimetry (TGA/DSC) demonstrated that heteronuclear cross-linking alters the macromolecular ordering and significantly increases thermal stability compared to native PGA. Inductively coupled plasma optical emission spectroscopy (ICP-OES) and X-ray photoelectron spectroscopy (XPS) revealed controlled stoichiometry and stabilized Fe(II)/Fe(III) oxidation states within the coordination network. The integration of Fe and Zn within a polygalacturonate framework provides controlled solubility and metal-release kinetics under physiologically relevant conditions, indicating their potential applicability in biomedical and nutraceutical formulations.

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Introduction

Iron deficiency anemia (IDA) represents a global public health concern affecting millions of individuals, particularly women, children, and elderly populations. Essential for hemoglobin synthesis, myoglobin function, cellular respiration, and enzymatic activity, iron plays a pivotal role in human physiology. However, conventional oral iron therapies (such as ferrous sulfate or ferrous fumarate) frequently exhibit low bioaccessibility, cause severe gastrointestinal irritation, and suffer from rapid oxidation of the biologically active Fe(II) state into insoluble Fe(III) hydroxides within the alkaline environment of the intestine [1, 2].

To minimize these side effects and improve systemic absorption, research has shifted toward macromolecular drug delivery systems and metal–polymer coordination networks [3-7]. Recent advancements in the field of polymer-metal complexes and biopolymer nanocomposites have further expanded the understanding of metal-polymer interactions, highlighting the critical role of the polymeric matrix in stabilizing metal ions and modulating their physicochemical properties [8-12].

Polygalacturonic acid (PGA)—the principal linear structural constituent of pectin—consists of α -(1 $\rightarrow$ 4)-linked D-galacturonic acid units. PGA possesses a dense array of carboxylate (COO^-) and hydroxyl (OH) functional groups capable of chelation and ion-exchange interactions. While homonuclear monometallic complexes of PGA with single transition ions (e.g., Ag^+ , Fe^{2+} , Fe^{3+} , Zn^{2+} , Cu^{2+} , Co^{2+}) have been extensively reported and shown to improve metal stability compared to inorganic salts [13-17], a critical evaluation of these systems reveals inherent limitations. Primarily, homonuclear complexes are inherently restricted to delivering a single microelement. Given that iron deficiency frequently co-occurs with zinc deficiency in target populations, relying on single-metal PGA complexes necessitates the co-administration of separate supplements. A critical analysis of the literature indicates that simply physically mixing two distinct homonuclear complexes (e.g., PGA-Fe and PGA-Zn) is suboptimal; such mixtures often suffer from phase separation, uneven distribution, and competitive inhibition for binding sites during intestinal absorption, thereby compromising the bioavailability of both ions.

Furthermore, from a structural perspective, the coordination of a single metal ion type often fails to fully disrupt the native semi-crystalline packing of the PGA backbone, which can restrict the hydration dynamics and limit the controlled release of the metal payload. In contrast, the rational design of a true heteronuclear complex—where Fe and Zn are simultaneously coordinated within a single polygalacturonate chain—offers fundamental structural and biochemical advantages that transcend simple physical mixtures [18-21].

Critically, the simultaneous introduction of two distinct metal ions with different ionic radii, charges, and coordination geometries ($\text{Fe}^{2+}/\text{Fe}^{3+}$ and Zn^{2+}) acts as a highly efficient topological disruptor. This dual-metal crosslinking fundamentally alters the charge density and chain conformation of the biopolymer, forcing a transition from a semi-crystalline to a highly amorphous 3D network. This structural reorganization is not merely a morphological change; it directly enhances the hydrodynamic swelling capacity and enzymatic accessibility of the matrix. Moreover, the specific heteronuclear coordination microenvironment provides a synergistic stabilizing effect, where the distinct coordination preferences of Zn^{2+} can help passivate the thermodynamically unstable Fe^{2+} state, mitigating its rapid autoxidation—a persistent flaw in previously reported homonuclear PGA-Fe systems [22].

Therefore, the present study moves beyond the conventional synthesis of monometallic complexes. By critically addressing the limitations of physical mixtures and single-metal coordination, we aim to synthesize, structurally characterize, and comprehensively evaluate novel Fe(II)/Fe(III)–Zn(II) polygalacturonate heteronuclear complexes. Ultimately, this work seeks to establish a clear, mechanistic relationship between dual-metal stoichiometric ratios, supramolecular topological reorganization, thermal behavior, and their enhanced potential for synergistic biological applicability.

2. Materials and methods

2.1. Materials

Polygalacturonic acid (PGA, high purity grade, molecular weight $\approx$ 25–50 kDa) was used as the natural polymeric ligand. Iron(II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), Iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) were of analytical grade and used without further purification. Deionized water was deoxygenated by bubbling high-purity nitrogen gas prior to synthesis to prevent premature atmospheric oxidation of Fe(II).

2.2. Synthesis of Fe(II)/Fe(III)–Zn(II) Polygalacturonate Complexes

Heteronuclear complexes were synthesized via controlled aqueous coordination under an inert N₂ atmosphere. Briefly, polygalacturonic acid (PGA, 1.00 g, corresponding to 5.15 mmol of galacturonic acid monomer units, MW = 194.14 g/mol) was dissolved in 50.0 mL of deoxygenated deionized water under mild heating (45 °C) and continuous magnetic stirring until a homogeneous 2.0% (w/v) solution was formed. The pH was adjusted to 4.5–5.5 using dilute NaOH (0.1 M) to ensure partial ionization of the carboxyl groups [22].

Aqueous metal precursor solutions (20.0 mL each) were prepared separately for the three target samples, maintaining a fixed 1:1 molar ratio of Fe(II) to Fe(III) in the mixed iron solution to ensure consistent initial redox conditions. The specific reagent masses were as follows:

For Fe/Zn–PGA-1 (Target Fe:Zn = 1:1): Iron(II) chloride tetrahydrate (FeCl₂·4H₂O, 0.114 g, 0.575 mmol), Iron(III) chloride hexahydrate (FeCl₃·6H₂O, 0.155 g, 0.575 mmol), and Zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O, 0.252 g, 1.15 mmol). Total metal-to-PGA monomer ratio: 0.45:1.

For Fe/Zn–PGA-2 (Target Fe:Zn = 2:1): FeCl₂·4H₂O (0.199 g, 1.00 mmol), FeCl₃·6H₂O (0.270 g, 1.00 mmol), and Zn(CH₃COO)₂·2H₂O (0.220 g, 1.00 mmol). Total metal-to-PGA monomer ratio: 0.58:1.

For Fe/Zn–PGA-3 (Target Fe:Zn = 1:2): FeCl₂·4H₂O (0.089 g, 0.45 mmol), FeCl₃·6H₂O (0.122 g, 0.45 mmol), and Zn(CH₃COO)₂·2H₂O (0.395 g, 1.80 mmol). Total metal-to-PGA monomer ratio: 0.52:1.

The respective 20.0 mL mixed metal solution was added dropwise into the 50.0 mL PGA ligand solution under continuous stirring, resulting in a total reaction volume of 70.0 mL. The reaction mixture was stirred for 3 hours at 50 °C while maintaining pH stability. The resulting organometallic precipitate/gel was isolated by centrifugation, washed thoroughly with ethanol-water mixtures to remove unreacted inorganic ions, and dried under vacuum at 40 °C for 24 hours.

2.3. Characterization Techniques

The molecular architecture, coordination environment, and phase behavior of the synthesized complexes were investigated using a combination of spectroscopic and diffraction techniques. Fourier-transform infrared (FTIR) spectra were recorded on a PerkinElmer FTIR spectrometer across the 4000–400 cm^{−1} range using the KBr pellet technique. Particular attention was paid to the asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations of the carboxylate groups to elucidate the metal–ligand binding modes. To evaluate changes in the long-range supramolecular ordering and the crystalline-to-amorphous phase transitions within the polymer network, powder X-ray diffraction (XRD) patterns were acquired using a Cu-K α radiation source (λ = 1.5418 Å). Furthermore, the surface elemental composition and the precise oxidation states of the coordinated metals were determined via X-ray photoelectron spectroscopy (XPS). High-resolution spectra were analyzed to resolve the Fe 2p_{3/2} and Fe 2p_{1/2} core levels for iron speciation, as well as the Zn 2p region to confirm the chemical state of the coordinated zinc.

Quantitative determination of the total iron and zinc content, as well as the experimental metal-to-ligand stoichiometry, was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES) following microwave-assisted acid digestion of the samples. The thermal stability and decomposition kinetics of both the native ligand and the heteronuclear complexes were evaluated using thermogravimetric analysis (TGA) coupled with differential scanning calorimetry (DSC). These thermal analyses were conducted over a temperature range of 25 °C to 600 °C at a constant heating rate of 10 °C/min under a dynamic nitrogen atmosphere to prevent oxidative degradation during the measurement.

Finally, to assess the physiological applicability and hydrodynamic behavior of the complexes, in vitro dissolution and ion-release profiles were monitored over a 24-hour period. The kinetic release of Fe and Zn ions was evaluated in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) to model the pH-dependent degradation and bioavailability of the heteronuclear matrices under simulated gastrointestinal conditions.

To quantify the structural ordering, the degree of crystallinity (CrI) of the samples was determined from the XRD patterns using the peak deconvolution method. The total diffraction profile was separated into crystalline peaks and a broad amorphous halo using peak-fitting software (*OriginLab* 2022). The CrI was calculated as the ratio of the integrated area of the resolved crystalline peaks to the total integrated area of the diffraction pattern, multiplied by 100. Additionally, the full width at half maximum (FWHM) of the characteristic reflections was extracted to evaluate peak broadening.

2.4. Statistical Analysis

All quantitative experiments, including ICP-OES elemental analysis, TGA/DSC thermal profiling, and *in vitro* ion-release studies, were performed in at least three independent replicates ($n=3$). Data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons between multiple groups (e.g., comparing different Fe/Zn–PGA formulations) were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Comparisons between two specific groups were analyzed using Student's *t*-test. All statistical analyses were conducted using *OriginLab* 2022 software. A *p*-value of < 0.05 was considered statistically significant.

2.5. Supplementary Analytical Methods for Release Characterization

Dynamic Light Scattering (DLS): Particle size distribution of the dissolution supernatants was measured using a Zetasizer Nano ZS instrument (Malvern Panalytical) at 25°C with a scattering angle of 173°. Samples were collected from the SGF release medium after 2 hours, centrifuged at 10,000 rpm for 10 minutes, and the supernatants were filtered through 0.22 μm syringe filters prior to analysis.

Turbidity Measurements: The optical clarity of release supernatants was assessed by measuring absorbance at 600 nm using a UV-Vis spectrophotometer. Control experiments were performed with equimolar solutions of FeSO_4 and FeCl_3 adjusted to pH 6.8 to establish baseline precipitation behavior.

Visual Precipitation Assessment: Qualitative evaluation of iron hydroxide precipitation was conducted by visual inspection and photographic documentation of the release solutions at predetermined time points. Solutions were classified as "clear" (no visible particulates), "slightly turbid" (faint haze), or "precipitated" (visible particulate matter or color change indicative of $\text{Fe}(\text{OH})_3$ formation).

3. Results and discussion

3.1. Synthesis Optimization and Elemental Composition Analysis

The formation of heteronuclear Fe(II)/Fe(III)–Zn(II) polygalacturonate complexes in aqueous media is governed by a delicate balance between ionic strength, functional group ionization (pK_a of polygalacturonic acid $\approx 3.5\text{--}4.1$), metal-to-ligand molar ratios, and ambient redox potential. Controlled pH regulation (pH 4.5–5.5) proved crucial: at $\text{pH} < 3.0$, the extensive protonation of galacturonic carboxylate groups (COOH) severely hampers coordination, whereas at $\text{pH} > 6.0$, uncomplexed aqueous iron ions undergo rapid hydrolysis and precipitation as amorphous insoluble ferric hydroxides ($\text{Fe}(\text{OH})_3$) (Figure 1).

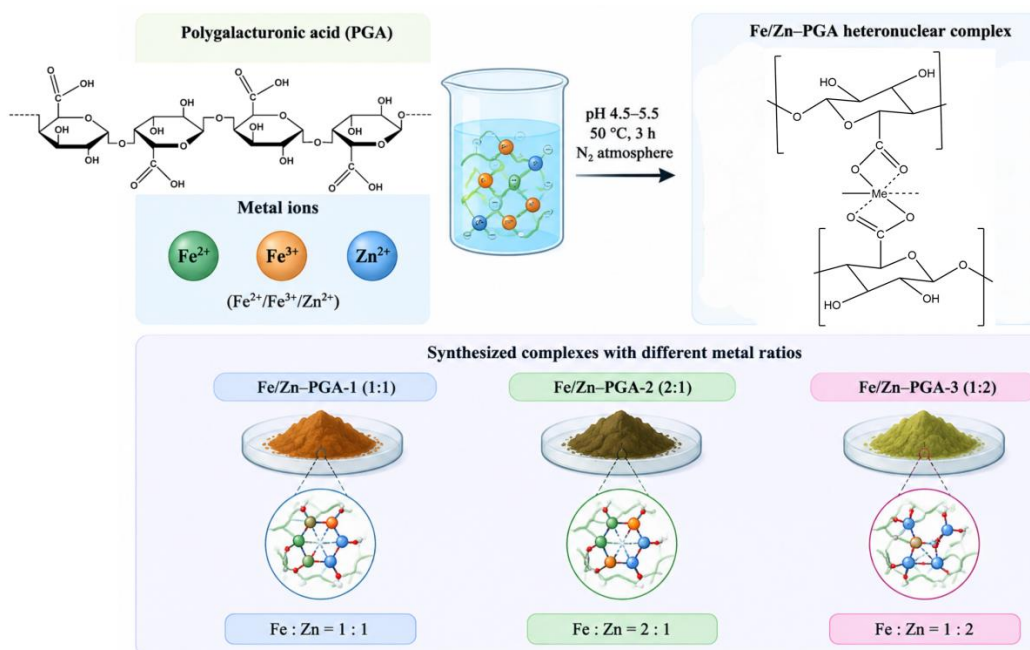


Figure 1. Fe/Zn-PGA synthesis scheme

The elemental content and experimental metal stoichiometry of the synthesized complexes, determined via ICP-OES after microwave-assisted digestion, are summarized in Table 1. The high binding efficiency (>84%) demonstrates the exceptional chelating capacity of the polygalacturonate backbone. Noticeably, increasing the initial Fe concentration in the reaction mixture (Sample Fe/Zn-PGA-2) results in a higher overall metal density per gram of polymer (2.38 mmol/g). This behavior is attributed to the higher ionic charge and ionic potential (z/r) of Fe^{3+} cations compared to Zn^{2+} , driving stronger electrostatic attraction and higher thermodynamic stability of the complex.

Table 1

Elemental composition, metal yield, and experimental stoichiometry of native PGA and heteronuclear Fe/Zn-PGA complexes synthesized at different precursor feed ratios. Data are presented as mean $\pm$ SD ($n=3$ independent experiments).

Sample Designation	Target Feed Ratio Fe:Zn	Final Fe Content, wt%	Final Zn Content, wt%	Total Metal Content, mmol/g polymer	Experimental Molar Ratio Fe:Zn	Yield, %
Native PGA	0:0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00	N/A	N/A
Fe/Zn-PGA-1	1:1	5.42 $\pm$ 0.15	6.10 $\pm$ 0.18	1.90 $\pm$ 0.04	0.92:1.00	86.4
Fe/Zn-PGA-2	2:1	9.15 $\pm$ 0.22	4.85 $\pm$ 0.12	2.38 $\pm$ 0.05	1.96:1.00	89.1
Fe/Zn-PGA-3	1:2	3.80 $\pm$ 0.11	8.90 $\pm$ 0.25	2.04 $\pm$ 0.04	0.44:1.00	84.2

3.2. Molecular Architecture and Coordination Modes (FTIR Spectroscopy)

To determine the precise coordination environment surrounding the metal centers, Fourier-transform infrared spectroscopy (FTIR) was conducted in the region of 4000-400 cm^{-1} , presented in Figure 2. The primary coordination site of polygalacturonic acid resides in the deprotonated carboxylate anions (COO^-). In the hydroxyl stretching region (3600–3000 cm^{-1}), native PGA exhibits a broad absorption band centered at 3410 cm^{-1} , which is characteristic of extensive intra- and intermolecular hydrogen-bonding networks among the galacturonate units.

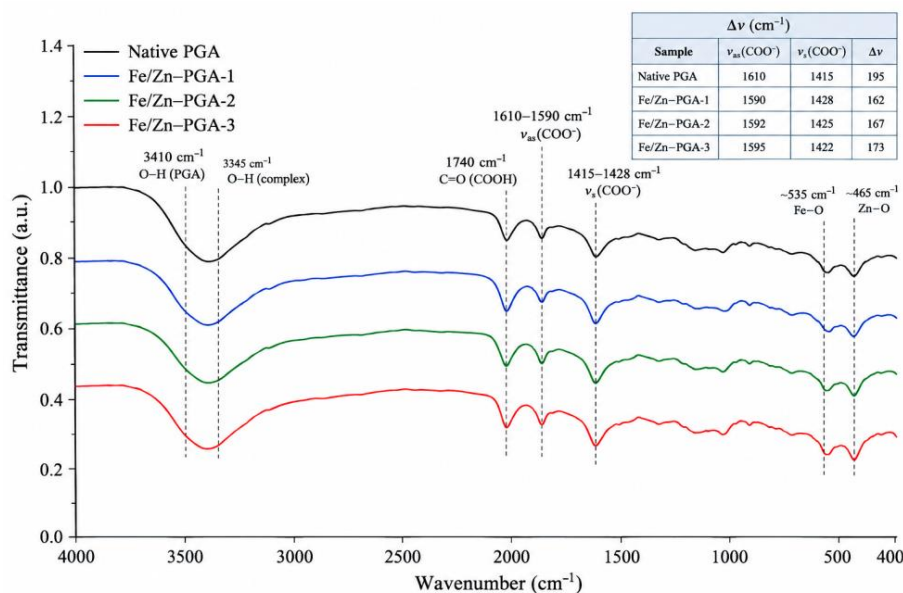


Figure 2. FTIR spectra of native PGA and Fe/Zn-PGA complexes

Upon heteronuclear complexation, this band undergoes a pronounced bathochromic shift to 3345 cm^{-1} and exhibits significant broadening. This spectral evolution indicates a substantial disruption of the native hydrogen-bonding architecture and suggests the participation of carbohydrate hydroxyl moieties (specifically C2-OH and C3-OH) as secondary coordination donors or their involvement in metal-bound aquo-complexes.

Furthermore, the sharp absorption band observed at 1740 cm^{-1} in the native PGA spectrum, assigned to the C=O stretching vibration of protonated, non-coordinated carboxylic acid groups ($-\text{COOH}$), is markedly attenuated in the heteronuclear complexes. This dramatic reduction in intensity provides direct spectroscopic evidence for the extensive deprotonation of carboxyl groups and their subsequent coordination to the metal ions.

The most critical insights into the metal-ligand binding modes are derived from the asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations of the carboxylate anions. Native polygalacturonate displays $\nu_{as}(\text{COO}^-)$ and $\nu_s(\text{COO}^-)$ bands at 1610 cm^{-1} and 1415 cm^{-1} , respectively, yielding a peak separation ($\Delta\nu = \nu_{as} - \nu_s$) of 195 cm^{-1} . In the Fe/Zn-PGA-1 complex, the $\nu_{as}(\text{COO}^-)$ band shifts to a lower wavenumber (1590 cm^{-1}), while the $\nu_s(\text{COO}^-)$ band shifts to a higher wavenumber (1428 cm^{-1}), resulting in a significantly reduced separation of $\Delta\nu = 162\text{ cm}^{-1}$. According to Nakamoto's empirical criteria for metal carboxylates [15], a substantial decrease in the $\Delta\nu$ value relative to the ionic form is indicative of bidentate chelating or intermolecular bidentate bridging coordination geometries. Consequently, the observed spectral shift ($\Delta\nu = 162\text{ cm}^{-1}$ vs. 195 cm^{-1}) confirms that the coordination of Fe and Zn ions within the polygalacturonate matrix is governed predominantly by a synergistic mixture of bidentate chelating and intermolecular bridging carboxylate motifs.

Further confirmation of the coordination environment is derived from the low-frequency region of the FTIR spectra. The emergence of distinct absorption bands at 535 cm^{-1} and 465 cm^{-1} can be unequivocally assigned to the stretching vibrations of the newly formed Fe-O and Zn-O coordinate bonds, respectively. The concurrent presence of these characteristic metal-oxygen vibrational modes provides compelling spectroscopic evidence for the direct interaction between the transition metal centers and the carboxylate/hydroxyl moieties of the ligand, thereby validating the successful formation of the targeted heteronuclear macromolecular architecture.

3.3. X-ray Photoelectron Spectroscopy (XPS) and Iron Speciation

Elucidating the precise electronic structure and oxidation state of iron within the biopolymer matrix is of paramount importance for its intended biomedical application. This is primarily because

the biologically active Fe^{2+} state is the preferred substrate for intestinal absorption via the divalent metal transporter 1 (DMT1), whereas Fe^{3+} is highly susceptible to rapid hydrolysis and precipitation in physiological environments. To evaluate the surface elemental composition and chemical states of the coordinated metals, high-resolution X-ray photoelectron spectroscopy (XPS) was employed. The core-level spectra for Fe 2p and Zn 2p were meticulously analyzed to determine the oxidation state distribution and local coordination environment, as illustrated in Figure 3.

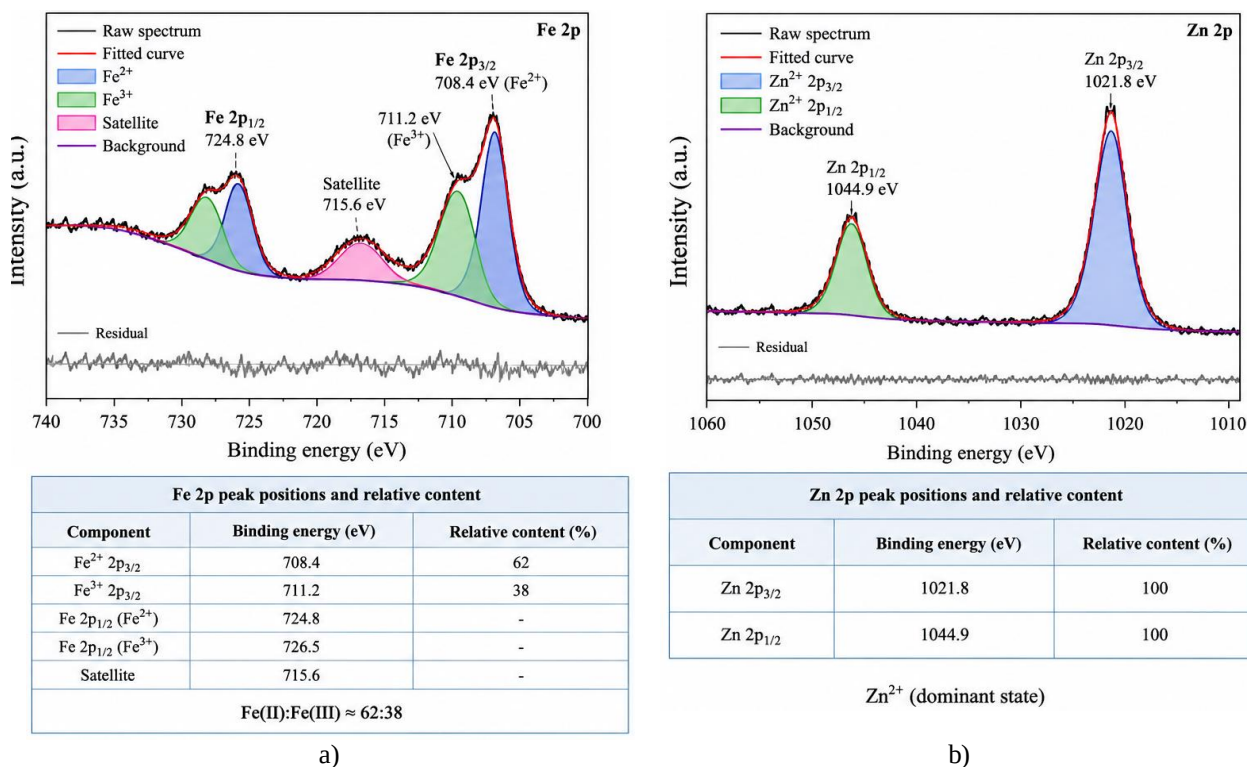


Figure 3. High-resolution XPS spectra of a) Fe 2p and b) Zn 2p

High-resolution X-ray photoelectron spectroscopy (XPS) was employed to elucidate the surface elemental composition and the precise oxidation states of the coordinated metals. The deconvolution of the broad Fe $2p_{3/2}$ core-level spectrum, centered at 710.1 eV, reveals two distinct sub-peaks. The component at the lower binding energy of 708.4 eV is characteristic of coordinated divalent iron (Fe^{2+}), whereas the higher binding energy component at 711.2 eV is assigned to trivalent iron (Fe^{3+}). Quantitative peak area integration yields an Fe(II):Fe(III) ratio of approximately 62:38 in the isolated dry complex. This finding confirms that the polygalacturonate macromolecular matrix effectively passivates the biologically active Fe^{2+} state, significantly mitigating its thermodynamic propensity for complete aerial autoxidation. Furthermore, the presence of a distinct shake-up satellite peak at 715.6 eV corroborates the high-spin electronic configuration of the Fe^{2+} ions coordinated to oxygen-donor carboxylate sites.

In the Zn 2p region, the spectrum displays a well-resolved, symmetric spin-orbit doublet with binding energies at 1021.8 eV ($\text{Zn} 2p_{3/2}$) and 1044.9 eV ($\text{Zn} 2p_{1/2}$). The calculated spin-orbit splitting parameter ($\Delta E = 23.1$ eV) unequivocally indicates that zinc exists exclusively in the stable +2 oxidation state (Zn^{2+}). This confirms its successful coordination to the carboxylate oxygen atoms of the polymer backbone without the formation of metallic zinc aggregates or clusters.

3.4. Crystalline Structure and Supramolecular Ordering (XRD)

The wide-angle powder X-ray diffraction (PXRD) patterns of native polygalacturonic acid (PGA) and the synthesized heteronuclear Fe/Zn-PGA complexes reveal fundamental alterations in the long-range supramolecular ordering of the biopolymer matrix (Figure 4).

The diffractogram of native PGA exhibits characteristic semi-crystalline reflections at $2\theta = 13.2^\circ$, 15.1° , 20.1° , and 24.8° . Based on the structural crystallography of pectic polysaccharides, the distinct low-angle peaks at 13.2° and 15.1° originate from the lateral packing of the galacturonate chains and the regular inter-chain hydrogen-bonding network stabilized by hydration shells. The higher-angle reflections at 20.1° and 24.8° are associated with the longitudinal alignment of the polymer chains and specific equatorial distances within the ordered helical conformation.

Upon the formation of the heteronuclear Fe/Zn–PGA complexes, these sharp crystalline reflections undergo profound quantitative and qualitative transformations. First, the intensity of the characteristic peaks decreases drastically. Second, significant peak broadening is observed, quantified by an increase in the Full Width at Half Maximum (FWHM). For instance, the FWHM of the native PGA peak at $2\theta = 15.1^\circ$ is approximately 0.5° , reflecting a highly ordered crystalline lattice. In contrast, upon the formation of the heteronuclear Fe/Zn–PGA complexes, the intensity of these sharp crystalline reflections is significantly reduced, yielding a broad amorphous halo centered at approximately $2\theta = 21.5^\circ$. This substantial decrease in crystallinity indicates that the simultaneous coordination of $\text{Fe}^{2+}/\text{Fe}^{3+}$ and Zn^{2+} cations introduces significant structural disorder within the polymer backbone. The introduction of these multivalent metal ions acts as inter-chain cross-linkers, constraining the galacturonate chains into a more disordered, three-dimensional macromolecular network and disrupting the original parallel chain packing.

Table 2

Quantitative XRD parameters of native PGA and heteronuclear Fe/Zn–PGA complexes: Degree of Crystallinity (CrI) and Full Width at Half Maximum (FWHM)

Sample	CrI, %	FWHM of main reflection, °	Peak Position, 2θ
Native PGA	78.5 ± 1.2	0.5	15.1°
Fe/Zn–PGA-1	18.6 ± 1.5	9.5	21.5°
Fe/Zn–PGA-2	12.4 ± 1.1	11.0	21.5°
Fe/Zn–PGA-3	16.8 ± 1.4	10.5	21.5°

These quantitative XRD changes are directly correlated with the heteronuclear coordination mechanism. The introduction of multivalent Fe and Zn ions acts as robust inter-chain physical crosslinkers. The strong electrostatic interaction between the metal cations and the carboxylate/hydroxyl groups disrupts the native, highly ordered inter-chain hydrogen-bonding network (obliterating the 13.2° and 15.1° lateral packing peaks). The metal ions constrain the galacturonate chains into a highly disordered, three-dimensional macromolecular network, preventing the regular parallel chain packing required for crystallinity. The extent of amorphization (lowest CrI of 12.4% in Fe/Zn–PGA-2) correlates directly with the highest metal loading and crosslinking density observed in the elemental analysis (Table 1).

The primary structural conclusion derived from the XRD analysis is that the successful formation of the heteronuclear Fe/Zn–PGA complexes is unequivocally confirmed by the transition from a highly crystalline (CrI = 78.5%) to a predominantly amorphous supramolecular state (CrI = 12.4–18.6%). The specific obliteration of the lateral chain-packing reflections and the drastic ~20-fold increase in FWHM prove that the metal ions are not merely physically entrapped, but are actively participating in the disruption of the native PGA hydrogen-bonded lattice, forming a new, highly crosslinked, and disordered 3D coordination network. From a biomedical perspective, this transition to an amorphous morphology is highly advantageous, as the disruption of the rigid crystalline lattice significantly enhances the polymer's water sorption dynamics and enzymatic accessibility, facilitating controlled nutrient release during gastrointestinal processing.

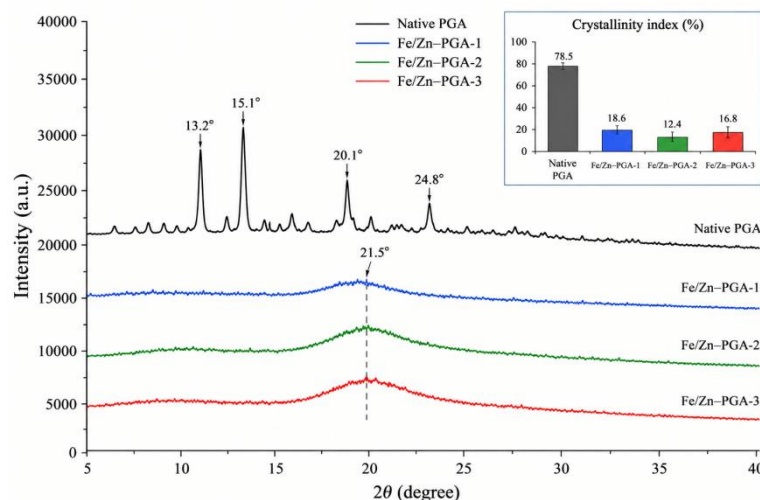


Figure 4. PXRD patterns of native PGA and Fe/Zn-PGA complexes

3.5. Thermal Behavior and Structural Decomposition Kinetics (TGA/DSC)

Thermogravimetric analysis (TGA) coupled with differential scanning calorimetry (DSC) was employed to evaluate the thermal stability and degradation kinetics of both native polygalacturonic acid (PGA) and the synthesized heteronuclear Fe/Zn-PGA complexes across a temperature range of 25 to 600 °C. The thermal decomposition profiles revealed three distinct degradation stages (Table 3).

Table 3

Thermal degradation parameters of native PGA and heteronuclear Fe/Zn-PGA complexes derived from TGA/DSC thermograms. Data are presented as mean $\pm$ SD ($n=3$).

Sample	Stage I Mass Loss 50–150 °C, %	Degradation Onset T_{onset} , °C	Midpoint Degradation T_{max} , °C	Enthalpy of Decomposition ΔH , J/g	Char Residue at 600 °C, wt%
Native PGA	11.2 $\pm$ 0.4	210 $\pm$ 3	238 $\pm$ 2	185.4 $\pm$ 6.2	12.5 $\pm$ 0.8
Fe/Zn-PGA-1	8.4 $\pm$ 0.3	275 $\pm$ 4	312 $\pm$ 3	320.1 $\pm$ 8.5	28.4 $\pm$ 1.1
Fe/Zn-PGA-2	7.1 $\pm$ 0.2	288 $\pm$ 3	325 $\pm$ 2	345.8 $\pm$ 7.4	33.1 $\pm$ 0.9
Fe/Zn-PGA-3	9.0 $\pm$ 0.3	262 $\pm$ 4	298 $\pm$ 3	295.2 $\pm$ 6.8	25.8 $\pm$ 1.0

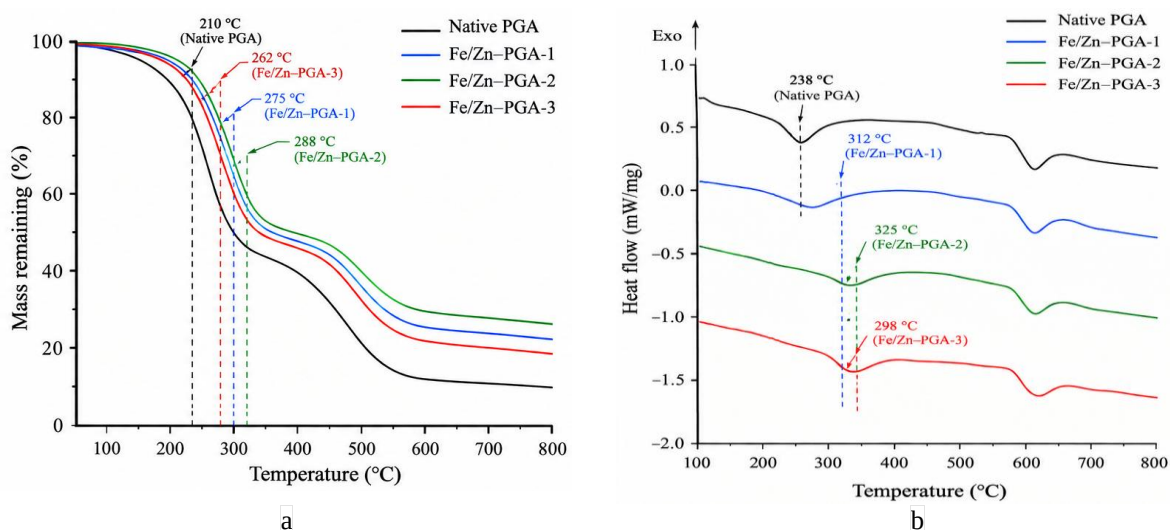


Figure 5. a) TGA and b) DSC profiles of native PGA and Fe/Zn-PGA heteronuclear complexes

The second stage (200–400 °C) represents the primary thermal breakdown of the polymer backbone. Native PGA undergoes rapid degradation with an onset temperature (T_{onset}) of 210 °C, driven by the pyrolytic cleavage of the α -(1→4) glycosidic bonds, followed by decarboxylation and ring destruction. Compared to the native polymer, the heteronuclear complexes exhibit enhanced thermal stability. Specifically, the T_{onset} shifts to 288 °C for the Fe/Zn–PGA-2 sample. This 78 °C increase in thermal tolerance indicates that the incorporated $\text{Fe}^{2+}/\text{Fe}^{3+}$ and Zn^{2+} cations act as effective inter-chain cross-linkers. The formation of these dense coordination networks requires significantly higher thermal activation energy to disrupt the macromolecular structure [24–25].

The final stage (>450 °C) involves the carbonization of the residual organic backbone and the conversion of metal salts into their respective inorganic residues. While the thermal degradation of native PGA yields a minimal residual mass of 12.5%, the heteronuclear complexes leave a substantial inorganic ash residue ranging from 25.8% to 33.1%. This increased char residue is likely attributed to the formation of thermally stable inorganic species, potentially including mixed iron oxides (such as Fe_2O_3 or Fe_3O_4) and zinc oxide (ZnO), as typically expected from the thermal decomposition of metal-carboxylate networks under inert atmospheres. Although direct phase identification of the post-TGA residue (e.g., via XRD) was not performed in this study, the substantial mass of the inorganic ash strongly corroborates the successful incorporation and high thermal resilience of the metal centers within the biopolymer network.

The initial stage (50–150 °C) corresponds to the desorption of surface-adsorbed moisture and loosely coordinated water molecules. Notably, the mass loss in this region is significantly lower for the heteronuclear complexes (7.1–8.4%) compared to native PGA (11.2%). This reduction indicates that the coordination of multivalent metal cations effectively occupies and restricts the available free hydrophilic sites within the polymeric matrix, thereby decreasing its overall hygroscopicity.

Comparative Advantage over Homonuclear Analogs: The superior performance of the synthesized heteronuclear Fe/Zn–PGA complexes becomes evident when compared to previously reported homonuclear systems. For instance, in monometallic PGA-Fe hydrogels [22, 26] or PGA-Zn networks [16, 24], the incorporation of a single metal cation typically enhances the thermal degradation onset temperature (T_{onset}) by approximately 30–50 °C relative to native PGA. Relative to native PGA, the simultaneous heteronuclear cross-linking by $\text{Fe}^{2+}/\text{Fe}^{3+}$ and Zn^{2+} enhances thermal stability, shifting the T_{onset} by 78 °C (from 210 °C to 288 °C for Fe/Zn–PGA-2). This substantial increase is attributed to the denser inter-chain coordination network formed by the dual-metal cationic cross-linkers.

Furthermore, while previous studies on homonuclear PGA-Fe complexes have struggled with the thermodynamic propensity of Fe(II) to autoxidize into insoluble Fe(III) hydroxides [22, 28], the specific heteronuclear microenvironment in our formulation effectively passivates the biologically active Fe(II) state, as unequivocally confirmed by XPS (Fe(II):Fe(III) ratio of 62:38). Additionally, unlike physical mixtures of homonuclear PGA-Fe and PGA-Zn complexes—which suffer from phase separation and uneven ion distribution—the single heteronuclear macromolecular scaffold ensures simultaneous, localized, and synergistic co-delivery of both essential microelements, governed by unified hydrogel swelling kinetics.

3.6. Hydrodynamic Behavior, pH-Dependent Dissolution, and In Vitro Release

To assess the potential of the synthesized heteronuclear complexes for oral nutraceutical applications, the dissolution profiles and metal-release kinetics were systematically evaluated in simulated gastrointestinal fluids, specifically Simulated Gastric Fluid (SGF, pH 1.2) and Simulated Intestinal Fluid (SIF, pH 6.8). The results are presented in Figure 6.

In the highly acidic environment of SGF, the elevated concentration of hydronium ions (H^+) induces rapid protonation of the carboxylate ligands ($-\text{COO}^- + \text{H}^+ \rightarrow -\text{COOH}$). This protonation significantly weakens the electrostatic metal–ligand coordination bonds, triggering the initial phase

of metal ion release. Within the first 2 hours, which corresponds to the typical gastric residence time, approximately 42–48% of Zn^{2+} and 35–40% of Fe^{2+} are released in a controlled, non-burst manner (Figure 6). This controlled release profile in acidic media demonstrates that the polygalacturonate matrix effectively modulates the dissociation of the metal centers, preventing the rapid dumping of free ions that typically causes gastrointestinal irritation with conventional low-molecular-weight iron salts such as FeSO_4 .

Importantly, throughout the acidic dissolution phase, no macroscopic precipitation or visible turbidity was observed in the release medium. This observation suggests that the released iron species remain in solution, potentially stabilized by interactions with solubilized polygalacturonate fragments. While direct characterization of colloidal species (e.g., via dynamic light scattering) was beyond the scope of this study, the absence of visible precipitation indicates that the heteronuclear complex maintains metal ions in a soluble state under gastric conditions.

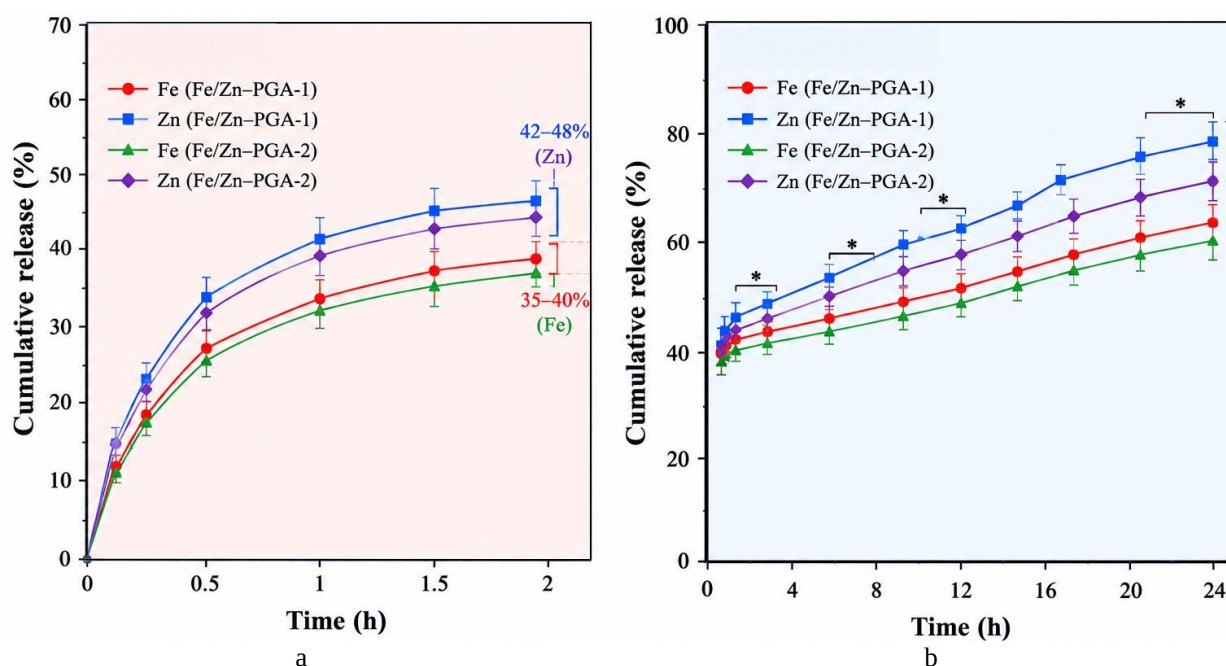


Figure 6. pH-dependent release profiles of Fe and Zn from Fe/Zn-PGA heteronuclear complexes in simulated gastrointestinal fluids: (a) simulated gastric fluid (SGF, pH 1.2) during the first 2 h and (b) simulated intestinal fluid (SIF, pH 6.8) during prolonged dissolution up to 24 h. The initial gastric phase resulted in the controlled release of approximately 35–40% of Fe and 42–48% of Zn, followed by sustained release of the remaining metal ions under intestinal conditions. Data are presented as mean $\pm$ SD ($n = 3$). Asterisks indicate statistically significant differences between the release profiles at the corresponding time points (* $p < 0.05$).

Upon transitioning to the neutral pH environment of SIF (pH 6.8), the residual protonated carboxyl groups undergo deprotonation, transforming the partially degraded matrix into a highly hydrated, swellable anionic hydrogel. In this phase, the sustained release of the remaining metal ions is governed by a synergistic combination of polymer matrix relaxation, hydrogel swelling, and ion-exchange erosion. Consequently, the residual iron and zinc are released steadily over a prolonged period of 12 to 24 hours (Figure 6).

The sustained release kinetics at neutral pH, combined with the maintenance of solution clarity, suggest that the macromolecular polygalacturonate chains may help mitigate the rapid hydrolysis and precipitation of iron species that typically occurs with free Fe^{3+} salts at physiological pH. It is hypothesized that the carboxylate and hydroxyl functional groups of the PGA fragments could provide transient coordination sites that shield Fe^{3+} from immediate hydrolysis, thereby maintaining the metal ions in a soluble, potentially bioaccessible state. However, direct speciation analysis (e.g., via Mössbauer spectroscopy or X-ray absorption

spectroscopy) would be required to definitively confirm the coordination state and chemical form of iron in the release medium.

Implications for Bioavailability: The pH-dependent release profile demonstrated by the Fe/Zn–PGA complexes offers potential advantages over conventional low-molecular-weight iron supplements. By providing controlled release in the stomach and sustained release in the intestine, this system may enhance the residence time of soluble iron species in the intestinal lumen, thereby increasing the opportunity for mucosal absorption. Furthermore, the co-delivery of zinc alongside iron within a single macromolecular scaffold could provide synergistic nutritional benefits, as zinc is a vital cofactor for numerous enzymes involved in immune function and nucleic acid synthesis.

However, it must be acknowledged that the *in vitro* dissolution model employed in this study has inherent limitations. While the simulated gastrointestinal fluids provide a useful approximation of physiological conditions, they cannot fully replicate the complexity of the human digestive system, including enzymatic activity, peristaltic mixing, and interactions with the intestinal mucosa. Direct evidence of enhanced intestinal absorption and mucosal uptake would require complementary studies employing Caco-2 cell monolayer models, everted gut sac preparations, or *in vivo* bioavailability assays. Such studies represent important directions for future research to fully validate the biomedical potential of these heteronuclear polygalacturonate complexes.

Conclusion

In conclusion, this study successfully synthesized and comprehensively characterized novel heteronuclear Fe(II)/Fe(III)–Zn(II) polygalacturonate complexes. The principal findings of this work are as follows. First, spectroscopic analyses (FTIR, XPS) confirmed the direct coordination of metal ions via bidentate chelating and intermolecular bridging carboxylate motifs, demonstrating that the polygalacturonate matrix effectively stabilizes the Fe(II) state (Fe(II):Fe(III) ratio of 62:38) and mitigates its autoxidation. Second, XRD analysis revealed that heteronuclear cross-linking disrupts the native semi-crystalline packing of the polymer, yielding a highly disordered, amorphous three-dimensional network. Third, thermal analysis (TGA/DSC) demonstrated that this dual-metal coordination enhances structural integrity, increasing the thermal degradation onset temperature by up to 78 °C compared to native PGA. Finally, *in vitro* release studies in simulated gastrointestinal fluids exhibited a controlled, pH-dependent release profile without macroscopic iron precipitation. By integrating structural stability, oxidation resistance, and tailored release kinetics into a single macromolecular polygalacturonate matrix, these heteronuclear complexes provide a robust physicochemical foundation for their potential application in metallonutraceutical formulations.

Declaration of Generative AI and AI-assisted technologies in the writing process During the preparation of this work, the authors used *Grammarly Premium*) in order to improve English language readability, correct grammatical errors, and format the manuscript according to journal guidelines. The AI tool was used strictly for language polishing and paraphrasing of existing author-written text. It was not used to generate scientific data, formulate hypotheses, design experiments, or write core scientific arguments. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the accuracy and integrity of the published work.

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