Iron Complexation Studies of Gallic Acid

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In this work, formation in the binary and ternary systems of the Fe^{III} metal ion with gallic acid and glycine was investigated by means of potentiometry, conductometry, polarography, and UV−visible absorption spectroscopy techniques at 25 °C and in $I = 0.10$ mol·dm^{-3} $NaNO_3$. The protonation equilibria of gallic acid and glycine were investigated and discussed. The acidity constants of gallic acid and glycine were determined and used for determining the stability constants of the binary and ternary complexes formed in the aqueous medium under the experimental conditions. The ternary complex formation was found to occur in a stepwise manner. The stability constants of these binary and ternary systems were calculated. The concentration distribution of the various complex species in solution was evaluated and discussed. The solid binary [Fe^{III}−gallic acid] and ternary [Fe^{III}−gallic acid−glycine] complexes were synthesized and characterized by elemental analysis, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy.

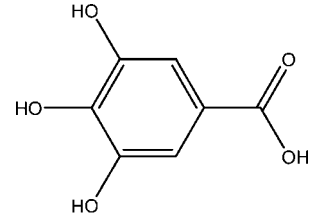
Introduction

Gallic acid, an organic acid, known as 3,4,5-trihydroxybenzoic acid ($C_6H_2(OH)_3COOH$) (Chart 1), is found widely throughout the plant kingdom. High gallic acid contents can be found in gallnuts, grapes, sumac, witch hazel, tea leaves, hops, and oak bark. Gallic acid exists in two forms as the free molecule and as part of tannins. Pure gallic acid is a colorless crystalline organic powder, while salts and esters of gallic acid are termed gallates. Despite its name, it does not contain gallium.[1] It has many applications in chemical research and industry such as being used as a standard for determining the phenol content of various analytes by the Folin−Ciocalteau assay[2] and also used for making dyes and inks.[3]

Gallic acid is commonly used in the pharmaceutical industry because many in vivo and in vitro studies in humans, animals, and cell culture have provided evidence for the following actions of gallic acid: (1) it shows cytotoxicity against cancer cells, without harming healthy cells;[4] (2) it can be used to treat albuminuria and diabetes;[5] (3) it seems to have antifungal and antiviral properties;[6] (4) used as an antioxidant and helps to protect human cells against oxidative damage;[7] (5) it can be used as a remote astringent in cases of internal hemorrhage;[8] (6) used to treat psoriasis and external hemorrhoids containing gallic acid.[9]

Tea consumption around the world is very high and ranks second only to water consumption. Tea is prepared from the dried leaves of *Camellia sinensis* and can be classified into black tea, oolong tea, and green tea.[10] Green tea is the most abundant source of tea phenolics, mostly in the form of simple hydroxybenzoic acids such as gallic acid and propyl gallate and its catechin derivatives (epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin). One of the most common moieties in the structure of tea phenolics, besides hydroxyl groups, is the gallic acid moiety. Green tea catechins and phenolic acids have been shown to demonstrate profound biochemical and pharmacological activities including antioxidant activities, modulation of carcinogen metabolism, and inhibition of cell proliferation.[11,12]

Chart 1. Molecular Structure of Gallic Acid



Gallic acid

Extracts derived from green tea have the potential to reduce the oxidation of food products and extend their shelf life. The antioxidant action was shown to be dependent on the ability of their constituent phenolic compounds to scavenge free radicals and to chelate metals.[13] Tea and other beverages rich in phenolic compounds, including coffee and wine, were shown to inhibit the absorption of nonheme iron, and a limited number of studies suggest that the effect is dose dependent.[14,15] Although the antioxidant effect is beneficial because of the reduced risk of spoilage, the inhibition of nonheme-iron absorption constitutes a potential adverse effect. Because iron deficiency is widespread and the mode of action of some antioxidants includes chelation of metals such as iron, it is prudent to examine the iron complexation of gallic acid, one of the most abundant compounds of tea extracts.

Iron exists in two distinct oxidation states—ferrous and ferric ions. Ferric ion (Fe^{3+}) is a relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on the conditions, particularly pH,[16] and oxidized back through Fenton-type reactions, with production of hydroxyl radicals or Haber−Weiss Cycle reactions with superoxide anions.[17,18] The production of these radicals can lead to lipid peroxidation, protein modification, and DNA damage. Chelating agents may inactivate metal ions and potentially inhibit the metal-dependent processes.[19] There are some ambiguous results in the literature concerning metal-chelation properties of polyphenols. Since they

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10.1021/je800441u CCC: $40.75
Published on Web 11/26/2008

may act as antioxidants and pro-oxidants, this may lead to a reduction in their antioxidant properties.[20,21] Iron(III) chelation is recognized by some authors as a minor mechanism in some polyphenols,[22] yet the contribution of free radical scavenging or of metal ion chelation to the antioxidative effect of polyphenols is not fully specified. The iron(III) chelating ability of polyphenols is related to the presence of *ortho*-dihydroxy polyphenols, i.e., molecules bearing catechol or galloyl groups.[23,24]

Evaluation of iron-chelation properties of phenolic acids is commonly done by means of UV−vis absorption spectroscopy, analyzing the shifts of UV bands I and II, which characterize polyphenolic spectra, and by potentiometry techniques.[25−38]

In the present work, we study the formation of binary and ternary complexes of the Fe^{III} metal ion with gallic acid and glycine in solution by using potentiometry, UV−visible absorption spectroscopy, conductometry, and polarography techniques at 25 °C and in $I = 0.10$ mol·dm^{-3} $NaNO_3$. Also, we present in this work the physicochemical properties obtained from the elemental analysis, ^{1}HNMR, ^{13}C NMR, and FT-IR spectroscopy techniques on the synthesized solid binary [Fe^{III}−gallic acid] and ternary [Fe^{III}−gallic acid−glycine] complexes.

Experimental Section

Materials and Solutions. The gallic acid used in this study was a commercially available chemical (TCI Co., LTD, Tokyo, Japan) and used without further purification. Glycine was provided by Merck, Germany. Carbonate-free sodium hydroxide pellets (titrant, prepared in 0.10 mol·dm^{-3} $NaNO_3$ solution) were standardized potentiometrically with KH−phthalate solution (Merck AG). Nitric acid, sodium hydroxide, iron chloride, and sodium nitrate were from Merck. Water used throughout the experiments was obtained from a NANO pure-Ultrapure water system that was distilled and deionized with resistance of 18.3 MΩ.

Potentiometric Measurements. pH-potentiometric titrations were performed using a Metrohm 686 titroprocessor with a 665 Dosimat and a 728 magnetic stirrer, coupled with a dosino burette model 700. pH titrations were carried out in an 80 cm^3 commercial double-walled glass vessel. The ionic strength of the solution was maintained at a constant level by using the desired concentration of $NaNO_3$ solution as supporting electrolyte, and the temperature was adjusted inside the cell at the desired value by circulating thermostatted water using an oil-thermostatted setup. A computer program (GLEE, glass electrode evaluation)[39] was used for the calibration of the glass electrode by means of a strong acid−strong base titration. This program provided an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential and slope of the electrode, and optionally, the concentration of the base and pK_W.

The metal-to-ligand ratios were 1:1 and 1:2.5 for the binary systems and 1:1:1 (Fe(III):gallic acid:glycine) for the ternary system. The investigated solutions (total volume of 50 cm^3) were prepared and titrated with potentiometrically standard carbon dioxide free NaOH (0.10 mol·dm^{-3}) solution. During the course of titrations, a stream of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of atmospheric carbon dioxide. Each solution was thermostatted at the required temperature with accuracy of ± 0.1 K, where the solutions were left to stand for about 15 min before titration. A magnetic stirrer was used during all titrations. Each titration was repeated at least 4 times under carefully controlled experimental conditions. Typically, more than 60 pH readings (points of potentiometric measurements) were collected and

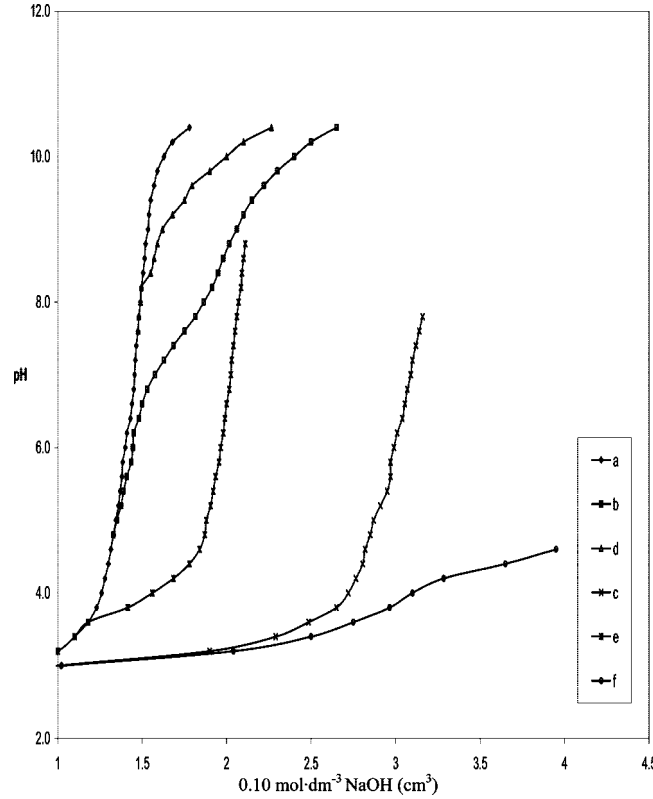
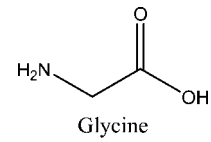


Figure 1. Potentiometric titration curves for the Fe(III) + gallic acid + glycine system at (298.15 ± 0.1) K and $I = 0.10$ mol·dm^{-3} $NaNO_3$. (a) $3 \cdot 10^{-3}$ mol·dm^{-3} HNO_3 + 0.10 mol·dm^{-3} $NaNO_3$. (b) Solution a + $1 \cdot 10^{-3}$ mol·dm^{-3} gallic acid; (c) solution b + $4 \cdot 10^{-4}$ mol·dm^{-3} iron(III); (d) solution a + $1 \cdot 10^{-3}$ mol·dm^{-3} glycine; (e) solution d + $4 \cdot 10^{-4}$ mol·dm^{-3} iron(III); (f) solution a + $1 \cdot 10^{-3}$ mol·dm^{-3} iron(III) + $1 \cdot 10^{-3}$ mol·dm^{-3} gallic acid + $1 \cdot 10^{-3}$ mol·dm^{-3} glycine.

Chart 2. Molecular Structure of Glycine



Glycine

taken into account for each titration (Figure 1). The complexes are quite stable up to high pH values. In all cases, no calculations have been performed beyond the precipitation point; hence, the hydroxyl species likely to be formed after this point could not be studied.

Calculations. An Irving and Rossotti pH titration technique with modifications was used to determine the protonation constants of the ligands and formation constants of the different binary and ternary iron(III) complexes as described before in our previous work.[40−43]

From the titration curves of the solutions a, b, c and a, d, e (Figure 1), the following parameters were calculated using the modified formula of Irving and Rossotti: $\bar{n}_H$, average number of protons associated with the ligands, gallic acid, and amino acid glycine (Charts 1 and 2); $\bar{n}_b$, average number of ligand molecules attached to a metal ion; and P_L, free ligand exponent at several pH values.

Eq 1 was used for calculation of the $\bar{n}_H$ values from the pH-metric titration curves a, b and a, d, where y is the number of dissociable protons ($y = 1$ in the case of glycine and $y = 2$ in the case of gallic acid).

$$\bar{n}_H = y - \frac{(V_b \text{ or } V_d - V_a)(E^o + N^o)}{(V_o + V_a)T_L^o} \quad (1)$$

V_a, V_b, and V_d are the volumes of NaOH consumed to reach the same pH values in curves a, b, and d, respectively. E^o and N^o are the concentrations of HNO_3 and NaOH, respectively. T_L^o is the initial total molar concentration of the primary ligand (gallic acid) and secondary ligand (glycine) studied in the titrated solution = $1.0 \cdot 10^{-3}$ mol·dm^{-3}, and V_o is the original volume (50 cm^3).

The average number of ligand molecules ($\bar{n}_b$) coordinated to the iron(III) ion and the free ligand exponent (P_L) at several pH values were calculated according to eqs 2 to 4, where V_c and V_e are the volumes of NaOH consumed to reach the same pH values in curves c and e, respectively. T_M^o is the initial total molar concentration of the iron(III) ion (Fe^{III}) used in the titrated solution in mol·dm^{-3}.

$$\bar{n}_b = \frac{(V_c - V_b) \text{ or } (V_e - V_d)[(E^o + N^o) + T_L^o(y - \bar{n}_H)]}{(V_o + V_b)(V_o + V_d)\bar{n}_H T_M^o} \quad (2)$$

Since $(E^\circ + N^\circ) \gg T_L^o$, thus

$$\bar{n}_b = \frac{(V_c - V_b) \text{ or } (V_e - V_d)(E^o + N^o)}{(V_o + V_b) \text{ or } (V_o + V_d)\bar{n}_H T_M^o} \quad (3)$$

Generally we obtain

$$P_L = \log\left\{ \sum_{y=0}^{1 \text{ or } 2} \frac{\beta_y (1/10^{pH})^y}{T_L^o - \bar{n} T_M^o} \cdot \frac{(V_o + V_c \text{ or } V_e)}{V_o} \right\} \quad (4)$$

βy represents the proton−ligand dissociation constants of the ligands. $\bar{n}_H$ values were available from the determination of the proton−ligand formation constant. It is worth mentioning that the values of $\bar{n}_b$ exceed 1.5 indicating the formation of both 1:1 and 1:2 binary complexes.

Experimental formation curves corresponding to the various ligands and their 1:1 or 1:2 binary iron(III) complexes were obtained by plotting $\bar{n}_b$ against P_L. The corresponding acid formation constant values and the stability constant values of their complexes were determined from the constructed n_H−pH and n_b−P_L curves, respectively.

On the other hand, the titration curves of the solutions c and f, $\bar{n}_{mix}$, the average number of moles of the secondary ligand (glycine), was calculated from the relationship

$$\bar{n}_{mix} = \frac{(V_f - V_c)[(E^o + N^o) + T_L^o(y - \bar{n}_H)]}{(V_o + V_c))[T^o_{M(\text{primary ligand})}]\bar{n}_H} \quad (5)$$

where V_f and V_c are the volumes of NaOH consumed to reach the same pH value in the curves f and c, respectively. [$T^o_{M(\text{primary ligand})}$] is the concentration of the binary (iron(III)−primary ligand) complex which is equivalent to the initial iron(III) ion concentration T_M^o; $\bar{n}_H$ is the average number of protons associated with the secondary ligand (these values are available at different pH values from the binary complexing system); T_L^o is the initial concentration of the secondary ligand; and y is the number of dissociable protons per molecule of the secondary ligand (glycine). V_o, E^o, and N^o have the same meaning as mentioned before.

Since $E^o + N^o \gg T_L^o$

$$\bar{n}_{mix} = \frac{(V_f - V_c)[E^o + N^o]}{(V_o + V_c)[T^o_{M(\text{primary ligand})}]\bar{n}_H} \quad (6)$$

From the values of $\bar{n}_{mix}$ so obtained, the free secondary ligand exponent, pL'_{mix} was calculated using the equation

$$pL'_{mix} = \log\left\{ \frac{1 + 10^{pK_{a2}}(1/10^{pH})}{T_L^o - n'_{mix} T_M^o} \cdot \frac{(V_o + V_f)}{V_o} \right\} \quad (7)$$

pK_{a2} represents the second dissociation constants value of glycine. All other terms have the same meaning as defined above.

Formation curves corresponding to the different ternary complexes under investigation were constructed by plotting $\bar{n}_{mix}$ versus pL'_{mix}. At $\bar{n}_{mix}$ equals 0.5, the pL'_{mix} value is the corresponding stability constant of the ternary complex formed in the solution (1:1:1).

Conductometric Measurements. Conductometric titrations were followed with a Techne conductivity meter (model 4510). The following mixture was titrated conductometrically against a 0.10 mol·dm^{-3} NaOH solution: $1 \cdot 10^{-2}$ mol·dm^{-3} Fe(III) + $1 \cdot 10^{-2}$ mol·dm^{-3} gallic acid + $1 \cdot 10^{-2}$ mol·dm^{-3} glycine.

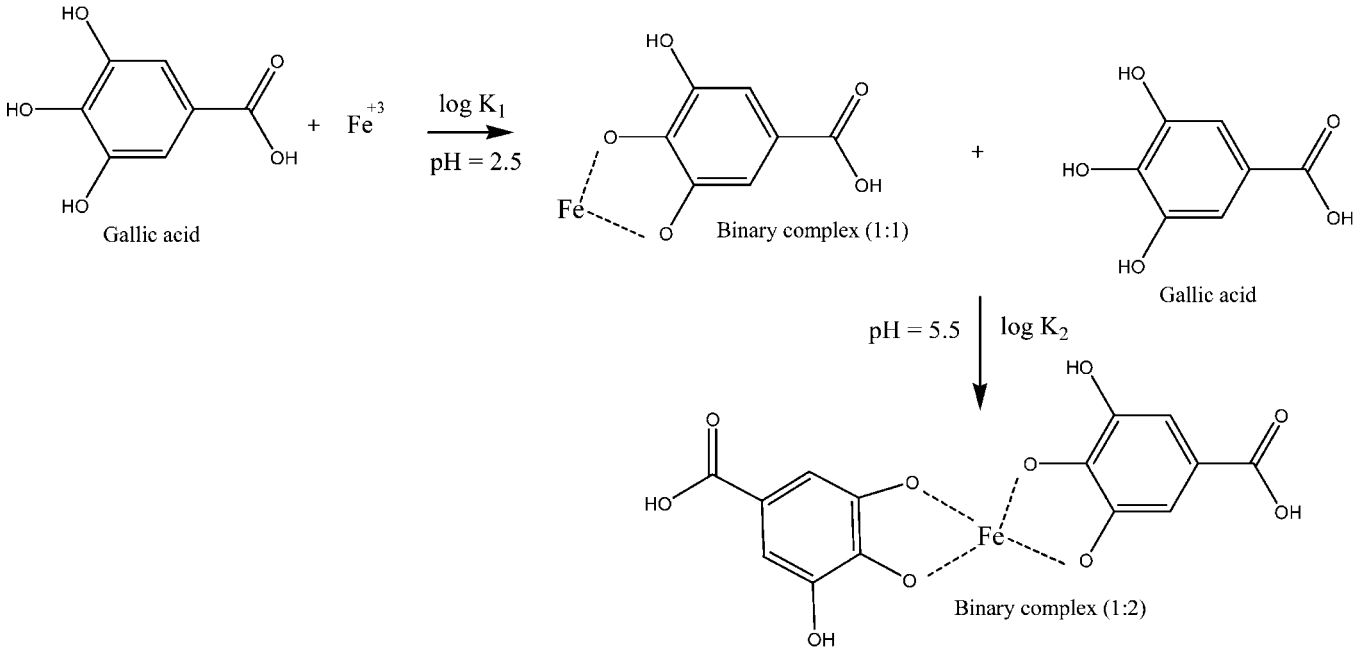
Electrochemical Measurements. The polarograms were recorded by a LP 60 polarograph at room temperature (20 °C). A saturated calomel electrode (SCE) was used as a reference electrode with an agar-saturated potassium chloride salt U-bridge. The short arm of the bridge was immersed in the KC1 solution of the calomel electrode and the long arm entering in tall glass tubing provided with a sintered glass disk of porosity IG 3 partly immersed in the electrolyte. The dropping mercury electrode (DME) capillary had a value of m = 3.65 mg·s^{-1} and t = 3.3 s·drop^{-1} in 1 mol·dm^{-3} perchloric acid at 0.0 V vs SCE. The height of the mercury column was 30 cm.

Spectrophotometric Measurements. Absorption spectra were obtained using UV−vis spectra (Perkin-Elmer; model Lambda 25).

Synthesis of the Iron(III)−Gallic Acid Binary Complex. The appropriate iron(III) salt (0.01 mmol) was dissolved in ethanol (10 mL) and added to a stirred solution of gallic acid (0.01 mmol) in 10 mL of ethanol. The resulting dark brown reaction mixture was gently heated, then stirred for 1/2 h and concentrated in a rotary evaporator until the volume was about 10 mL. Ethanol was added to the solution, and the resulting solid products obtained were isolated by allowing the solution mixture to stand in air at room temperature for slow evaporation and dried under vacuum. Dark brownish violet crystals were obtained and washed by ethanol and then dried by using a rotary evaporator (yield, 311 mg). Anal. Calcc for $C_7H_4O_3Fe \cdot 2H_2O$ (MW: 172): C, 72.4; H, 4.2; O, 3.9; Fe, 5.4 %. Found: C, 72.0; H, 4.5; Fe, 6.0 %. Yield: 83 %. IR (KBr, cm^{-1}): ν(−OH) 3150−3550; $\nu(-COO)_{asym}$ weak, 1590; $\nu(-COO)_{sym}$ weak, 1700, ν(phenolic, C−O) weak, 1252, 1609, ν(CC), 770, 863, ν(C−H), 624, 1089, 1105, 1119. ^{1}H NMR (d_6-TMS) δ (ppm): 12.21 (s, 1H, −COOH); 8.86−9.17 (s, 3H, −OH); 6.92 (s, 2H, −CH). ^{13}C NMR (d_6-TMS) δ (ppm): 120.50, 108.85 (2C, phenyl), 145.36, 137.95, 145.36 (3C−O, phenol), 108.85 (1C−C), 167.48 (1C═O, carboxylic).

Synthesis of Iron(III)−Gallic Acid−Glycine Ternary Complex. The appropriate iron(III) salt (0.01 mmol) was dissolved in ethanol (10 mL) and added to a stirred solution of gallic acid (0.01 mmol) in ethanol. Then, the solution of glycine (0.01 mmol) was added once. The resulting dark brown reaction mixture was gently heated and stirred for 5 h and concentrated in a rotary evaporator until the volume was about 10 mL. Ethanol was added to the solution, and the resulting solid products obtained were isolated by allowing the solution mixture to stand in air at room temperature for slow evaporation and dried under vacuum. Dark brownish crystals were obtained and washed by ethanol and then dried by using a rotary evaporator (yield, 426 mg). Anal. Calcd for $C_9H_7O_5NFe \cdot 2H_2O$ (MW: 172):

Chart 3. Proposed Stepwise Reaction Mechanism for the Binary Complex Formation of Iron(III) and Gallic Acid



$$\text{Fe(II)} + (\text{gallic acid})^{-2} \underset{\text{pH = 2.5}}{\overset{\log K_1}{\rightleftharpoons}} [\text{Fe(gallic acid)}]^{+}$$

$$[\text{Fe(gallic acid)}]^{+} + (\text{gallic acid})^{-2} \underset{\text{pH =5.5}}{\overset{\log K_2}{\rightleftharpoons}} [\text{Fe(gallic acid)}_2]^{-}$$

Table 1. Protonation and Stability Constants for Fe^{III} Binary and Ternary Complexes of Gallic Acid and Glycine at (25 ± 0.10) °C and I = 0.1 mol·dm^{-3} $NaNO_3$

compound	pK_{a1}	pK_{a2}	log K_1	log K_2	log K^{MA}_{MAL}	log β^{MA}_{MAL}	Δ log K	log X
gallic acid (A)	4.10 ± 0.01[a]	8.38 ± 0.01[a]	14.73 ± 0.02[d]	11.93 ± 0.02[d]	-----	-----	-----	-----
glycine (L)	-----	9.63 ± 0.06[b]	10.83 ± 0.03[c]	9.65 ± 0.04[c]	10.03 ± 0.02	24.76	−4.7	4.63

[a] Ref 40. [b] Ref 41. [c] Ref 42. [d] Ref 43.

C, 74.4; H, 6.2; O, 3.9; N, 1.3 Fe, 5.4 %. Found: C, 75.4; H, 6.4; O, 4.0; N, 1.2 Fe, 5.7 %. Yield: 85 %. IR (KBr, cm^{-1}): ν(−OH) strong and broadband 2600−3650; $\nu(-COO)_{asym}$ strong, 1550; $\nu(-COO)_{sym}$ strong, 1750, ν(phenolic, C−O) strong, 1300, 1650, ν(CC), 760, 863, ν(C−H), 624, 1100, 1105, 1150, $\nu(NH_2)$, 420, ν(Fe−N), 640. ^{1}H NMR (d_6-TMS) δ (ppm): 12.21 (s, 1H, −COOH); 8.86−9.17 (s, 2H, −OH); 6.92 (s, 2H, −CH); 3.54 (s, 1H, −CH). ^{13}C NMR (d_6-TMS) δ (ppm): 41.87 (1−CH (glycine)) 120.50, 108.85 (2C, phenyl); 145.36, 137.95, 145.36 (3C−O, phenol), 108.85 (1C−C), 167.48 (1C═O, Carboxylic), 170.21 (1C═O, carboxylic (glycine)).

FT-IR and NMR spectra give enough information to elucidate the way of bonding of the ligands (gallic acid and glycine) to iron(III). The most characteristic vibrations are selected by comparing the IR spectra of the ligands with those of their iron complexes.

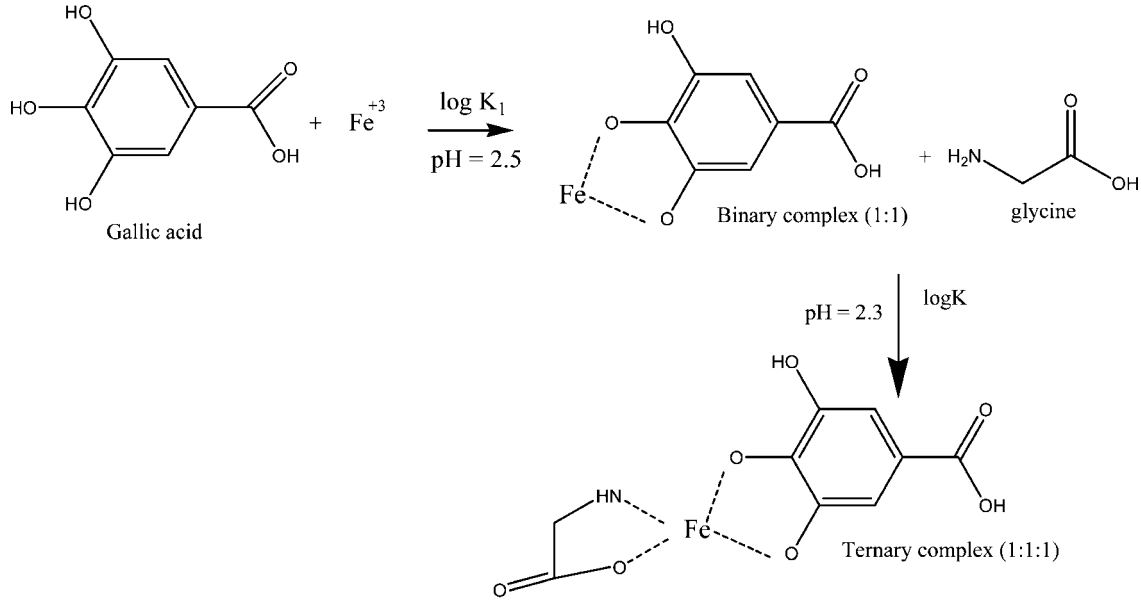
The elemental analyses (C, H, and N) were performed on a LECO-CHSNO-9320 type elemental analyzer. A Fourier transform infrared spectrometer (FTIR), model, Bio-Rad Digilab, FTS-3500, and solid state nuclear magnetic resonance spectrometer (400 MHz) were used to investigate the synthesized complexes.

Results and Discussion

Protonation Constants. The first and second proton dissociation constants (pK_{a1} and pK_{a2}) of gallic acid were calculated from curves a and b in Figure 1. The acid−base behavior of gallic acid, in aqueous solution and in different solvent mixtures, has been previously studied by us,[40] but using different experimental conditions and method of calculation. The proton dissociation constants of glycine have also been determined potentiometrically from curves a and d in Figure 1. The value of pK_{a2} for the monocarboxylic amino acid, although already reported in a previous work,[41] has been redetermined at (298.15 ± 0.1) K and I = 0.10 mol·dm^{-3} $NaNO_3$ to obtain a value using the same experimental procedures as used in the study of binary and ternary systems and is in agreement with data found in the literature. It is worth mentioning that the pK_{a1} value of glycine is low (≤ 2.30)[41,44] and exists only in strongly acidic solutions. Therefore, this value is not used in calculations because the pH-metric data are measured in the range of 2.6 ≤ pH ≤ 11.

Binary Systems. An analysis of complexed ligand curves c and e, as shown in Figure 1, indicates that the addition of iron(III) to the free-ligand solutions shifts the buffer region of the ligand to lower pH values. This shows that the complexation

Chart 4. Proposed Stepwise Reaction Mechanism for the Ternary Complex Formation of Iron(III) and Gallic Acid and Glycine



$$\text{Fe(II)} + (\text{gallic acid})^{-2} \underset{\text{pH = 2.5}}{\overset{\log K_1}{\rightleftharpoons}} [\text{Fe(gallic acid)}]^{+}$$

$$[\text{Fe(gallic acid)}]^{+} + (\text{glycine})^{-} \underset{\text{pH =2.3}}{\overset{\log K^{MA}_{MAL}}{\rightleftharpoons}} [\text{Fe(gallic acid)(glycine]}$$

reaction proceeds by releasing protons from such ligands (Charts 2 and 3). Generally, it was observed that the binary iron complexes of gallic acid and glycine begin to form in the pH ranges of 2.0 to 3.6 and 3.0 to 5.0, respectively. The stability constants of the 1:1 and 1:2 binary complexes of gallic acid with iron (Table 1) have been determined at (298.15 ± 0.1) K and in $I = 0.1$ mol·dm^{-3} $NaNO_3$. The values obtained are more or less in good agreement with literature data.[40−43]

Ternary System. When a solution contains two different ligands and a metal ion, they may exist in equilibria in which either (i) both the ligands may combine with the metal ion simultaneously or (ii) the two ligands may be combined one by one at different pH. As is evident from the titration curves in the present study, the addition of two ligands is stepwise (Chart 4). It was deduced that gallic acid interacts first with the iron(III) ion, followed by the interaction of the glycine amino

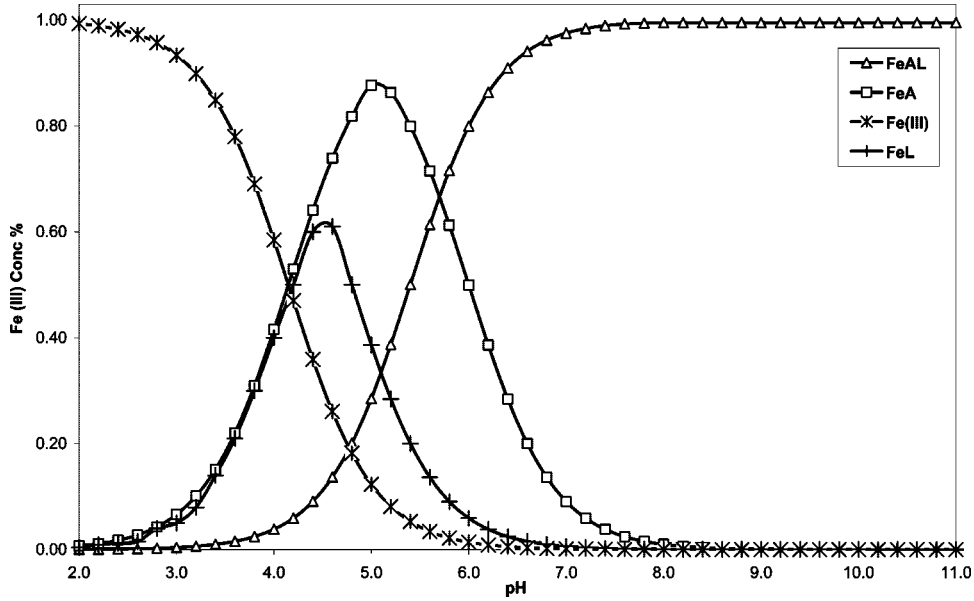


Figure 2. Concentration distribution curves as a function of pH calculated for the Fe^{III} + gallic + glycine system in the ratio 1:1:1 at (298.15 ± 0.1) K, I = 0.1 mol·dm^{-3} ($NaNO_3$). Concentration: $C_{Fe(III)} = 1 \cdot 10^{-3}$ mol·dm^{-3}; $C_{\text{gallic acid}} = 1 \cdot 10^{-3}$ mol·dm^{-3}; $C_{\text{glycine}} = 1 \cdot 10^{-3}$ mol·dm^{-3}.

acid; that is, the ternary complex formation could be considered in stepwise complexation equilibria, i.e., the formation of a ternary complex (Chart 4) can be represented by the stepwise equilibrium;

$$M + A \rightleftharpoons MA \quad K^{M}_{MA} = [MA]/[M][A]$$

$$MA + L \rightleftharpoons MAL \quad K^{M}_{MAL} = [MAL]/[MA][L]$$

where M = F^{III}; A = gallic acid; and L = glycine.

The overall stability constant β^{M}_{MAL} may be represented by the following equations

$$M + A + L \rightleftharpoons MAL$$

$$\beta^{M}_{MAL} = [MAL]/[M][A][L] = K^{M}_{MAL} + K^{M}_{MA}$$

The relative stability of the ternary complexes, as compared to that of the corresponding binary systems, can be quantitatively expressed in different ways. A review of those methods has shown that, for a variety of reasons, the most suitable comparison is in terms of $\Delta \log K$, as defined by the equation

$$\Delta \log K = \log K^{MA}_{MAL} - \log K^{M}_{ML}$$

It is to be noted here that if the $\Delta \log K$ values are positive the ternary complexes are more stable than the corresponding

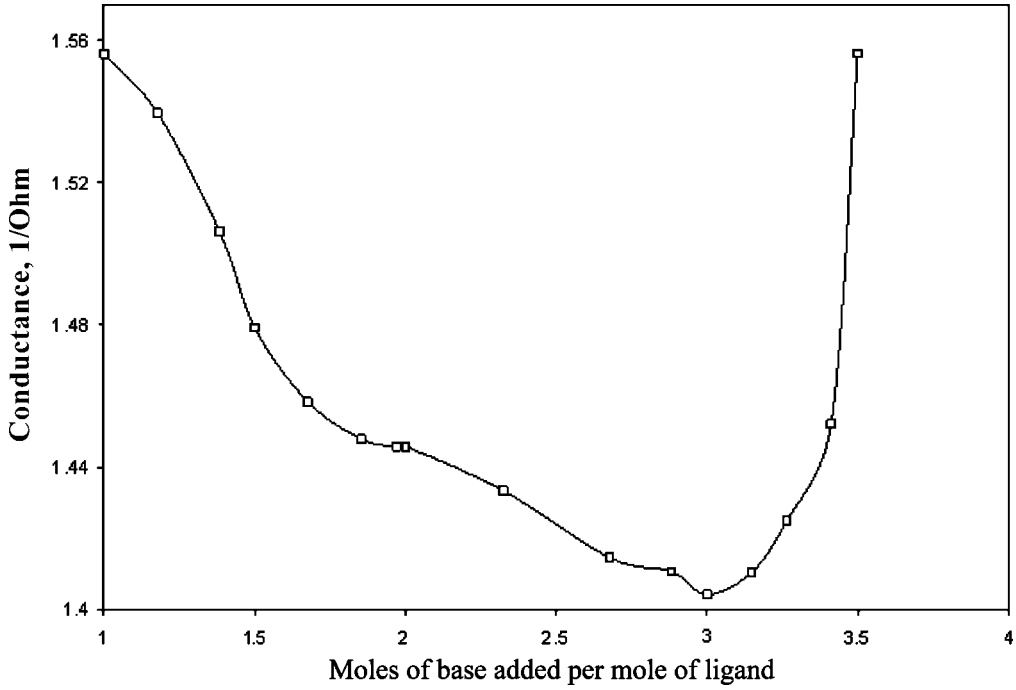


Figure 3. Conductometric titration curve for Fe^{III}−gallic−glycine systems at I = 0.1 mol·dm^{-3} ($NaNO_3$), (298.15 ± 0.1) K, 1·10^{-2} mol·dm^{-3} Fe(III) + 1·10^{-2} mol·dm^{-3} gallic acid + 1·10^{-2} mol·dm^{-3} glycine.

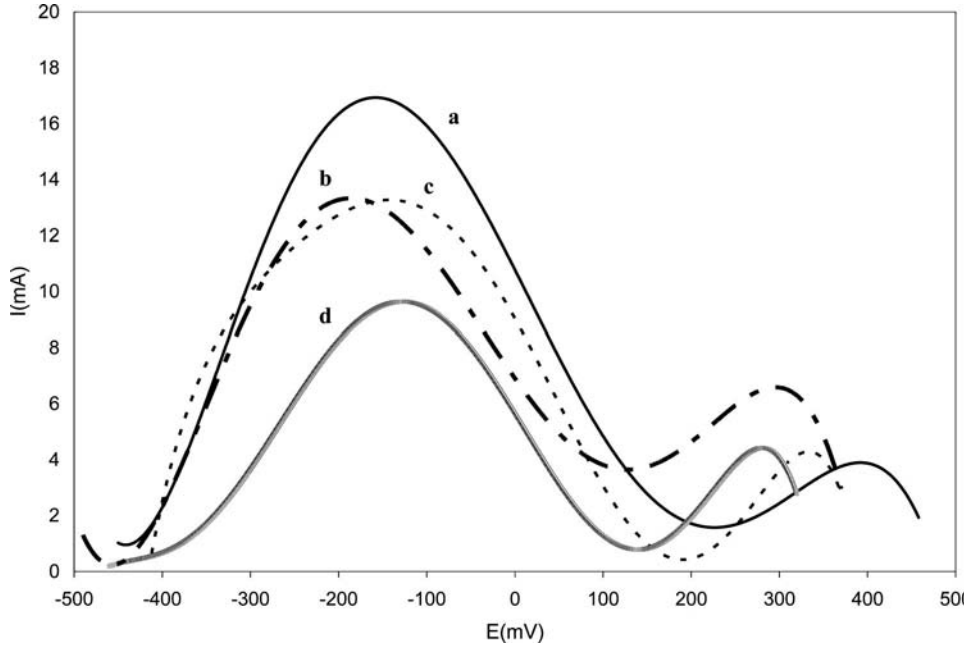


Figure 4. Differential pulse polarograms for the iron(III) + gallic acid + glycine system at I = 0.1 mol·dm^{-3} $NaNO_3$, pH = 2.3, and (298.15 ± 0.1) K. (a) 0.001 mol·dm^{-3} Fe^{III}; (b) 0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} gallic acid; (c) 0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} glycine; (d) 0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} gallic acid + 0.001 mol·dm^{-3} glycine.

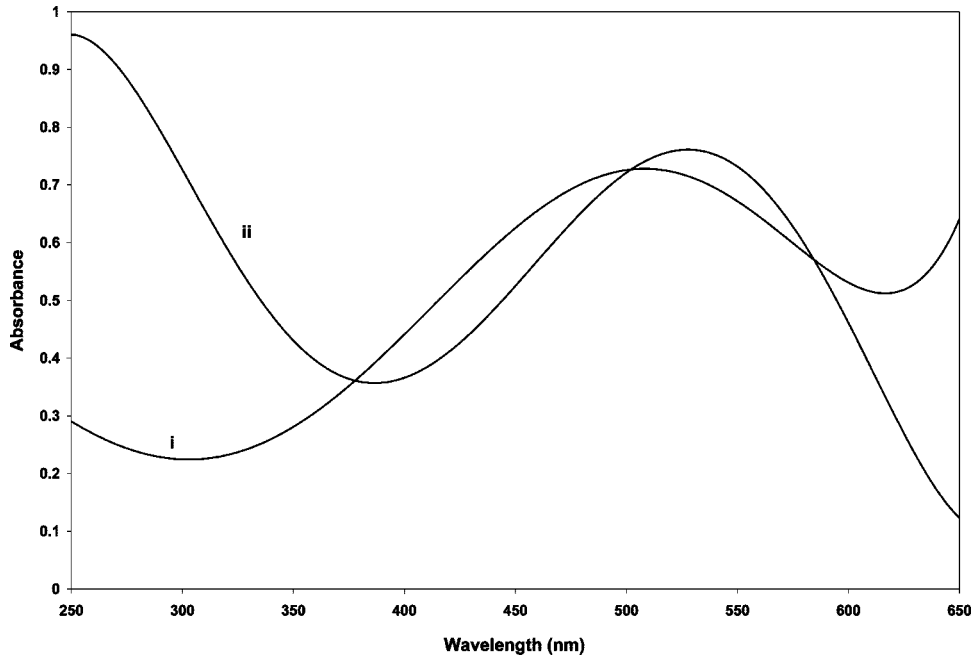


Figure 5. Absorbtion spectra for (i) the binary system (0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} gallic acid at pH = 2.5); (ii) the ternary system (0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} gallic acid + 0.001 mol·dm^{-3} glycine at pH = 2.3).

binary complexes, while if the values for $\Delta \log K$ are negative, the reverse is true. However, the negative values of $\Delta \log K$ do not preclude the formation of ternary complexes in solution. Thus, the $\Delta \log K$ values clearly emphasize the amount of extra stabilization in these complexes.

A parameter, known as log X, is frequently used to characterize the stability of ternary or mixed complexes. It measures the tendency of one mole each of the binary complexes MA_2 and ML_2 to form 2 moles of MAL; i.e.

$$MA_2 + ML_2 \rightleftharpoons 2MAL$$

$$X = [MAL]^2/[MA_2][ML_2]$$

It is therefore calculated by

$$\log X = 2 \log \beta_{MAL} - (\log \beta_{MA2} + \log \beta_{ML2})$$

The value of the constant X expected on statistical grounds is 4. Whenever it deviates from this value, it must be the result of intraligand electronic and/or steric interactions.

From the calculated $\Delta \log K$ and log X values listed in Table 1, one can conclude that, in most ternary systems, $\Delta \log K$ is found to be positive and the values of log X were high, demonstrating a significant stabilization of these mixed ligand complexes. The higher stability constants of ternary complexes compared with the binary systems may be attributed to the interligand interactions or some cooperative coordination between the coordinate ligands, possibly H-bond formation.

The concentration distribution of various complex species existing in solution as a function of pH can be obtained by means of the so-called SPECIES program.[45] The species distribution for the iron(III)−gallic acid−glycine system, taken as representative, is given in Figure 2.

In Figure 3, a conductometric titration curve for the ternary complex of iron(III) with gallic acid and glycine is displayed. The titration curve shows an initial decrease and an inflection at $a = 2$ (a = moles of base added per mole of ligand), which probably corresponds to the neutralization of H^+ ions resulting from the formation of the iron(III)−gallic acid binary complex. Between $2 < a < 3$, the conductance decreases slightly due to the formation of the ternary complex and is associated with the release of a proton from the secondary ligand (glycine). Beyond $a = 3$, the conductance increases more uniformly due to the presence of excess NaOH.

Confirmation of the ternary complex iron(III) + gallic acid + glycine in solution has been carried out using differential pulse polarography (DPP). A differential pulse polarogram for the system iron(III) + gallic acid + glycine is given in Figure 4. The differential pulse polarograms of the Fe(III) solution show one cathodic peak at $E_p = -69$ mV. This peak may be described as a result of the reduction of Fe(III) to Fe (in a two-electron-transfer process) at the glassy carbon electrode.

UV−visible absorption spectra are known to be useful to prove the formation of both binary and ternary systems of iron(III) and to characterize the coordination site of iron(III) complexes of gallic acid. Figure 5 shows the visible absorption spectra of both the iron(III)−gallic acid binary complex and the iron(III)−gallic acid−glycine ternary complex at given pH value. The spectra of the ternary systems are quite different from those of the binary systems, emphasizing the formation of the former in solution. Figure 5 reveals that the absorption spectra of the iron(III)−gallic acid binary complex gives a strong absorption peak at 535 nm with absorbance of 0.73, while the absorption spectra of the iron(III)−gallic acid−glycine ternary complex gives a strong absorption peak at 500 nm with absorbance of 0.68.

Literature Cited

(1) Rajalakshmi, D.; Narasimban, S. Food antioxidants: sources and methods of evaluation. In *Food antioxidants*; Madhavi D. L., Deshpande, S. S., Salunkhe, D. K., Eds.; Marcel Dekker: New York, 1996; pp 65−157.

(2) Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. *Methods Enzymol.* **1999**, *299*, 152–178.

(3) Martín-Gil, J.; Ramos-Sánchez, M. C.; Martín-Gil, F. J.; José-Yacamán, M. Chemical composition of a fountain pen ink. *J. Chem. Educ.* **2006**, *83*, 1476–1478.

(4) Elvira, G. M.; Chandra, S.; MarcoVinicio, R. M.; Wenyi, W. Catalytic inhibition of human DNA topoisomerase by phenolic compounds in

Ardisia compressa extracts and their effect on human colon cancer cells. *Food Chem. Toxicol.* **2006**, *44*, 1191–1203.
(5) Chiu-Lan, H.; Yuh-Charn, L.; Gow-Chin, Y.; Hui-Yin, C. Preventive effects of guava (Psidium guajava L.) leaves and its active compounds against α-dicarbonyl compounds-induced blood coagulation. *Food Chem.* **2007**, *103*, 528–535.
(6) Misao, U.; Hisashi, Y.; Yukiko, K.; Masanori, H.; Tomihiko, H.; Koyama, A. Antiviral effect of octyl gallate against DNA and RNA viruses. *Antiviral Res.* **2007**, *73*, 85–91.
(7) Jittawan, K.; Sirithon, S. Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. *Food Chem.* **2008**, *110*, 881–890.
(8) Hurrell, R. F.; Reddy, M.; Cook, J. D. Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br. J. Nutr.* **1999**, *81*, 289–295.
(9) Cook, J. D.; Reddy, M. B.; Hurrell, R. F. The effect of red and white wines on nonheme-iron absorption in humans. *Am. J. Clin. Nutr.* **1995**, *61*, 800–804.
(10) Min, Z.; Colin, W. B.; Lee, A. H. Tea consumption and ovarian cancer risk. A case-control study in China. *Cancer Epidemiol., Biomarkers Prev.* **2002**, *11*, 713–718.
(11) Barbosa, D. S. Green tea polyphenolic compounds and human health. *J. Verbraucherschutz Lebensmittelsicherheit* **2007**, *7*, 407–413.
(12) Kuroda, Y.; Hara, Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutat. Res.* **1999**, *436*, 69–97.
(13) Morck, T. A.; Lynch, S. R.; Cook, J. D. Inhibition of food iron absorption by coffee. *Am. J. Clin. Nutr.* **1983**, *37*, 416–420.
(14) Strlic, M.; Radovic, T.; Kolar, J.; Pihlar, B. Anti- and prooxidative properties of gallic acid in Fenton-type systems. *J. Agric. Food Chem.* **2002**, *50*, 6313–6317.
(15) Kehrer, J. P. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* **2000**, *149*, 43–50.
(16) Wong, Y. Y.; Kitts, D. D. An iron binding assay to measure activity of known food sequestering agents: studies with buttermilk solids. *Food Chem.* **2001**, *72*, 245–254.
(17) Finefrock, E.; Bush, A. I.; Doraiswamy, P. M. Current status of metals as therapeutic targets in Alzheimer's disease. *J. Am. Geria. Soc.* **2003**, 1143–1148.
(18) Keceli, A.; Gordon, M. H. Ferric ions reduce the antioxidant activity of the phenolic fraction of virgin olive oil. *J. Food Sci.* **2002**, *67*, 943–947.
(19) Salonen, J. T.; Nyyssonen, K.; Korpela, H.; Tuomilehto, J.; Seppanen, R.; Salonen, R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* **1992**, *86*, 803–811.
(20) Miura, S.; Watanabe, J.; Tomita, T.; Sano, M.; Tomita, I. The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low density lipoprotein. *Biol. Pharm. Bull.* **1994**, *17*, 1567–1572.
(21) Asakura, T.; Nakamura, Y.; Inoue, N.; Murata, M.; Homma, S. Characterization of zinc chelating compounds in instant coffee. *Agric. Biol. Chem.* **1990**, *54*, 855–862.
(22) Disler, P. B.; Lynch, S. R.; Torrance, J. D.; Sayers, M. H.; Bothwell, T. H.; Charlton, R. W. The mechanism of the inhibition of iron absorption by tea. *Afr. J. Med. Sci.* **1975**, *40*, 109–116.
(23) Morck, T. A.; Lynch, S. R.; Cook, J. D. Inhibition of food iron absorption by coffee. *Am. J. Clin. Nutr.* **1983**, *37*, 416–420.
(24) Moran, J. F.; Klucas, R. V.; Grayer, R. J.; Abian, J.; Becana, M. Complexes of iron, with phenolic compounds from soybean oxidant properties. *Free Radical Biol. Med.* **1997**, *22*, 861–870.
(25) Moridani, M. Y.; Pourahmad, J.; Bui, H.; Siraki, A.; O'Brien, P. J. Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. *Free Radical Biol. Med.* **2003**, *34*, 243–253.
(26) Paiva-Martins, F.; Gordon, M. H. Effects of pH and ferric ions on the antioxidant activity of olive polyphenols in oil-in-water emulsions. *J. Am. Oil Chem. Soc.* **2002**, *79* (6), 571–576.
(27) Brenes, M.; Garcia, P.; Garrido, A. Effect of pH on the color formed by Fe-phenolic complexes in ripe olives. *J. Sci. Food Agric.* **1995**, *67*, 35–41.
(28) Paiva-Martins, F.; Gordon, M. H. Interactions of ferric ions with olive oil phenolic compounds. *J. Agric. Food Chem.* **2005**, *53*, 2704–2709.
(29) Sørensen, A.-D. M.; Haahr, A.-M.; Becker, E. M.; Skibsted, L. H.; Bergenståhl, B.; Nilsson, L.; Jacobsen, C. Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-enriched oil-in-water emulsions. *J. Agric. Food Chem.* **2008**, *56*, 1740–1750.
(30) Mirjana, A.; John Van, C.; BrunoDe, M.; Griet, D.; Carmen, S.; Marc, V.; Roland, V. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.* **2006**, *98*, 23–31.
(31) Samuel, B.; Beate, B.; Michael, M.; Hynes, J. Reaction of iron(III) with theaflavin: complexation and oxidative products. *J. Inorg. Biochem.* **2004**, *98*, 657–663.
(32) Koch, S.; Ackermann, G.; Lindner, P. Application of redox reactions in spectrophotometry—II Detection and spectrophotometric determination of phenolic compounds with the iron(III)/1,10-phenanthroline complex. *Talanta* **1992**, *39*, 693–696.
(33) Petronio, B. M.; Laganà, A.; Andrea, G. D. Applications of ligand-exchange—III: Preparation and properties of phenol-formaldehyde-based resin in the iron(III) form. *Talanta.* **1984**, *31*, 357–360.
(34) Petronio, B. M.; Laganà, A.; Andrea, G. D.; Russo, M. V. Some applications of ligand-exchange—I. Recovery of phenolic compounds from water. *Talanta.* **1981**, *28*, 215–220.
(35) Bailey, N. A.; Cummins, D.; McKenzie, E. D.; Worthington, J. M. Iron(III) compounds of phenolic ligands. The crystal and molecular structure of iron(III) compounds of the sexadentate ligand N,N′-ethylene-bis-(o-hydroxyphenylglycine). *Inorg. Chim. Acta* **1981**, *50*, 111–120.
(36) Hideo, I.; Toshihiro, I.; Ryojin, S.; Ryo, K. The effects of caffeic acid and its related catechols on hydroxyl radical formation by 3-hydroxyanthranilic acid, ferric chloride, and hydrogen peroxide. *Arch. Biochem. Biophys.* **1990**, *276*, 242–247.
(37) Arif Kazmi, S.; Saqib Qureshi, M.; Maqsood, Z. Reactivity of an iron(III) complex of gallic acid. *Inorg. Chim. Acta* **1987**, *137*, 151–154.
(38) Elinany, G. A.; Ebeid, F. M.; Zahra, A. M.; Ziedan, F. I. Polarography of metal-gallic complexes. *J. Electroanal. Chem.* **1976**, *72*, 363–369.
(39) Gans, P.; Sullivan, O.; Glee, B. Glee, a new computer program for glass electrode calibration. *Talanta* **2000**, *51*, 33–37.
(40) Fazary, A. E.; Ju, Y. H. Non aqueous solution studies on the protonation equilibria of some phenolic acids. *J. Solution Chem.* **2008**, *37*, 1305–1319.
(41) Fazary, A. E.; Mohamed, A. F.; Lebedeva, N. Protonation equilibria studies of the standard α-amino acids in $NaNO_3$ solutions in water and in mixtures of water and dioxane. *J. Chem. Thermodyn.* **2006**, *38*, 1467–1473.
(42) Khalil, M. M.; Fazary, A. E. Potentiometric studies on binary and ternary complexes of di- and trivalent metal ions involving some hydroxamic acids, amino acids, and nucleic acid components. *Monatsh. Chem.* **2004**, *135*, 1455–1474.
(43) Powell, H. K. J.; Taylor, M. C. Interactions of iron(II) and iron(III) with gallic acid and its homologues: a potentiometric and spectrophotometric study. *Aust. J. Chem.* **1982**, *35*, 739–756.
(44) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1982; Vol. 5.
(45) Gans, P.; Vacca, A.; Miniquad, A. General computer programme for the computation of formation constants from potentiometric data; 1974; Vol. 21, pp 54−57.

Received for review June 18, 2008. Accepted October 18, 2008.

JE800441U