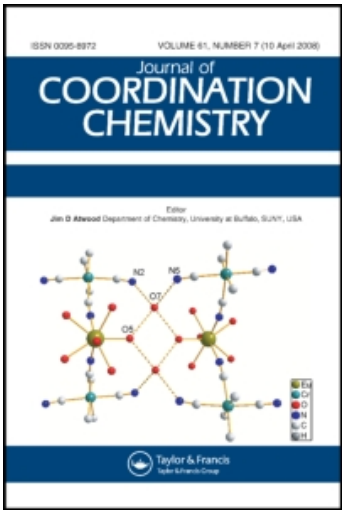

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The kinetics and mechanisms of the reactions of iron(III) with naringenin, hesperetin, 3-hydroxychromone and 3-hydroxyflavone. A comparison of the coordination power of 3-hydroxychromone and 5-hydroxychromone

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Kinetics and mechanisms of the reactions of naringenin, hesperetin, 3-hydroxychromone and 3-hydroxyflavone with a pseudo first order excess of iron(III) have been investigated in aqueous solution at 25°C and an ionic strength of 0.5 M. Mechanisms have been proposed which account satisfactorily for the kinetic data. These are consistent with a mechanism in which a 1 : 1 metal : ligand complex is formed in each case. For naringenin and hesperetin, the main reaction path involves reaction of the ligand with the monohydroxy species $Fe(OH)^{2+}$, however the iron hydroxo dimer $Fe_2(OH)_2^{4+}$ proved to be most reactive in the reactions with 3-hydroxyflavone and 3-hydroxychromone. The 1 : 1 iron(III) : naringenin and iron(III) : hesperetin complexes did not undergo subsequent electron-transfer based decomposition while both the iron(III) : 3-hydroxyflavone and iron(III) : 3-hydroxychromone complexes decompose rather slowly in an electron-transfer step. The reactions were monitored using UV–visible spectrophotometry. The suggested mechanisms and calculated rate constants are supported by calculations carried out using global analysis of time dependant spectra.

Keywords: Kinetics; Mechanisms; Flavonoids; Electron transfer; Hydroxychromone; Iron

1. Introduction

Polyphenolic compounds are a topic of interest as a result of their numerous biological effects including radical scavenging and metal chelation. A correlation between high dietary intakes of phenolics and reduced risk of cardiovascular disease and cancer has been shown by numerous epidemiological studies [1–7]. Flavanones such as naringenin and hesperetin have a more restricted distribution than other flavonoids and are for the most part specific to citrus fruits. Naringenin is mostly found in grapefruit, however low concentrations are also found in tomatoes and tomato-based products. Hesperetin is commonly found in oranges, mandarins and all orange juices. Naringenin is known to reduce the extent of cisplatin induced nephrotoxicity in rats [8] and both naringenin and hesperetin exhibit estrogenic [9], anticarcinogenic [10–13] and antioxidative properties [14, 15].

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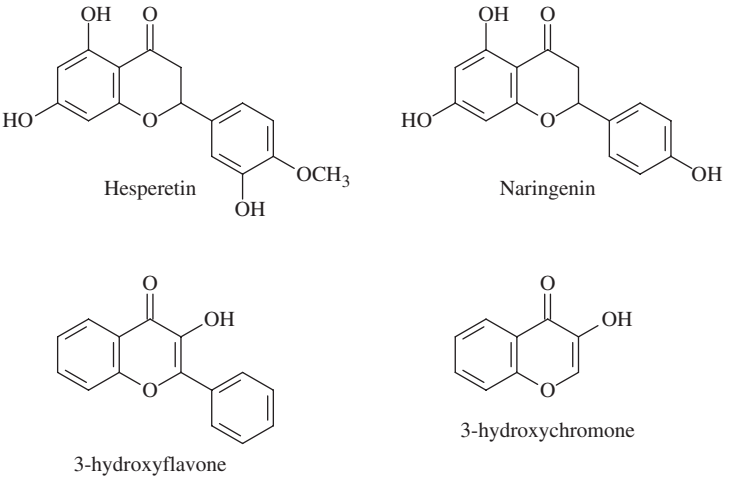

Figure 1. Ligand structures.

Previous studies have suggested that in acidic media the 3-hydroxychromone site possesses greater complexing power than either 5-hydroxychromone or catechol sites [16, 17]. The monotopic ligands used in this study were chosen to conclusively determine which hydroxychromone site is involved in iron binding reactions with polyphenols where two potential sites are available. The structures of the ligands investigated are shown in figure 1. Several studies of the kinetics and mechanisms of the interactions of naturally occurring catecholates with iron(III) have been reported, involving a variety of different mechanisms [18–28].

Reactions were studied in aqueous solution at 0.5 M $NaClO_4$ ionic strength and 25°C, in the pH range 1–3, similar to the pH found in the human stomach and in acid root soils where phenolic ligands have been known to increase the bioavailability of micronutrients. The pK_a of $[Fe(H_2O)_6]^{3+}$ to form the monohydroxy species is 2.77, thus in this pH range the two main iron(III) species present in aqueous solution are Fe^{3+} and $Fe(OH)^{2+}$ (water molecules are omitted). The solvent exchange rates on these two species are quite different with $[Fe(H_2O)_6]^{3+}$ having a water exchange rate of $160\,s^{-1}$ while that on $[Fe(H_2O)_5(OH)]^{2+}$ is $1.2 \times 10^5\,s^{-1}$ [29]. While $Fe[H_2O)_6]^{3+}$ is believed to react by an I_a mechanism with a $\Delta V^{\ddagger}$ of $-5.4\,cm^3\,M^{-1}$, $[Fe(H_2O)_5(OH)]^{2+}$ reacts by an I_d mechanism with $\Delta V^{\ddagger}$ of $+7\,cm^3\,M^{-1}$ [29]. However, when high concentrations of iron are used, consideration must also be given to the hydrolytic dimeric species $[Fe_2(OH)_2]^{4+}$ as it begins to form in the pH range of the present studies [30]; a solvent exchange rate is not available for this species. Lente and Fabian [31] have reported that the forward and reverse rate constants for the following reactions are $0.35\,s^{-1}$ and $1.3 \times 10^2\,M^{-1}\,s^{-1}$, respectively, while

$$Fe_2(OH)_2^{4+} = 2Fe(OH)^{2+}$$

Shi *et al.* [32] assert that the reactivity of the dimer is similar to that of $[Fe(H_2O)_5(OH)]^{2+}$.

Iron(III) reactions with 3-hydroxyflavone were studied in 70 : 30 vol/vol methanol–water due to the insolubility of the ligand in water. The value of the

hydrolysis constant of $[Fe(H_2O)_6]^{3+}$ to form the monohydroxy species is much greater ($\log K_h = -1.79 \pm 0.01$ [33]) in this solvent mixture. Thus at pHs greater than 1, the monohydroxy species $[Fe(H_2O)_5(OH)]^{2+}$ becomes an important reacting species. All other iron(III) reactions were studied in water.

2. Experimental

2.1. *Reagents*

Naringenin (Aldrich) ($C_{15}H_{12}O_5$), hesperetin (Sigma) ($C_{16}H_{14}O_6$) and 3-hydroxyflavone (Extrasynthese) ($C_{15}H_{10}O_3$) were of the highest quality available and used as supplied. 3-Hydroxychromone was synthesized from chromone (Aldrich) using niobium pentachloride (Aldrich) [34].

Iron(III) solutions were prepared from reagent grade iron(III) nitrate nonahydrate, $Fe(NO_3)_3 \cdot 9H_2O$ (Merck) and standardized by titration with EDTA using variamine blue indicator. Solutions of the required final pH were made up from deoxygenated stock solutions of the ligands and $Fe(NO_3)_3 \cdot 9H_2O$ using perchloric acid (BDH) as the source of hydrogen ions. All solutions were adjusted to an ionic strength of 0.5 M with sodium perchlorate (Aldrich). 3-Hydroxyflavone had very low solubility in water, so solutions of this ligand were prepared using 70 : 30 methanol : water vol/vol.

2.2. *Instrumentation*

An AGB 3000 pH meter equipped with an AMAGRUSS Refex combination electrode was used to measure pH. The filling solution in the reference compartment was 3 M sodium chloride. The linearity of the electrode was established using buffers of pH 4.0 and 7.0. The electrode was calibrated to read hydrogen ion concentration by titrating solutions of perchloric acid (0.001–0.002 M) with standard sodium hydroxide solutions [35]. Titrations were carried out in a jacketed titration vessel through which water at 25°C was circulating. Endpoints were determined using the method of Johansson [36, 37].

Kinetic data for faster reactions were acquired with a HI–TECH SF–20 stopped flow apparatus interfaced to a PC via an ADC–216 16-bit (1 in 65536 or 1.5×10^{-3}% resolution) digital oscilloscope (PICO Technology Limited) and a custom Windows interface [38]. This allowed up to 1,000 data points to be recorded for each kinetic run. A xenon lamp was used as light source in the 250–800 nm region. The reactant reservoirs and the mixing chamber were thermostated at 25°C. Pseudo first order rate constants were calculated from the experimental data using the OLIS KINFIT routines [39]. The reported rate constants are the average of at least three determinations. All reactions were studied under conditions where the metal was in large excess and so the presence of oligomeric iron(III) hydrolytic species was taken into account. The lowest pK_a value for all of the ligands investigated was sufficiently high as to allow the involvement of any deprotonated ligand species to be neglected.

UV/visible spectral data and kinetic runs for the slowest reactions were obtained using a HP 8453A diode array spectrophotometer equipped with a HI–TECH SFA-20

rapid kinetics stopped flow accessory. All spectra and kinetic runs were recorded at 25°C. Wavelengths which afforded large absorbance changes were selected for the single-wavelength kinetic investigations.

2.3. *Global kinetic analysis*

Spectral-kinetic data were processed as described previously [28] by using SPECFIT/32^{TM} (Spectrum Software Associates, Chapel Hill, North Carolina), which is based on the published works of Zuberbühler *et al.* [40–43].

A non-linear least squares curve fitting routine based on the subroutine NIHH23, written by J.E. Fletcher and R.I. Shrager at the Division of Computer Research and Technology, National Institute of Health, Bethesda, MD, USA, was utilized [44] to fit the kinetic data to the proposed mechanisms. During the fitting procedure, the individual concentrations of the iron(III) species present, Fe^{3+}, $Fe(OH)^{2+}$ and $[Fe_2(OH)_2]^{4+}$ were calculated at each data point. Initial modelling runs included all possible iron(III) species; those making no significant contributions to the overall reaction were then identified and eliminated from the model in a stepwise fashion.

2.4. *Bipyridyl tests*

The fact that Fe^{2+} reacts with 2,2′–bipyridyl to give a highly colored red $[Fe^{II}(bipy)_3]^{2+}$ product can be used to determine the number of iron(III) atoms reduced per mol of ligand as previously described [27]. The molar absorptivity of $[Fe^{II}(bipy)_3]^{2+}$ was determined by addition of an excess of bipy to a known concentration of Fe^{2+} and was found to be 8,200 $M^{-1}\,cm^{-1}$. The number of Fe^{2+} ions produced in each solution was calculated from equation (1),

$$\text{No. of } Fe^{2+} \text{ ions produced} = \frac{A}{\varepsilon bc} \tag{1}$$

where A is the absorbance obtained when an excess of bipy is added to the reduced solution, ε is the molar absorptivity of $[Fe^{II}(bipy)_3]^{2+}$, b is the pathlength (1 cm) and c is the phenol concentration. The number of Fe^{2+} ions produced is indicative of the number of electrons transferred and hence the number of electron-transfer reactions [25].

3. Results

For all four ligands investigated, reaction of a solution of the ligand with an excess of iron(III) resulted in formation of a 1 : 1 metal : ligand complex. For both 3-hydroxyflavone and 3-hydroxychromone electron-transfer based decomposition of the complexes followed, resulting in the formation of the corresponding benzoquinone type products [18–23, 25–28]. The iron(III) complexes of naringenin and hesperetin showed no evidence of electron-transfer based decomposition over a period of 30 min.

$$Fe^{3+} + H_2O \overset{K^{MOH}}{\rightleftharpoons} Fe(OH)^{2+} + H_3O^+$$

$$2Fe(OH)^{2+} \overset{K^{D}}{\rightleftharpoons} Fe_2(OH)_2{}^{4+}$$

$$Fe(OH)^{2+} + HL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Fe(L)^{2+} + H_2O$$

$$Fe^{3+} + HL \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} Fe(L)^{2+} + H_3O^+$$

Scheme 1. Mechanism for reaction of iron(III) with naringenin and hesperetin.

3.1. *The complex formation reactions of iron(III) with naringenin and hesperetin*

Since both hesperetin and naringenin possess the same single 5-hydroxychromone binding site it was expected that they would interact similarly with iron(III); this indeed proved to be the case as is evident from the kinetic data. When reacted with a pseudo-first order excess of iron(III), an absorbance increase was observed in both cases, ascribed to formation of a 1 : 1 iron(III) : ligand complex. The reactions were monitored at 500 nm by single wavelength spectrophotometry and over a range of wavelengths by diode array spectrophotometry. Since only a single reaction was observed, electron-transfer based decomposition of the complexes was absent. This was confirmed by the bipy test which showed no evidence for iron(II).

The presence of a single 5-hydroxychromone site on these ligands greatly simplifies interpretation of the kinetic data. The kinetic traces at 500 nm show good fits to a single exponential indicating that simple first order kinetics are operative. Scheme 1 was found to best describe the kinetic data for the reaction of iron(III) with both ligands. For the mechanism outlined in scheme 1, k_{obs} for the formation of the iron(III) ligand complex has the form of equation (2).

$$k_{obs} = k_2[Fe(OH)^{2+}] + k_3[Fe^{3+}] + k_{-2} + k_{-3}[H^+] \quad (2)$$

Fitting the kinetic data to equation (2) showed that the k_3 reaction pathway does not contribute significantly to complex formation and so can be excluded from consideration. The k_{-2} reverse reaction can also be excluded as its contribution is negligible, thus reducing equation (2) to equation (3) which accounts well for the kinetic data, giving values of 711(±8.9) and 5.54(±0.8) $M^{-1}s^{-1}$ for k_2 and k_{-3}, respectively.

$$k_{obs} = k_2[Fe(OH)^{2+}] + k_{-3}[H^+] \quad (3)$$

Plots of the kinetic data for complex formation are shown in figure 2. A similar approach yielded values of 732(±9) and 4.4(±0.9) $M^{-1}s^{-1}$ for k_2 and k_{-3}, respectively, when the iron(III) – hesperetin kinetic data were fitted to equation (3). The kinetic data are plotted in figure 3. Not unexpectedly, these results are very similar to those obtained from the iron(III) naringenin data.

The iron(III) complexes formed contain stable six-membered chelate rings and do not show any evidence for subsequent electron-transfer reactions of the type observed for

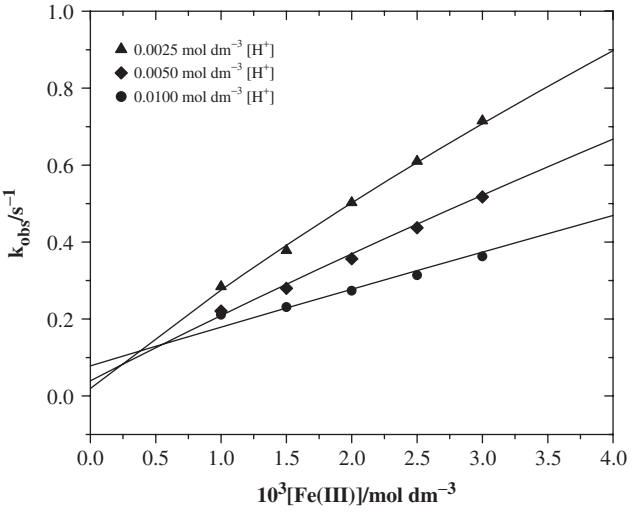

Figure 2. Plot of the kinetic data for complex formation of iron(III) with naringenin at 25°C, I = 0.5 M $NaClO_4$, $[Naringenin]_{total} = 1.0 \times 10^{-4}$ M and $\lambda = 500$ nm. Solid lines were calculated on the basis of equation (3). ▲, $[H^+] = 0.0025$ M; ◆, $[H^+] = 0.005$ M; ●, $[H^+] = 0.010$ M.

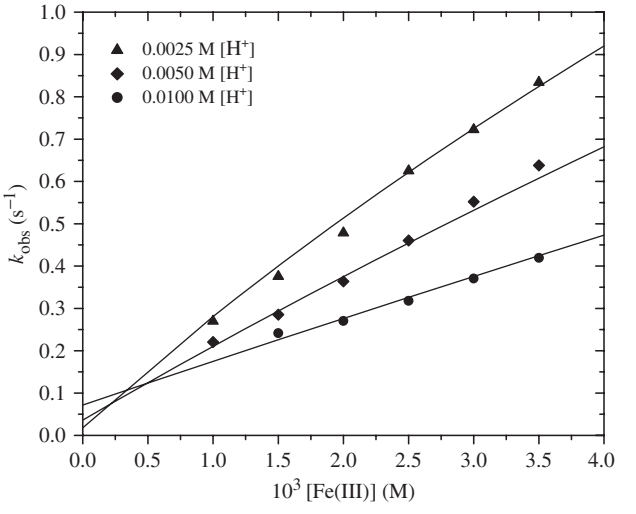

Figure 3. Plot of the kinetic data for complex formation of iron(III) with hesperetin at 25°C, I = 0.5 M $NaClO_4$, $[Hesperetin]_{total} = 1.0 \times 10^{-4}$ M and $\lambda = 500$ nm. Solid lines were calculated on the basis of equation (3). ▲, $[H^+] = 0.0025$ M; ◆, $[H^+] = 0.005$ M; ●, $[H^+] = 0.010$ M.

other iron(III) complexes with ligands containing phenol groups [27, 28]. Thus, it can be concluded that redox active metals such as iron(III) bound to the 5-hydroxychromone site do not undergo electron transfer subsequent to iron(III) binding under the present conditions of concentration and pH. Naringenin and hesperetin may be efficient scavengers of free iron(III), but, unlike other complexes with phenol-based ligands, the iron(III) is not reduced.

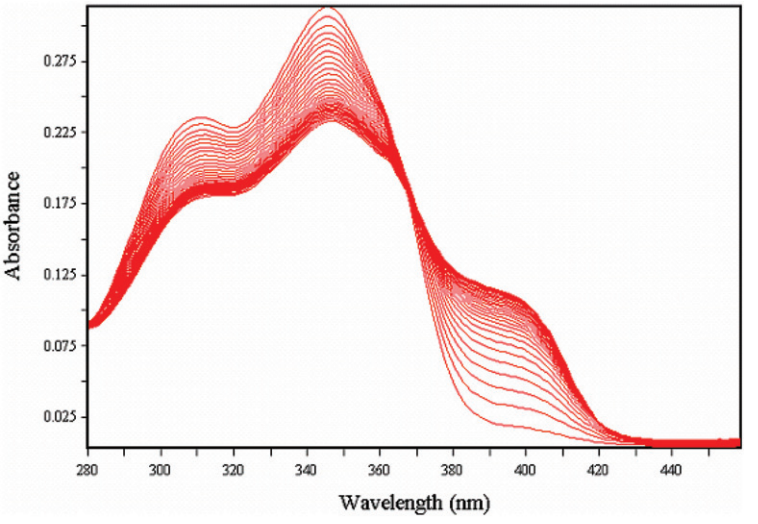

Figure 4. Two-dimensional time-dependent spectra for complex formation of iron(III) with 3-hydroxyflavone at 25°C, $I = 0.5$ M $NaClO_4$, pH = 2.00, $\Delta T = 60$ s, $[Fe(III)]_{total} = 1.0 \times 10^{-4}$ M and $[3\text{-hydroxyflavone}]_{total} = 1.0 \times 10^{-4}$ M.

3.2. *The complex formation reaction of iron(III) with 3-hydroxyflavone*

Given that the ligand possesses a single binding site, reaction of a pseudo first order excess of iron(III) with 3-hydroxyflavone involved a single complex formation reaction. Under conditions of pseudo first order excess of iron(III) the complex formation reaction proved too fast to be monitored by methods other than single wavelength stopped-flow spectrophotometry. A 1 : 1 iron(III) : ligand ratio was therefore used to allow the spectral data to be subjected to global analysis. Spectral data for this reaction are shown in figure 4.

A single isosbestic point at 370 nm suggests the presence of only one reaction during the complex formation time domain. A typical stopped-flow trace for the reaction is shown in figure 5. The trace features a rapid absorbance decrease (~0.1 s) prior to the increase ascribed to complex formation. This initial decrease is more apparent at low $[H^+]$ and high iron(III) concentrations and consequently can only be interpreted as the disappearance of $[Fe_2(OH)_2]^{4+}$ since redox reactions at this stage of the reaction can be excluded. Mixing of the reactants in the stopped-flow apparatus will result in a decrease in the concentration of $[Fe_2(OH)_2]^{4+}$. As outlined by Lente, this will only be apparent if the reaction is being monitored at a wavelength at which the dimer absorbs [45]. The trace fits well to a two exponential equation. The rate constant for the absorbance increase was ascribed to complex formation. The kinetic data can be described by scheme 2.

For the mechanism in scheme 2, k_{obs} for the reaction of iron(III) with 3-hydroxyflavone has the form of equation (4).

$$k_{obs} = k_1\left[Fe_2(OH)_2^{4+}\right] + k_2[Fe(OH)^{2+}] + k_3[Fe^{3+}] + k_{-1}[Fe(OH)^{2+}] + k_{-2} + k_{-3}[H^+] \quad (4)$$

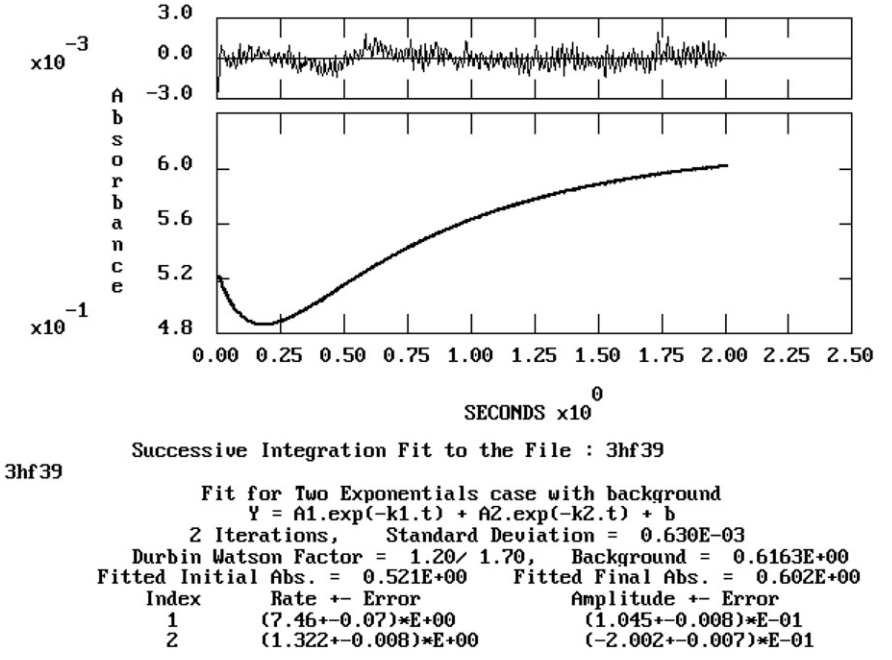

Figure 5. Stopped-flow trace of the reaction of 1.5×10^{-3} M iron(III) with 5.0×10^{-5} M 3-hydroxyflavone in aqueous solution at 25°C, $I = 0.5$ M $NaClO_4$, pH = 2.00 and $\lambda = 420$ nm.

$$Fe^{3+} + H_2O \underset{}{\overset{K^{MOH}}{\rightleftharpoons}} Fe(OH)^{2+} + H_3O^+$$

$$2Fe(OH)^{2+} \overset{K^{D}}{\rightleftharpoons} Fe_2(OH)_2^{4+}$$

$$Fe_2(OH)_2^{4+} + HL \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Fe(L)^{2+} + Fe(OH)^{2+} + H_2O$$

$$Fe(OH)^{2+} + HL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Fe(L)^{2+} + H_2O$$

$$Fe^{3+} + HL \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} Fe(L)^{2+} + H_3O^+$$

Scheme 2. Mechanism of reaction of iron(III) with 3-hydroxyflavone.

The absence of a hydrogen ion dependent intercept in figure 6 indicates that the k_{-1} and k_{-3} reverse reactions can be excluded as they do not contribute significantly to k_{obs} and as a result, equation (4) can be reduced to equation (5) which accounts satisfactorily for the kinetic data.

$$k_{obs} = k_1\left[Fe_2(OH)_2^{4+}\right] + k_2[Fe(OH)^{2+}] + k_3[Fe^{3+}] + k_{-2} \quad (5)$$

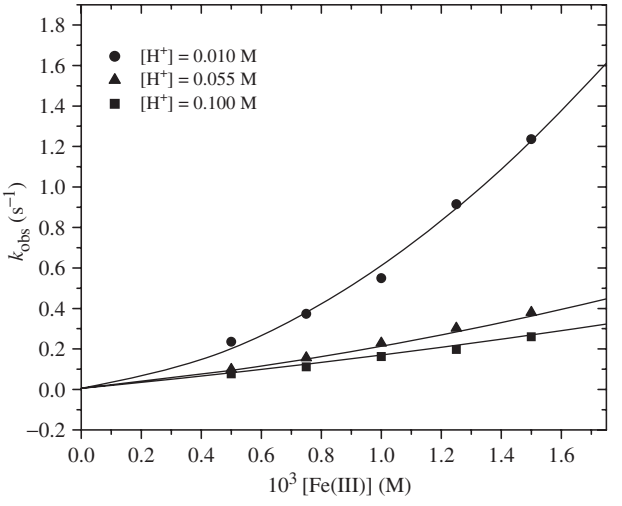

Figure 6. Plot of the kinetic data for complex formation of iron(III) with 3-hydroxyflavone at 25°C, $I = 0.5$ M $NaClO_4$, $[3\text{-hydroxyflavone}]_{total} = 5.0 \times 10^{-5}$ M and $\lambda = 420$ nm. Solid lines were calculated on the basis of equation (5). ●, $[H^+] = 0.010$; ◆, $[H^+] = 0.055$ M; ■, $[H^+] = 0.100$ M.

Fitting the kinetic data to equation (5) yields values of $3.4(\pm0.3) \times 10^4$, $177(\pm90)$, $140(\pm20)\,M^{-1}\,s^{-1}$ and $6.0(\pm0.2) \times 10^{-3}\,s^{-1}$ for k_1, k_2, k_3 and k_{-2}, respectively. A plot of the kinetic data together with the fitted data is shown in figure 6. There is a large contribution from the dimer in the reaction as evident from the rate constants; the $Fe(OH)^{2+}$ contribution is significantly reduced as a result.

Arising from proton ambiguity, the kinetic data may also be described by an alternative mechanism in which the dimer $\left[Fe_2(OH)_2^{4+}\right]$ pathway is replaced by a $[Fe(OH)^{2+}]^2$ pathway. This mechanism was found to describe the data as effectively as that shown in scheme 2 giving values of $200(\pm100)$, $130(\pm20)\,M^{-1}\,s^{-1}$ and $7.8(\pm0.2) \times 10^{-3}\,s^{-1}$ for k_2, k_3 and k_{-2}, respectively. The rate constant for the $[Fe(OH)^{2+}]^2$ pathway was found to be $1.3(\pm0.1) \times 10^6\,dm^6\,mol^{-2}\,s^{-1}$.

A previous study [28] of the reactions of iron(III) with quercetin and morin has shown that when both 3-hydroxychromone and 5-hydroxychromone sites are in competition a second-order iron(III) effect is observed, which may be ascribed to a contribution from the $\left[Fe_2(OH)_2^{4+}\right]$ dimer. Thus, it can be reasonably assumed that when the two sites are in competition on the same ligand, the iron(III) is bound at the 3-hydroxychromone site as the same second-order dependency on iron(III) concentration observed only when the 3-hydroxychromone site is present. Further evidence for this comes from the occurrence of an electron-transfer step at the 3-hydroxychromone site and the absence of one at the 5-hydroxychromone site.

Absorbance decreases in the spectral data for the reaction of iron(III) with 3-hydroxyflavone together with a positive result for the presence of iron(II) using the bipy test provide conclusive evidence for the occurrence of an electron-transfer based decomposition of the iron(III) 3-hydroxyflavone complex. A single wavelength trace at 400 nm is shown in figure 7. The trace shown illustrates the evolution of two distinct reactions. The initial absorbance increase ascribed to complex formation is followed by a significantly slower absorbance decrease ascribed to decomposition of the complex in an electron-transfer step.

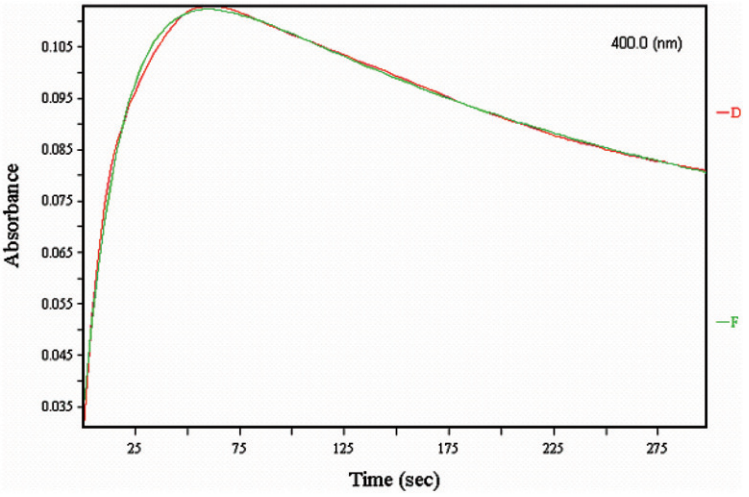

Figure 7. Single wavelength SPECFIT/32^{TM} derived trace of the reaction of iron(III) with 3-hydroxyflavone at 25°C, $I = 0.5$ M $NaClO_4$, $\Delta T = 250$ s, $[Fe(III)]_{total} = 1.0 \times 10^{-3}$ M and $[3\text{-hydroxyflavone}]_{total} = 1.0 \times 10^{-4}$ M. D, experimental data; F, fitted data.

Kinetic data for the electron-transfer step proved unreliable giving irreproducible rate constants. The reaction proceeds slowly even at low $[H^+]$ and high iron(III) concentrations. Global analysis using SPECFIT/32^{TM} also failed to resolve the kinetic data in a meaningful manner and the details of the kinetic data are not reported here. The complicated nature of the electron-transfer kinetic data is probably due to the slowness of the electron-transfer reaction, accompanied by subsequent oxidation reactions [26] that take place following the initial electron transfer from the ligand.

3.3. *The reactions of iron(III) with 3-hydroxychromone*

As with 3-hydroxyflavone, the reaction of a pseudo first order excess of iron(III) with 3-hydroxychromone leads to formation of a 1 : 1 metal : ligand complex which subsequently decomposes in a slow electron-transfer step. A fast absorbance increase followed by a slower decrease at 350 nm demonstrates the presence of two distinct reactions. Figure 8 demonstrates that when the data are fitted to a two exponential equation there is good agreement between the experimental (D) and the fitted data (F). It can therefore be concluded that two separate reactions are indeed present.

The initial absorbance decrease (~0.1 s) observed in the iron(III) 3-hydroxyflavone reaction traces is evident here and is again ascribed to disappearance of the dimer, which absorbs in this region [45]. A two exponential equation was therefore used to fit the kinetic data and the rate constant for the absorbance increase was ascribed to complex formation. The kinetic complex formation data can be represented by scheme 3 and equation (6).

$$k_{obs} = k_1\left[Fe_2(OH)_2^{4+}\right] + k_2[Fe(OH)^{2+}] + k_{-1}[Fe(OH)^{2+}] + k_{-2} \quad (6)$$

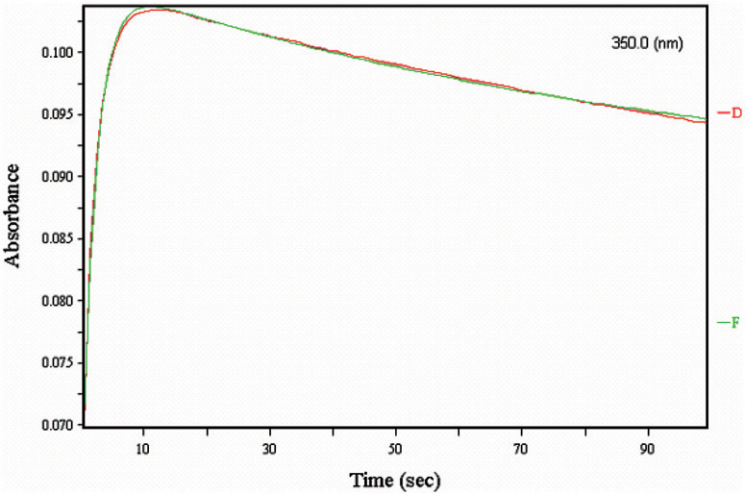

Figure 8. Single wavelength SPECFIT/32^{TM} derived trace for the reaction of iron(III) with 3-hydroxychromone at 25°C, $I = 0.5\,M$ $NaClO_4$, $\Delta T = 100\,s$, $[Fe(III)]_{total} = 1.0 \times 10^{-3}\,M$ and $[3\text{-hydroxychromone}]_{total} = 1.0 \times 10^{-4}\,M$. D, experimental data; F, fitted data.

$$Fe^{3+} + H_2O \underset{}{\overset{K^{MOH}}{\rightleftharpoons}} Fe(OH)^{2+} + H_3O^+$$

$$2Fe(OH)^{2+} \overset{K^{D}}{\rightleftharpoons} Fe_2(OH)_2^{4+}$$

$$Fe_2(OH)_2^{4+} + HL \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Fe(L)^{2+} + Fe(OH)^{2+} + H_2O$$

$$Fe(OH)^{2+} + HL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Fe(L)^{2+} + H_2O$$

Scheme 3. Mechanism for reaction of iron(III) with 3-hydroxychromone.

The absence of a significant increase in the absorbance change in the kinetic runs as the Fe(III) concentration increased suggests that the reaction goes to completion. Therefore, the intercept seen in figure 9 cannot be associated with the reverse reactions (k_{-1} and k_{-2}) and the reverse reactions can be excluded reducing equation (6) to equation (7).

$$k_{obs} = k_1[Fe_2(OH)_2^{4+}] + k_2[Fe(OH)^{2+}] + \text{constant} \tag{7}$$

The data were fitted to equation (7) giving values of 3300(±800) $M^{-1}\,s^{-1}$, 1600(±100) $dm^3\,mol^{-1}\,s^{-1}$ and 0.28(±0.01) s^{-1} for k_1, k_2 and the constant, respectively. The constant in equation (7) is representative of the intercept in figure 9. In this instance, due to the fact that the reactions were monitored at 350 nm where $Fe_2(OH)_2^{4+}$ has appreciable absorption, the intercept is attributed to dissociation of this species when the reactant solutions are mixed in the stopped flow apparatus. The value obtained, 0.28 s^{-1}, compares well with the reported dimer dissociation value of 0.35 s^{-1} [31].

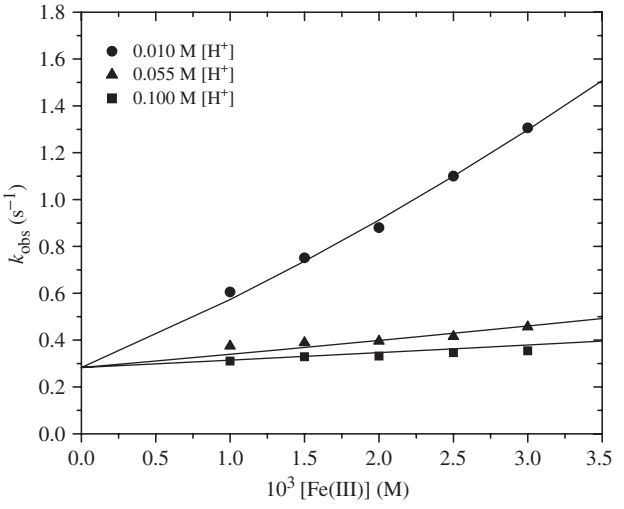

Figure 9. Plot of the kinetic data for complex formation of iron(III) with 3-hydroxychromone at 25°C, $I = 0.5\,M$ $NaClO_4$, $[3\text{-hydroxychromone}]_{total} = 1.0 \times 10^{-4}\,M$ and $\lambda = 350\,nm$. Solid lines were calculated on the basis of equation (7). ●, $[H^+] = 0.010$; ◆, $[H^+] = 0.055\,M$; ■, $[H^+] = 0.100\,M$.

It is important to note that an alternative complex formation mechanism exists in which the dimer pathway can be replaced by a $[Fe(OH)^{2+}]^2$ pathway. This mechanism was found to describe the data as effectively as that shown in scheme 3 giving values of $k_2 = 1600(\pm 100)\,M^{-1}\,s^{-1}$ and $0.28(\pm 0.01)\,s^{-1}$ for the constant. The rate constant for the $[Fe(OH)^{2+}]^2$ pathway was found to be $1.3(\pm 0.1) \times 10^6\,M^{-2}\,s^{-1}$.

Data for the electron transfer based decomposition reaction are not reported as they proved irreproducible and unreliable, as was the case for the analogous 3-hydroxyflavone electron-transfer reaction.

4. Discussion

The goal of this study was to investigate the reactions of iron(III) with a number of flavonoid ligands and to identify differences in the interactions of iron(III) with 3-hydroxychromone and 5-hydroxychromone sites. This information was necessary to determine which of the aforementioned sites preferentially binds iron(III) when both are in competition, as is the case for many polyphenolic ligands such as quercetin and morin [28]. The flavanone ligands, naringenin and hesperetin, each possess a single 5-hydroxychromone metal-binding site while both 3-hydroxyflavone and 3-hydroxychromone possess a single 3-hydroxychromone site. Therefore, the ligands selected were ideal for the present investigation.

When naringenin and hesperetin reacted with a pseudo first order excess of iron(III), 1 : 1 metal : ligand complexes were formed with no subsequent decomposition. The main contributing species to the complex formation was $Fe(OH)^{2+}$. When reacted with iron(III), 3-hydroxyflavone and 3-hydroxychromone also formed 1 : 1 metal : ligand complexes. However, subsequent decomposition of the complexes occurs through

Table 1. Summary of the complex formation rate constants of naringenin, hesperetin, 3-hydroxyflavone (3-HF) and 3-hydroxychromone (3-HC). $k_1 = Fe_2(OH)_2^{4+}$ pathway; $k_2 = Fe(OH)^{2+}$ pathway; $k_3 = Fe^{3+}$ pathway.

Rate constant ($M^{-1}s^{-1}$)	Naringenin	Hesperetin	3-HF	3-HC
k_1	n/a	n/a	$3.4(\pm0.3) \times 10^4$	3200(±800)
k_2	711(±9)	732(±9)	180(±90)	1600(±100)
k_3	n/a	n/a	140(±20)	n/a

a relatively slow electron transfer, confirmed by bipy experiments. A second-order iron(III) effect was found to be present in complex formation with the main contributing species to the reaction being the iron hydroxo dimer $[Fe_2(OH)_2]^{4+}$. The complex formation reaction rate constants obtained are summarized in table 1.

The rate constants for the reaction of iron(III) with naringenin and hesperetin are almost identical as expected, considering their structural similarities. Rate constants for the reaction of iron(III) with 3-hydroxyflavone and 3-hydroxychromone would also be expected to be relatively similar, however they are quite different, as can be seen from table 1. Dimer and Fe^{3+} contributions are important for 3-hydroxyflavone while the 3-hydroxychromone reaction shows increased contribution from the $Fe(OH)^{2+}$ pathway. This may arise from solvent factors since the 3-hydroxyflavone reactions were carried out in 70 : 30 methanol : water, while the 3-hydroxychromone reactions were carried out in pure water. Although there are a number of differences in rate constants between the iron(III) 3-hydroxyflavone and 3-hydroxychromone reactions, both reactions involve a significant contribution from the iron hydroxo dimer species $[Fe_2(OH)_2]^{4+}$.

Previous investigations have shown that reactions of iron(III) with quercetin and morin involve a major contribution from the iron dimer [28]. With both ligands, the complexes formed have been shown to undergo electron transfer subsequent to the formation of an iron(III) complex under the conditions used. In the present investigation, the iron(III) bound at the 5-hydroxychromone sites in hesperetin and naringenin did not undergo a subsequent electron transfer. Given the results obtained with quercetin and morin [28], this leads one to conclude that the 3-hydroxychromone site has greater chelating power and preferentially binds iron when in competition with the 5-hydroxychromone type site, in accord with previous suggestions [16, 17]. Demonstration that the 5-hydroxychromone site is redox inactive is important as is the sluggish nature of the electron transfer with 3-hydroxychromone compared to catechol sites.

For solvated metal ions that react by D or I_d mechanisms in accord with the Eigen–Wilkins mechanism, the rate of complex formation may be estimated using equation (8),

$$k_f = \left(\frac{3}{4}\right) K_o k_s \tag{8}$$

where k_f is the experimental rate constant for complex formation, K_o is the outer-sphere association constant, k_s is the rate of solvent exchange and 3/4 is a statistical factor that takes account of the fact that not all solvent exchanges lead to complex formation [46].

Table 2. O–O and O···H bond distances and charges in the ligands used in this investigation calculated at the B3LYP/6.31G* level using SPARTAN'06.

Ligand	O–O (Å)	O···H (Å)	Charge on O–H	Charge on =O
Salicylic acid	2.594 (2.590[a])	1.707 (1.69[a])	−0.552	−0.463
Naringenin	2.608	1.704	−0.552	−0.541
Hesperetin	2.609	1.707	−0.549	−0.533
3-Hydroxyflavone	2.596	1.939	−0.493	−0.486
3-Hydroxychromone	2.658	2.039	−0.485	−0.490

Note: [a]From crystal structure determination [50].

Arising from this, a factor R can be elaborated, equation (9), as an indicator of whether or not the reaction is

$$R = k_f \Big/ \left(\frac{3}{4} K_o k_s\right) \tag{9}$$

'normal'. For reactions in which solvent exchange is the rate determining step, R should be of the order of 1. Under the experimental conditions of the current work, all the ligands are neutral so that K_o is given by equation (10) [47],

$$K_o = 10^{-3}\left[\left(\frac{4}{3}\right)\pi a^3 N_A\right] \tag{10}$$

where a is the distance of closest approach of the reactants (m) and N_A is Avogadro's number (where a is 5 Å, K_o is approximately 0.3). Thus for reaction of a neutral ligand with $Fe(OH)^{2+}$, a 'normal' reaction should give a complex formation rate constant of $2.7 \times 10^4\,M^{-1}\,s^{-1}$. However, various factors can significantly reduce this. Perhaps the most important is intramolecular hydrogen bonding of the type encountered in ligands such as β-diketones [48]. In order to ascertain the degree of intramolecular hydrogen bonding in the ligands investigated here, we have carried out calculations at the B3LYP/6.31G* level using SPARTAN'06 [49], table 2. In order to validate the calculations, we included data for salicylic which is known to have strong intramolecular hydrogen bonding and for which a crystal structure determination is available [50]. It is clear that apart from 3-hydroxychromone, the reactive sites of all four ligands are strongly hydrogen bonded and, as a result, their reactions would be expected to be retarded. This results in R values significantly less than unity.

The four ligands can be divided into two classes, those where the donor atoms reside on adjacent rings and those where the donor atoms are on the same ring. Naringenin and hesperetin belong to the former and are clearly the most strongly hydrogen bonded. Given their structures and the data in table 2, it would be expected that they would react at approximately the same rate and that the reactions would be strongly retarded. This is indeed the case and the rate constants are of similar magnitude to those obtained for similar reactions with β-diketones [48]. As is frequently observed for complex formation reactions of iron(III) in aqueous solution, the reactive metal species in each case is $Fe(OH)^{2+}$.

Both the 3-hydroxyflavone and 3-hydroxychromone species exhibit high reactivity towards $[Fe_2(OH)_2]^{4+}$. Previous investigations in which the presence of this species

was inferred include its reactions with simple inorganic ligands [51] and 5-nitrotropolone [52]. The rate constant for its reaction with 5-nitrotropolone, $3 \times 10^4\,M^{-1}\,s^{-1}$, is very similar to that for its reaction with 3-hydroxyflavone. However, it is some ten times less reactive towards 3-hydroxychromone, and while the degree of hydrogen bonding in 3-hydroxychromone is less than in 3-hydroxyflavone, the O–O distance is greater in the former and indeed is also considerably larger than in salicylic acid. This may result in a slower rate of ring closure and a smaller overall rate constant.

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