

# Mechanistic studies on oxidation of L-ascorbic acid by an oxo-bridged diiron complex in aqueous acidic media†

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$[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})_2]^{4+}$  (**1**) (Fig. 1, phen = 1,10-phenanthroline) equilibrates with  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})(\text{OH})]^{3+}$  (**2**) and  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{OH})_2]^{2+}$  (**3**) in aqueous solution in the presence of excess phen, where no phen-releasing equilibria from **1**, **2** and **3** exist. **1** quantitatively oxidizes ascorbic acid ( $\text{H}_2\text{A}$ ) to dehydroascorbic acid ( $\text{A}$ ) in the pH range 3.00–5.50 in the presence of excess phen, which buffers the reaction within 0.05 pH units and ensures complete formation of end iron product ferriox,  $[\text{Fe}(\text{phen})_3]^{2+}$ . The reactive species are **1**, **2** and  $\text{HA}^-$  and the reaction proceeds through an initial 1 : 1 inner-sphere adduct formation between **1** and **2** with  $\text{HA}^-$ , followed by a rate limiting outer-sphere one electron one proton (electroprotic) transfer from a second  $\text{HA}^-$  to the ascorbate-unbound iron(III).

## Introduction

Binuclear oxo-bridged iron species have received much attention in bio-inorganic chemistry due to the wide occurrence of such unit(s) in different organisms and their performance of a range of biological activities such as the storage and transport of dioxygen (in hemerythrin,<sup>1</sup> Hr), hydroxylation of alkanes (in methylmonooxygenase,<sup>2</sup> MMOH), phosphate ester hydrolysis (in purpleacidphosphatase,<sup>3</sup> PAP) and DNA synthesis (in ribonucleotidoreductase,<sup>4</sup> RNR). A series of model systems<sup>5</sup> have also been developed in parallel to contribute better understanding of the mechanism of action of the bio-molecules and eventually to mimic their activities. Complexes capable of acting as functional model for the diiron non-heme proteins must have either a free coordination site or a ligand (unsaturated metal center), which can be replaced easily by an incoming substrate molecule.

Despite the fact that a handful of structural models for oxo-bridged diiron proteins have appeared in literature during the last two decades, reactivity<sup>6</sup> and mechanistic<sup>7</sup> studies of these model complexes are not so common. The title  $\mu$ -oxo diiron(III,III) complex,  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})_2](\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$ ,<sup>8</sup> (**1**) (Fig. 1) with two labile water ligands, is an excellent Raman spectroscopic model for the binuclear iron site in RNR and metHr (oxidized form of Hr).<sup>8</sup> The hydroxo-aquo diiron(III,III) base of (**1**),  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})(\text{OH})]^{3+}$ , (**2**) is a possible functional model of PAP.<sup>9</sup> The diiron complex (**1**) is freely soluble in water, retains its dinuclearity in aqueous solution and is stable against self-decomposition in the acidity range pH 3.0–7.0 in presence of added phen. The title complex **1** thus meets several criteria for examining its mechanistic pathways of electron transfer.

Aqueous solutions of ascorbic acid have been widely used by chemists because of the convenience of ascorbate as a reducing

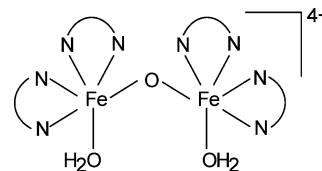


Fig. 1 Schematic drawing of  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})_2]^{4+}$ . The N–N ligand is 1,10-phenanthroline.

agent. Ascorbic acid, a weak dibasic acid in aqueous solution, has a rich redox chemistry and simultaneous proton transfer reactions in ascorbate redox introduces an additional subtlety.<sup>10</sup> The kinetics of ascorbate reductions for a wide range of metal complexes are available, where particular attention has been paid to outer-sphere processes because they are most commonly observed.<sup>11</sup> In some of its reactions, ascorbate binds with the metal centres prior to electron transfer,<sup>12</sup> when the metal centres allow rapid substitution of ligands like the water molecules in **1**. The title complex is not a powerful oxidant ( $E_{\text{red}}^\circ$  for  $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}} = -0.18 \text{ V}$  versus NHE),<sup>7c</sup> yet it rapidly oxidises ascorbic acid. To examine if the increased basicity of the  $\mu$ -oxo bridge of the reduced **1** is responsible for simultaneous electron proton transfer that could reduce the activation barriers<sup>5a,13</sup> for the apparently unfavourable oxidation of ascorbic acid ( $E_{\text{red}}^\circ$  for  $\text{HA}^\bullet/\text{HA}^- = 0.70 \text{ V}$  and that for  $(\text{A} + 2\text{H}^+)/\text{H}_2\text{A} = 0.40 \text{ V}$  (both versus NHE;  $\text{H}_2\text{A}$  = ascorbic acid,  $\text{HA}^-$  = ascorbate mono anion,  $\text{A}$  = dehydroascorbic acid)<sup>10,14</sup> is an obvious question. We also note that the mechanistic aspects of mononuclear iron(III) (aquated<sup>15</sup> or complexed<sup>16</sup>) oxidation of L-ascorbic acid are of considerable interest to chemists due to their mechanistic versatility.<sup>17</sup> How dimerisation affects the mechanistic features of monomeric metal complexes is also a potential interest of this study.

The intricate interaction between iron and ascorbic acid in food or in digestive systems have been of considerable significance for investigation because it directly imparts on the availability and utilization of these nutrients in human and/or animal bodies.<sup>18</sup> It is also known that ascorbic acid significantly enhances the iron uptake in the intestine.<sup>19</sup> The dinuclear iron(III,III) ascorbate

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redox may thus have a potential biochemical significance and this study, to the best of our knowledge, is the first of its kind describing the reduction of an oxo-bridged diiron(III,III) complex by ascorbic acid.

## Experimental

### Materials

All solutions were prepared in doubly distilled, freshly boiled water. Crystals of the title complex  $[\text{Fe}_2(\mu\text{-O})(\text{phen})_4(\text{H}_2\text{O})_2](\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$  were prepared using a reported method<sup>8</sup> and gave satisfactory CHN analyses and identical optical spectra as reported.<sup>8</sup> Stock solutions of L-Ascorbic acid (G. R., E. Merck) of known concentrations were prepared by accurately weighing out the required amount and dissolving this quickly in deoxygenated water. Fresh solutions were prepared prior to each experiment and diluted as per need. Solutions of recrystallised  $\text{NaNO}_3$  (G. R., E. Merck) and  $\text{NaClO}_4$  (G. R., E. Merck) were standardised as described earlier.<sup>20</sup> 1,10-phenanthroline (G. R., E. Merck) and  $\text{D}_2\text{O}$  (Aldrich, 99.9 atom% D) were used as received. All other chemicals were of reagent grade. Chromium(II) scrubbed dinitrogen gas was used for deoxygenating the reacting solutions while studying the effect of dissolved oxygen on the reaction rate. All the reported kinetic and equilibrium data are at  $25.0 (\pm 0.1)^\circ\text{C}$  and at  $I = 1.0 \text{ M}$  maintained by  $\text{NaNO}_3$ .

### Physical measurements and kinetics

The kinetics were monitored using a Biologic SFM-3/QS Stopped-flow spectrophotometer with a DT-2801 data transmission system at 510 nm—the visible absorption maximum of the product iron species,  $[\text{Fe}(\text{phen})_3]^{2+}$ .<sup>21</sup> Spectrophotometric titration and spectral observations were recorded with a Shimadzu (1601 PC) spectrophotometer using 1 cm quartz cells in the electrically-controlled thermostatted ( $25.0 \pm 0.1$ )  $^\circ\text{C}$  cell housing (CPS-240A). Solution pH values (3.00–5.50) were measured with an Orion pH meter (model 710 A) with calibrated electrodes as described earlier.<sup>22</sup> The relation,  $\text{pD} = \text{pH}_{\text{measured}} + 0.40$ <sup>23</sup> was used while working in  $\text{D}_2\text{O}$  media, where  $\text{pH}_{\text{measured}}$  is the pH meter reading in  $\text{D}_2\text{O}$ . Excess (at least ten-fold) reducing agent ( $[\text{H}_2\text{A}]_{\text{T}} = [\text{H}_2\text{A}] + [\text{HA}^-]$ ), 0.50–5.0 mM and 1,10-phenanthroline ( $C_{\text{phen}} = [\text{Hphen}^+] + [\text{phen}]$ ), 3–10 mM were used over the complex concentration (mostly 0.05 mM) in all the kinetic runs.

### Equilibrium studies

The acid dissociation constant of ascorbic acid was determined by pH-metric titration using a Metrohm (736 GP Titrino) autotitrator in 95%  $\text{D}_2\text{O}$  media as described earlier.<sup>7c</sup>

### Stoichiometry and reaction products

The stoichiometry of the overall reaction was determined by spectrophotometric titration (Fig. 2) at 510 nm with 0.05 mM complex solution and varying concentrations of ascorbic acid under the same conditions under which kinetics were studied.

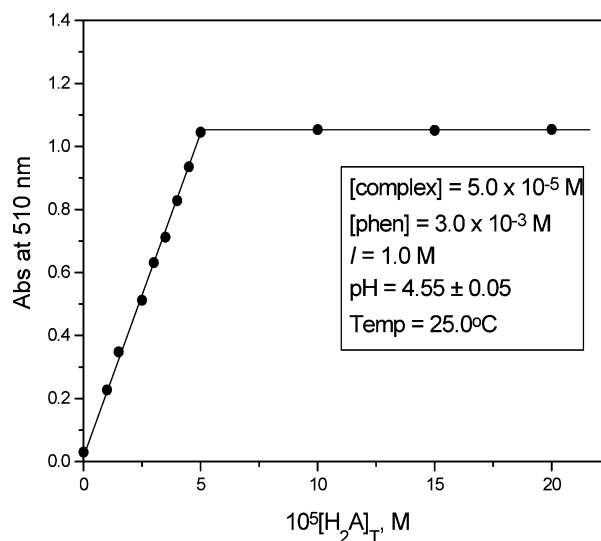
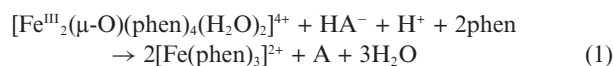


Fig. 2 Plot of absorbance versus  $[\text{H}_2\text{A}]_{\text{T}}$  during spectrophotometric titration at 510 nm with varying  $[\text{H}_2\text{A}]_{\text{T}}$ .

## Results and discussion

### Stoichiometry and reaction products

Spectrophotometric titration (Fig. 2) showed that the stoichiometry of the title reaction obeys eqn. (1) where  $\text{HA}^-$  is the ascorbate mono anion and A is the dehydroascorbic acid.



The UV-vis spectra of the product solution confirmed quantitative ( $99 \pm 2$ )% formation of  $[\text{Fe}(\text{phen})_3]^{2+}$  as the only iron end-product with absorption maxima at 510 nm ( $\epsilon = 1.11 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ).

Although the dominance of ascorbate redox results in the formation of dehydroascorbic acid, in certain situations oxalate, tryhydroxy butyric acid or even carbon dioxide are formed as the final oxidation products.<sup>24</sup> The present study conclusively shows that the oxidation of ascorbic acid by the title oxidant leads to the formation of dehydroascorbic acid as the sole oxidation product. The title complex, an overall two-electron oxidant, oxidises ascorbic acid through a free radical mechanism (*vide infra*). No oxalate could be detected in the product solutions (by  $\text{CaCl}_2$  at  $\text{pH} \sim 4$ ), which further supports the non-occurrence of a single two-electron transfer in the title redox.

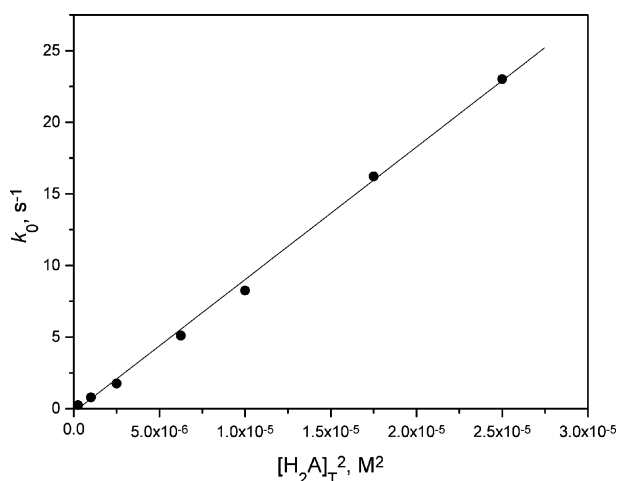
### Equilibrium studies

The acid dissociation constant of ascorbic acid in 95%  $\text{D}_2\text{O}$  media was found to be  $4.28 \pm 0.10$  at  $25.0^\circ\text{C}$ ,  $I = 1.0 \text{ M}$  while the reported value in  $\text{H}_2\text{O}$  media is  $4.03$ .<sup>25</sup>

### Kinetics

An isosbestic point (at  $\sim 388 \text{ nm}$ ) exists among the family of spectra (Fig. 3) of the equilibrium mixtures of the title complex with several stoichiometrically deficient amounts of ascorbic acid probably suggests formation of precursor 1 : 1 complex between ascorbic acid and the diiron(III,III) complex. The spectrum of





**Fig. 5** Plot of  $k_0$  versus  $[\text{H}_2\text{A}]_{\text{T}}^2$  at  $[\text{Fe}^{\text{III}}_2] = 0.05$  mM,  $\text{pH} = 4.11 \pm 0.02$ ,  $C_{\text{phen}} = 3.0$  mM,  $T = 25.0$  °C,  $I = 1.0$  M ( $\text{NaNO}_3$ ).

**Table 1** Some representative first-order rate constants for the oxidation of ascorbic acid by the dinuclear iron(III,III) complex<sup>a</sup> at  $C_{\text{phen}} = 3.0$  mM,  $T = 25.0$  °C,  $I = 1.0$  M ( $\text{NaNO}_3$ )

pH	$10^4[\text{H}_2\text{A}]_{\text{T}}/\text{M}$	$k_0/\text{s}^{-1}$	CV (in %) <sup>b</sup>
3.00	5	0.020	4.5
3.45	5	0.086	4.7
3.86	5	0.189	4.9
4.11	5	0.245	4.6
4.55	5	0.208	4.8
5.08	5	0.079	4.8
5.47	5	0.029	4.7
4.09	10	0.786	4.5
4.10	25	5.102	4.3
4.13	50	23.01	4.4
3.01	2.5	0.005	4.3
5.45	5	0.046 <sup>c</sup>	4.9
5.44	5	0.062 <sup>d</sup>	4.6
5.45	5	0.030 <sup>e</sup>	4.7
4.52	5	0.208 <sup>f</sup>	4.6
4.54	5	0.206 <sup>g</sup>	4.5

<sup>a</sup>  $[\text{Fe}^{\text{III}}_2] = 0.05$  mM unless otherwise stated. <sup>b</sup> CV was measured using the relation:  $\text{CV} = \text{sd} \times 100/x$ , where  $\text{sd}$  = standard deviation of the measurements of  $k_0$  and  $x$  = average  $k_0$ . See ref. 28. <sup>c</sup> At  $I = 0.50$  M ( $\text{NaNO}_3$ ). <sup>d</sup> At  $I = 0.05$  M ( $\text{NaNO}_3$ ). <sup>e</sup> In presence of 0.20 mM  $[\text{Fe}(\text{phen})_3]^{2+}$ . <sup>f</sup> In presence of 6.0 mM phen. <sup>g</sup>  $[\text{Fe}^{\text{III}}_2] = 0.025$  mM.

wavelengths (440–530 nm). Averages of  $k_0$  values (Table 1) from at least three independent runs were taken and the average coefficient of variation (CV)<sup>28</sup> for these measurements were within 5%.

The kinetic observations stated above can be well understood by the reactions (2)–(8) shown in Scheme 1, and it leads to the rate law given by eqn. (9), where rate is defined by eqn. (10). In deriving eqn. (9), we approximated both  $K_1$  and  $K_2$  much less than unity. An estimated maximum value of  $K_1$  lies at around 0.1 (see the ESI†) and thus  $K_2$  should be even less than this purely on charge grounds.

In the proposed scheme, we have considered only  $\text{HA}^-$  as the active reducing species. Remarkable superior reactivity of  $\text{HA}^-$  over  $\text{H}_2\text{A}$  in redox has many precedences<sup>12c,16b,f,29</sup> and in the acidity range employed in the present investigation  $[\text{A}^{2-}]$  is negligibly small ( $\text{p}K_{\text{a}}$  of  $\text{HA}^-$  is 11.93).<sup>25</sup>

**Table 2** Composite rate constants<sup>a</sup> for the oxidation of ascorbic acid by the dinuclear iron(III,III) complex at  $T = 25.0$  °C,  $I = 1.0$  M ( $\text{NaNO}_3$ ),  $C_{\text{phen}} = 3.0$  mM

Reaction path	Composite rate constant in $\text{H}_2\text{O}/\text{M}^{-2} \text{s}^{-1}$	Composite rate constant in $\text{D}_2\text{O}/\text{M}^{-2} \text{s}^{-1}$
$k_1K_1(\text{I}_1 + \text{HA}^-)$	$(1 \pm 0.05) \times 10^7$	$(7.7 \pm 0.1) \times 10^6$
$k_2K_2(\text{I}_2 + \text{HA}^-)$	$(1 \pm 0.07) \times 10^5$	$(5.9 \pm 0.4) \times 10^4$

<sup>a</sup> The  $k_1$  and  $k_2$  values are for overall reaction and are therefore equal to twice the rate constant for eqn (12) shown in Scheme 2.

Non-reactivity of **3** in the redox is probably due to absence of any labile water molecules that inhibit any kind of pre-equilibrium adduct formation prior to the electron transfer.

A plot of left hand side of eqn. (9) versus  $1/[\text{H}^+]$  resulted in a good linear fit ( $r \geq 0.98$ ) with  $(k_1K_1) = 1 \times 10^7$  and  $(k_2K_2) = 1 \times 10^5$  (both in  $\text{M}^{-2} \text{s}^{-1}$ ) (Table 2). These values regenerate the experimental  $k_0$  values within  $\pm 15\%$ . We wish to note here that the reaction of  $\text{H}_2\text{A}$  with **2** (not shown in Scheme 1) is proton-ambiguous with the reaction of  $\text{HA}^-$  with **1** and thus they are not separable. However, overwhelming dominance of reactivity of  $\text{HA}^-$  over  $\text{H}_2\text{A}$  in outer sphere reactions (the rate steps  $k_1$  and  $k_2$  shown in Scheme 1 as eqn (7) and (8) are outer sphere, *vide infra*) is well-established<sup>16b,f,29</sup> and the so-far-known reactivity order of the protolytic species of the title oxidant is  $\mathbf{1} > \mathbf{2} > \mathbf{3}$ .<sup>7c,7d,7e</sup> It thus appears that the  $\text{H}_2\text{A}$  reaction with **2** might be less contributive than  $\text{HA}^-$  reaction with **1** towards their combined rate.

$$\frac{k_0([\text{H}^+]^2 + K_{\text{a1}}[\text{H}^+] + K_{\text{a1}}K_{\text{a2}})(K_{\text{a}} + [\text{H}^+])}{([\text{H}^+][\text{H}_2\text{A}]_{\text{T}}K_{\text{a}})^2} = k_1K_1 + k_2K_2K_{\text{a1}} \frac{1}{[\text{H}^+]}$$

(9)

$$\text{Rate} = -d[\text{Fe}^{\text{III}}_2]/dt = 2d[\text{Fe}(\text{phen})_3^{2+}] = k_0 [\text{Fe}^{\text{III}}_2]$$

(10)

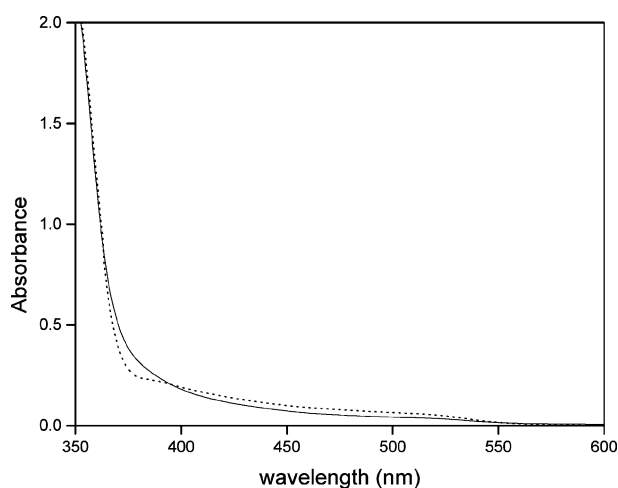
## Mechanism

The observed trend in composite constants,  $k_1K_1 > k_2K_2$ , is indicative of superior reactivity of protonated oxidant over their conjugate deprotonated form.<sup>7,30</sup> In addition to the charge effect, a lesser statistical probability of replacing one  $\text{H}_2\text{O}$  ligand by ascorbate in **2** than in **1** is also responsible for  $K_2$  being less than  $K_1$ . An estimated upper limit of  $K_1$  is 0.1 (see ESI†) and the value of  $K_2$  is expected to be much lower. Oxidation of ascorbic acid by  $\text{Fe}^{3+}_{\text{aq}}$ <sup>15a</sup> (with six replaceable  $\text{H}_2\text{O}$  molecules) also proceeds through an initial 1 : 1 pre-equilibrium adduct formation between the redox agents—the equilibrium constant for adduct formation lies at  $\sim 10^3$  level, where ascorbate acts as a bidentate ligand, much higher than the estimated value for the adduct formation between  $\text{Fe}(\text{III,III})$  and ascorbate (acting as monodentate) in the present investigation. The dinuclear complex **1** has only one water molecule on each  $\text{Fe}(\text{III})$  center. Consequently, little chance is left for the formation of an ascorbate chelate, but only a mono-associated complex at either  $\text{Fe}(\text{III})$  center appears feasible. The distance between the two oxygen atoms of the two water molecules in **1** is 524.4 pm<sup>8</sup> (calculated from the crystal structure of **1** using the programme G. M. SHELXL-93; a programme for crystal structure refinement, University of Gottingen, Gottingen, Germany, 1993) whereas the bite distance of the coordinating oxygen atoms in ascorbate is nearly 300 pm,<sup>31</sup> which does not allow ascorbate to

act as a bidentate fashion of binding with **1**. The thermodynamic stability of **I**<sub>1</sub> and **I**<sub>2</sub> should thus be much smaller than that for the [Fe(HA)]<sup>2+</sup>. The upper limit estimated for *K*<sub>1</sub> and *K*<sub>2</sub> thus appears reasonable for inner-sphere binding. Moreover, the first coordination sphere of Fe<sup>III</sup> centers in **1** are already nearly saturated with strong electron-donating ligands.

It has been shown that the solution of [L<sub>4</sub>Cl<sub>2</sub>(μ-O)Fe<sup>III</sup>]<sub>2</sub><sup>2+</sup> rapidly aquates to generate [L<sub>4</sub>(H<sub>2</sub>O)<sub>2</sub>(μ-O)Fe<sup>III</sup>]<sub>2</sub><sup>4+32</sup> and also instantaneously produces [L<sub>4</sub>(SCN)<sub>2</sub>(μ-O)Fe<sup>III</sup>]<sub>2</sub><sup>2+</sup> on adding KSCN solution<sup>32,33</sup> (L = 2,2'-bipyridine or 1,10-phenanthroline) that clearly indicates labile nature of the H<sub>2</sub>O ligands bound to high-spin d<sup>5</sup> Fe(III).<sup>34</sup> In some instances, spectral evidence was collected for the generation of a transient blue intermediate [Fe(HA)<sub>*n*</sub>]<sup>*m*+<sub>aq</sub></sup> (*n* = 1, *m* = 2 or *n* = 2, *m* = 1) during oxidation of ascorbic acid by [Fe(H<sub>2</sub>O)<sub>6</sub>]<sup>3+</sup> in highly acidic media.<sup>15a,b,c</sup> Also, a coloured 1 : 1 adduct formation between μ-oxo binuclear Fe(III) species, [Cl<sub>3</sub>Fe–O–FeCl<sub>3</sub>]<sup>2-</sup> with ascorbate has been reported.<sup>15b</sup>

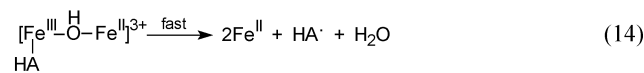
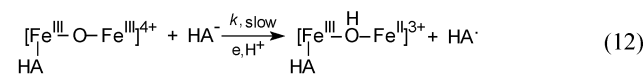
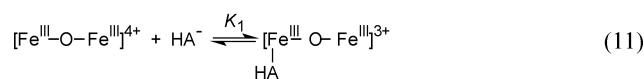
In an attempt to detect the existence of any such likely precursor complex, we rapidly mixed reagent solutions, all pre-equilibrated at pH 3.00 (±0.02) and at 25.0 °C, *I* = 1.0 M, in quartz cells kept in the cell compartments so that the desired concentrations (2.5 × 10<sup>-4</sup> M in ascorbic acid, 5 × 10<sup>-5</sup> M in diiron complex, 3 × 10<sup>-3</sup> M in phen) are reached after mixing. The absorbance data were then collected immediately after mixing. The process was executed at various wavelengths ranging between 350–600 nm. The plot of the absorbances thus found at these very initial times with λ when compared to the spectrum of the pure diiron(III,III) complex (Fig. 6) reflects a noticeable difference, *albeit small*, but the position of the isosbestic point (388 nm, Fig. 3) was maintained (not shown in Fig. 6, see ESI†). These observations clearly infer the formation of a precursor complex and the isosbestic at 388 nm is due to this precursor and the end iron product, [Fe(phen)<sub>3</sub>]<sup>2+</sup>, the UV-vis spectrum of which maintains this isosbestic (Fig. 3).



**Fig. 6** The plot of the absorbances at initial times (dotted line) at various wavelengths (see text) shows a noticeable change when compared to the spectrum of the pure diiron(III,III) complex (solid line).

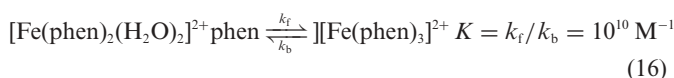
The title redox reaction is initialized by rapid inner-sphere 1 : 1 adduct formation between the oxo-bridged binuclear complex and ascorbate followed by a slow rate-limiting outer-sphere electron transfer step involving another ascorbate anion forming one-electron-reduced unstable [Fe<sup>III</sup>–O–Fe<sup>II</sup>]<sup>3+</sup> and HA<sup>•</sup> radical.

A subsequent fast electron transfer from the iron(III) bound ascorbate would lead to the products (Scheme 2).



**Scheme 2**

We propose the rate determining steps to be one-electron transfer from a free HA<sup>-</sup> to the HA<sup>-</sup>-unbound iron(III) centers in **I**<sub>1</sub> and **I**<sub>2</sub> to form the respective Fe<sup>III</sup>(HA)<sup>-</sup>O–Fe<sup>II</sup> dimers, which quickly collapse either by aquation or by further reduction to Fe(II) and Fe(III) monomers. Martell and coworkers,<sup>35</sup> and subsequently many authors,<sup>36</sup> explained that the {Fe<sub>2</sub>O}<sup>4+</sup> core unit gains stability from the superexchange of two d<sup>5</sup> high-spin Fe(III) centers linked by an oxo-bridge. However, the high-spin iron(II) and iron(III) are probably less strongly bound to oxide since both the oxidation states having two antibonding electrons are directed towards the formal bond axes. This would impart weaker Fe<sup>II</sup>–O–Fe<sup>III</sup> bonds, both of which should be rapidly broken by aquation, or the bound ascorbate quickly reduces Fe<sup>III</sup>. The HA<sup>-</sup> bound iron(III) centers in **I**<sub>1</sub> and **I**<sub>2</sub> should bear less formal positive charge than the other iron(III) center as HA<sup>-</sup> is much more basic than H<sub>2</sub>O<sup>37</sup> and thus the HA<sup>-</sup>-unbound iron(III) in **I**<sub>1</sub> and **I**<sub>2</sub> is expected to be reduced first. The iron(III) monomer [Fe(phen)<sub>2</sub>(HA)(H<sub>2</sub>O)]<sup>2+</sup> is further reduced to the iron(II) monomer [Fe(phen)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]<sup>2+</sup> by the bound HA<sup>-</sup>—the process is expected to occur rapidly as the reduction of [Fe(phen)<sub>3</sub>]<sup>2+</sup> by HA<sup>-</sup> is known to be very fast (*k* > 10<sup>9</sup> M<sup>-1</sup> s<sup>-1</sup>).<sup>16a</sup> Formation of two moles of [Fe(phen)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]<sup>2+</sup> from **I**<sub>1</sub> and **I**<sub>2</sub> by two successive (slow and fast) one-electron reductions at the two iron centers thus seems feasible. Excess phenanthroline, present in the reaction media rapidly forms the end iron product, [Fe(phen)<sub>3</sub>]<sup>2+</sup> (eqn. 16).



The equilibrium constant (*K* = 10<sup>10</sup> M<sup>-1</sup>) for reaction (16) can be calculated from the known formation constants<sup>38</sup> of [Fe(phen)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]<sup>2+</sup> (10<sup>11</sup> M<sup>-2</sup>) and [Fe(phen)<sub>3</sub>]<sup>2+</sup> (10<sup>21</sup> M<sup>-3</sup>) from Fe<sup>2+</sup><sub>aq</sub> and phen. The formation rate constant (*k*<sub>f</sub>) of [Fe(phen)<sub>3</sub>]<sup>2+</sup> from its bis analogue and phen could be calculated as 7.5 × 10<sup>5</sup> M<sup>-1</sup> s<sup>-1</sup> from the reported *k*<sub>b</sub> (7.5 × 10<sup>-5</sup> M<sup>-1</sup> s<sup>-1</sup>).<sup>39</sup> Compared with the value of *k*<sub>f</sub> (~10<sup>8</sup> M<sup>-2</sup> s<sup>-1</sup>, Table 2, assuming (*K*<sub>1</sub>)<sub>max</sub> ≈ 0.1, *vide supra*), it appears at a first approximation that formation of the end product [Fe(phen)<sub>3</sub>]<sup>2+</sup> from its bis analogue should limit the rate of the title redox, and the observed rate should be independent on ascorbate concentration. The observed dependency of rate on ascorbate concentration along with a purely first-order nature of observed rate (when [Fe<sup>III</sup>]<sub>0</sub> ≪ [H<sub>2</sub>A]<sub>T</sub>) rules out any rate

determining step of the reaction that does not involve ascorbate. The rate of formation of  $[\text{Fe}(\text{phen})_3]^{2+}$  by eqn. (16) will be:

$$\text{Rate} = k_r[\text{Fe}(\text{phen})_2(\text{H}_2\text{O})_2]^{2+}[\text{phen}] \quad (17)$$

Assuming at least  $1 \times 10^{-5}$  M (1/5th of the initial diiron(III) complex)  $[\text{Fe}(\text{phen})_2(\text{H}_2\text{O})_2]^{2+}$  is formed from the collapse of the  $[\text{Fe}^{\text{III}}(\text{HA})\text{--O--Fe}^{\text{II}}]^{2+}$  intermediate, the formation rate of  $[\text{Fe}(\text{phen})_3]^{2+}$  under the chosen experimental condition ( $[\text{phen}]_{\text{minimum}} = 1$  mM) would be  $7.5 \times 10^{-3}$  M s<sup>-1</sup>. It thus appears that the formation of 0.1 mM  $[\text{Fe}(\text{phen})_3]^{2+}$  (most of the kinetic runs were performed with initially  $[\text{Fe}^{\text{III}}] = 0.05$  mM) requires only around 1/100th of a second. ( $d[\text{ferroin}] = 0.1 \times 10^{-3}$  M (=  $7.5 \times 10^{-3}$  M s<sup>-1</sup>  $\times$  1/75 s)). The time requirements for the observed reactions are much larger (~0.1–400 seconds). A fast (much faster than the rate step of the title redox) formation of  $[\text{Fe}(\text{phen})_3]^{2+}$  is therefore conceived. Thus, use of excess  $C_{\text{phen}}$  was essential also to ensure rapid and quantitative formation of the tris(phenanthroline) complexes,  $[\text{Fe}(\text{phen})_3]^{3+}$  and  $[\text{Fe}(\text{phen})_3]^{2+}$ , from any transient bis intermediates. Additionally, it has been established that chlorite,<sup>40</sup> peroxydiphosphate<sup>41</sup> and hydrogen peroxide<sup>42</sup> oxidize  $[\text{Fe}(\text{phen})_3]^{2+}$  to a  $\{\text{Fe}_2\text{O}\}^{4+}$  species, possibly **I**, in the absence of excess phenanthroline.

The HA<sup>\*</sup> radical produced in steps 12 and 14 is strongly acidic ( $\text{p}K_{\text{a}} = -0.045$ )<sup>43</sup> and thus eqn (13) is a better representation of the radical and quick disprotonation (eqn. (15)) (second-order rate constant  $> 10^8$  M<sup>-1</sup>s<sup>-1</sup>)<sup>44</sup> of A<sup>\*-</sup> produces dehydroascorbic acid (A) and one molecule of ascorbic acid (H<sub>2</sub>A).

The exclusive second order dependence of  $k_0$  on  $[\text{H}_2\text{A}]_{\text{T}}$  indicates the simultaneous presence of two ascorbate anions at the rate step but replacement of two H<sub>2</sub>O ligands in **1** by two ascorbate anions forming bis ascorbate species is not possible. The distance between the two oxygen atoms of the two water molecules in **1** is 524.4 pm,<sup>8</sup> whereas twice the ionic radius of HA<sup>-</sup> is greater than 700 pm ( $r_{\text{H}_2\text{A}}$  is 350 pm<sup>16a</sup>). Accommodation of two ascorbate anions in the inner sphere of the complex **1** replacing two water molecules thus seems to be sterically unfavourable.

The unstable mixed-valent binuclear iron(III,II) species formed at the rate step should bear an oxo-bridge with enhanced basicity in comparison to its diiron(III,III) precursor, and hence the electron transfer in the rate determining step must be associated with a proton transfer to the oxo-bridge. Highly acidic HA<sup>\*</sup> formed simultaneously with iron(III,II) should be the proton transferring agent, if not the bulk solvent. The reaction rate in D<sub>2</sub>O (Table 2) is reduced considerably, suggesting an electroprotonic mechanism. We like to note here that the available evidences<sup>45</sup> on hemerythrin strongly suggest that protonation of the oxo-bridge is not a prerequisite for one-electron reduction of the diferric site in methemerythrin. Protonation at some point after one-electron reduction would be favoured due to the expected increase in basicity<sup>13,46</sup> of the oxo-bridge that could remove thermodynamic and/or kinetic barriers to further reduction.<sup>5a</sup> In this context we recall that the off (deoxygenation) rate constant for the oxygen binding to hemerythrin show a 19% decrease in D<sub>2</sub>O media compared to that in H<sub>2</sub>O media,<sup>47</sup> suggesting proton coupled electron transfer from bound peroxide to Fe(III,III), as evidenced by a much weaker antiferromagnetic coupling between the two iron atoms in deoxyhemerythrin compared to its oxy form due to the presence of the  $\mu$ -hydroxy group in the former.<sup>48</sup> The proposed rate step (eqn. 11, Scheme 2) of the present investigation

is thus mechanistically reminiscent to the deoxygenation process for the oxygen binding to hemerythrin. Reversible oxygenation to deoxyhemerythrin, disproportionation/reduction of mixed-valent forms of diiron sites in hemerythrin,<sup>45a,48a,b,49</sup> reaction of NO,<sup>50</sup> HNO<sub>2</sub><sup>51</sup> or H<sub>2</sub>O<sub>2</sub><sup>52</sup> with deoxyhemerythrin unambiguously demonstrates that oxo-bridge protonation is an essential prerequisite for the above noted redox processes.

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