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Complex Formation Followed by Internal Electron Transfer: The Reaction Between Cysteine and Iron(III)**

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Summary. A kinetic study of the anaerobic oxidation of cysteine (H_2L) by iron(III) has been performed over the *pH*-range 2.5 to 12 by use of a stopped-flow high speed spectrophotometric method. Reaction is always preceded by complex formation. Three such reactive complex species have been characterized spectrophotometrically: FeL^+ (λ_{max} = 614 nm, ε = 2 820 $M^{-1}cm^{-1}$); $Fe(OH)L$ (λ_{max} = 503 nm; shoulder at 575 nm, ε = 1 640 $M^{-1}cm^{-1}$); $Fe(OH)L_2^{2-}$ (λ_{max} = 545 nm; shoulder at 445 nm, ε = 3 175 M^{-1} cm^{-1}). Formation constants have been evaluated from the kinetic data: $Fe^{3+} + L^{2-} \rightleftarrows FeL^+$: $\log K_1^M = 13.70 \pm 0.05$; $Fe(OH)^{2+} + L^{2-} \rightleftarrows Fe(OH)L$: $\log K_1^{MOH} = 10.75 \pm 0.02$; $Fe(OH)L + L^{2-} \rightleftarrows Fe(OH)L_2^{2-}$; $\log K_2^{MOH} = 4.76 \pm 0.02$. Furthermore the hydrolysis constant for iron(III) was also obtained: $Fe(OH)^{2+} + H^+ \rightleftarrows Fe_{aq}^{3+}$: $\log K^{FeOH} = 2.82 \pm 0.02$). Formation of the mono-cysteine complexes, FeL^+ and $Fe(OH)L$, is via initial reaction of $Fe(OH)^{2+}$ with H_2L ($k = 1.14 \cdot 10^4\,M^{-1}s^{-1}$), the final product depending on the *pH*. FeL^+ (blue) formed at low *pH* decomposes following protonation with a second-order rate constant of $1.08 \cdot 10^5\,M^{-1}s^{-1}$. $Fe(OH)L$ (purple) decomposes with an apparent third order rate constant of $k = 3.52 \cdot 10^9\,M^{-2}s^{-1}$ via $2\,Fe(OH)L + H^+ \rightarrow$ products, which implies that the actual (bimolecular) reaction involves initial dimer formation. Finally, $Fe(OH)L_2^{2-}$ (purple) is remarkably stable and requires the presence of $Fe(OH)L$ for electron transfer. A rate constant of $8.36 \cdot 10^3\,M^{-1}s^{-1}$ for the reaction between $Fe(OH)L$ and $Fe(OH)L_2^{2-}$ is evaluated.

Keywords. Cysteine oxidation; Iron(III)-complexes; Electron transfer reactions.

Komplexbildung mit nachfolgendem internen Elektronenübergang: Die Reaktion von Cystein mit Eisen (III)

Zusammenfassung. Die Kinetik der Oxidation von Cystein (H_2L) mit Fe^{3+} unter Ausschluß von Sauerstoff wurde für den *pH*-Bereich von 2.5 bis 12 mittels einer spektralphotometrischen stopped flow-Methode untersucht. Die Reaktion erfolgt unter Komplexbildung, wobei drei reaktive Komplexverbindungen spektroskopisch nachgewiesen wurden: FeL^+ (λ_{max} = 614 nm, ε = 2 820 M^{-1} cm^{-1}); $Fe(OH)L$ (λ_{max} = 503 nm; Schulter bei 575 nm, ε = 1 640 M^{-1} cm^{-1}); $Fe(OH)L_2^{2-}$ (λ_{max} = 545 nm; Schulter bei 445 nm, ε = 175 M^{-1} cm^{-1}). Bildungskonstanten wurden aus den kinetischen Ergebnissen ermittelt: $Fe^{3+} + L^{2-} \rightleftarrows FeL^+$: $\log K_1^M = 13.70 \pm 0.05$; $Fe(OH)^{2+} + L^{2-} = \rightleftarrows Fe(OH)L$: $\log K_1^{MOH} = 10.75 \pm 0.02$; $Fe(OH)L + L^{2-} \rightleftarrows Fe(OH)L_2^{2-}$; $\log K_2^{MOH} = 4.76 \pm 0.02$. Weiters ergab sich für die Hydrolyse-Konstante des Eisen(III): $Fe(OH)^{2+} + H^+ \rightleftarrows Fe_{aq}^{3+}$: $\log K^{FeOH} = 2.82 \pm 0.02$.

** Dedicated to Prof. Dr. mult. Viktor Gutmann on the occasion of his 70th birthday

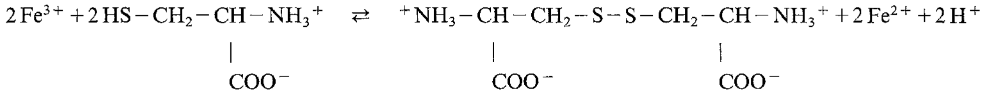
Die Bildung der Mono-Cystein Komplexe FeL^+ und $Fe(OH)L$ erfolgt über eine Reaktion von $Fe(OH)^{2+}$ mit H_2L ($k = 1.14 \cdot 10^4\,M^{-1}\,s^{-1}$), wobei das gebildete Produkt vom jeweiligen *pH*-Wert abhängt. FeL^+ (blau), welches bei niederen *pH*-Werten gebildet wird, zerfällt nach Protonierung mit einer Geschwindigkeitskonstante zweiter Ordnung von $1.08 \cdot 10^5\,M^{-1}\,s^{-1}$. $Fe(OH)L$ (purpur) zerfällt mit einer scheinbaren Geschwindigkeitskonstante dritter Ordnung von $3.52 \cdot 10^9\,M^{-2}\,s^{-1}$ entsprechend $2\,Fe(OH)L + H^+ \rightarrow$ Produkte. Dies läßt auf anfängliche Bildung eines Dimeren schließen, welches in einer bimolekularen Reaktion zerfällt. $Fe(OH)L_2{}^{2-}$ (purpur) erscheint bemerkenswert stabil und benötigt $Fe(OH)L$, um die Redoxreaktion einzugehen. Eine Geschwindigkeitskonstante von $8.36 \cdot 10^3\,M^{-1}\,s^{-1}$ wurde für die Reaktion zwischen $Fe(OH)L$ und $Fe(OH)L_2{}^{2-}$ ermittelt.

Introduction

For many readily oxidizable ligands, complex formation with a reducible metal ion is followed by internal electron transfer resulting in the reduction of the metal ion and concomitant oxidation of the ligand. Many of these systems are reversible and the presence of the reduced form of the metal ion results in the attainment of an equilibrium, but suitable choice of initial concentrations of ligand and metal ion can often be made such that the reaction goes essentially to completion, thus facilitating a study of the underlying kinetics. A further attraction of these kinetic studies is that they often enable formation constants for the (unstable) complexes to be determined from the kinetic data. Such a system is the iron(III)-cysteine system [1 – 9]. The overall reaction is

$$2\,Fe^{3+} + 2\,\text{cysteine} \rightleftarrows 2\,Fe^{2+} + \text{cystine} + 2\,H^+$$

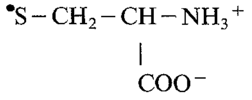
i.e.



$$2\,Fe^{3+} + 2\,HS-CH_2-\underset{\underset{\textstyle COO^-}{|}}{CH}-NH_3^+ \rightleftarrows {}^+NH_3-\underset{\underset{\textstyle COO^-}{|}}{CH}-CH_2-S-S-CH_2-\underset{\underset{\textstyle COO^-}{|}}{CH}-NH_3^+ + 2\,Fe^{2+} + 2\,H^+$$

(I) (II)

which presumably takes place via the free-radical (**III**) which appears



$$^\bullet S-CH_2-\underset{\underset{\textstyle COO^-}{|}}{CH}-NH_3^+$$

(**III**)

to dimerize without further involvement of metal ions. A particularly valuable property of the iron(III) – cysteine system is that the three main complexes that are formed exist in almost wholly independent *pH*-ranges with little overlap. Thus suitable choice of *pH*-ranges enabled the formation and decomposition of $[FeL]^+$, $[Fe(OH)L]$, and $[Fe(OH)L_2]^{2-}$ to be examined independently.

Nomenclature

The following abbreviations will be used throughout this paper: The zwitterion of cysteine is referred to as H_2L, total concentrations of iron(III) and cysteine as $[Fe]_T$ and $[Cys]_T$, respectively, and initial concentrations as $[Fe]_0$ and $[Cys]_0$. The equilibrium constants employed are defined as follows:

$$\begin{array}{lll}
L^{2-} + 2\,H^+ \rightleftarrows H_2L\ , & & \beta_2^H(=K_1^HK_2^H)\ , \\
Fe^{3+} + L^{2-} & \rightleftarrows FeL^+\ , & K_1^M\ , \\
Fe(OH)^{2+} + L^{2-} & \rightleftarrows Fe(OH)L\ , & K_1^{MOH}\ , \\
Fe(OH)L + L^{2-} & \rightleftarrows Fe(OH)L_2^{\,2-}\ , & K_2^{MOH}\ , \\
Fe(OH)^{2+} + H^+ & \rightleftarrows Fe_{aq.}^{\ 3+}\ , & K^{FeOH}\ .
\end{array}$$

Concentration units are $mol\,l^{-1}$ ($mol\,dm^{-3}$) written as M.

Experimental

All kinetic measurements were carried out using stopped-flow techniques. The high speed spectrometer, storing some 1 016 spectral data points for each spectrum in a multichannel analyzer, was supplied by Applied Photophysics Ltd. (London). The path length of the reaction cell was 2.00 cm. Cysteine solutions were made up with *L*-cysteine (pure) from Merck, and iron(III) solutions from iron(III)-chloride hexahydrate (pro analysi) from Merck and standarized with *edta*.

Solutions of the required final *pH* were made up from deoxygenated stock solutions of cysteine and of iron(III) that contained calculated amounts of HCl and sufficient KCl to maintain the final ionic strength at $0.10\,mol\,dm^{-3}$ and including, where necessary, an appropriate buffer (see text). The *pH* was measured with a Schott C-6818-*pH*-meter directly after each kinetic run. Note that $[H^+]$ was obtained from the experimental *pH* values by use of the empirical relationship: $[H^+] = 10^{-\{(pH-0.131)/0.982\}}$ obtained by titrating HCl with KOH [10].

All the reaction solutions were transferred to the stopped-flow apparatus from an oxygen-free glove box, where they had been prepared, in sealed syringes.

Results and Discussion

The spectra of the three complexes of interest, namely $[FeL]^+$, $[Fe(OH)L]$, and $[Fe(OH)L_2]^{2-}$ and the *pH*-ranges over which they singly predominate are given in Fig. 1.

In the case of the two iron(III)-hydroxy complexes the molar absorbance was obtained by extrapolation of the linear $(\text{absorbance})^{-1}$ vs. time plots for the relatively slow second-order decay curves involved to zero time, and assuming complete conversion when this absorption became constant with increase in *pH*. For Fe(OH)*L* the low absorbances found when the complex was not fully formed (at low *pH*) were used to construct a "formation curve" that yielded a value for the formation constant (see below). An analogous method could not be used to obtain the molar absorbance for FeL^+ since the decomposition reaction was too fast with respect to the formation reaction: curvefitting of the kinetic data gave the required value in this case (see below).

All data were obtained with cysteine in large excess which enabled them to be treated in terms of reduced pseudo-orders of reaction. This means that $[Cys]_T$ can in general be replaced by $[Cys]_0$. However, in equations below we use the correct terms to keep in mind their chemical origin. Chloride was chosen as the background electrolyte because of the considerably greater stability of chloride-containing acid stock solutions of iron(III), having first ascertained in each case that the same rate constants were obtained in either chloride, nitrate or perchlorate media. This is not to say that chloro-complexes play no part in the reaction, but that under the conditions pertaining to the present study these are minimal. Nitrogen was used to purge the reacting solutions of oxygen and the *pH* recorded immediately after

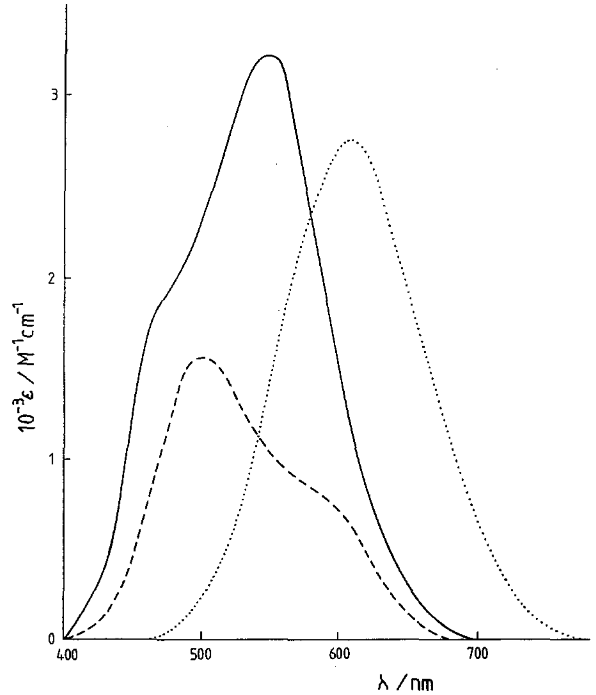


Fig. 1. Electronic spectra of cysteine complexes of iron(III) scaled to indicate relative molar absorptions. FeL^+: ...(pH 2.7 − 4.5); $Fe(OH)L$: ...(pH 5.1 − 6.7); $Fe(OH)L_2^{2-}$: ...(pH 8.6 − 11.6)

each kinetic run. The kinetics pertaining to each complex are treated as separate problems in what follows.

The pH Range 2.7 – 4.5: Formation and Decomposition of FeL^+

In acid solution iron(III) reacts with cysteine to give a bright blue colour which then rapidly fades. The colour is not returned either by adding excess iron(II) or

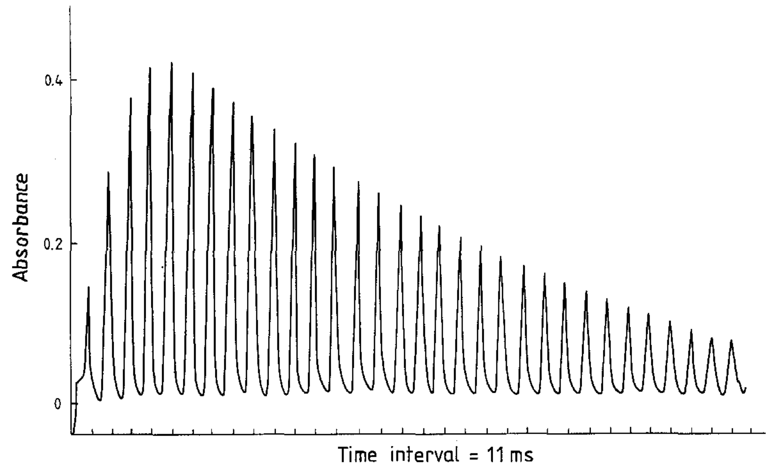


Fig. 2. Kinetic run recorded as a set of sequential complete spectra (spectral scan = 405 − 960 nm) taken at a scan rate of 11 ms/spectrum (pH = 2.97)

by passing O_2 through the solution. This might merely reflect the relative rates of the redox reactions iron(II) with dioxygen and iron(III) with cysteine.

A typical example of a stopped-flow run is shown in Fig. 2 and the component spectra were shown to consist of a single broad band with a maximum at 614 nm (Fig. 1). The shape of the kinetic curves suggested that we were following the formation of an intermediate B in a reaction scheme of the type (1) (the reaction is expressible as two first-order steps since cystein is present in large excess)

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C\,. \qquad (1)$$

A value of the rate constant, k_2^{obs}, for each run was obtained directly from the decay part of each curve, and typical values are listed in Table 1. It can be shown [11] that [B] varies with time as in Eq. (2) in which $[A]_0$ is the initial concentration of A,

$$[B] = [A]_0 k_1 \{\exp(-k_1 t) - \exp(-k_2 t)\}/(k_2 - k_1). \qquad (2)$$

Table 1. Typical results of first- and second-order rate constants for the formation and decomposition of FeL^+ ($[Fe]_0 = 2.5 \cdot 10^{-4}$ M)

pH	$10^5[H^+]$ (M)	k_1^{obs} (s^{-1})	k_2^{obs} (s^{-1})	$10^4/k_1'^{a}$ (M s)	$10^3 k_1'^{b}$ (M s)	ε (M^{-1} cm^{-1})
(i) $[Cys]_0 = 0.0125$ M						
2.82	173.0	63.8	4.38	1.96	2.85	2 800
3.16	79.4	88.5	8.96	1.41	2.09	2 820
3.56	32.1	93.4	6.31	1.34	1.96	2 840
3.90[c]	14.5	104.0	7.09	1.21	1.76	2 850
4.24[c]	6.63	96.2	10.3	1.30	1.23	2 810
4.54[c]	3.99	-	9.33	-	-	-
4.86[c]	1.58	-	14.1	-	-	-
(ii) $[Cys]_0 = 0.01$ M						
2.71	235.0	44.8	3.07	2.23	3.26	2 790
2.78	199.0	49.3	3.39	2.03	2.95	2 790
2.86	165.0	56.9	3.73	1.76	2.68	2 820
2.99	122.0	62.9	4.29	1.59	2.33	2 820
3.28	61.9	81.1	5.33	1.23	1.88	2 820
(iii) $[Cys]_0 = 0.005$ M						
2.98	120.0	31.2	2.13	1.60	2.35	2 850
3.32	55.0	38.8	2.67	1.29	1.87	2 840
3.66	25.1	42.9	2.94	1.17	1.70	2 820
3.90	14.5	46.2	3.09	1.08	1.62	2 790

[a] $k_1' = k_1^{obs}/[Cys]_0$

[b] $k_2' = k_2^{obs}/[Cys]_0$

[c] Spectrum changed during course of run

In terms of ε, the molar absorbance of B (the only absorbing species over the wavelength employed) and D, the measured absorbance, this becomes (3) which is solvable by least square methods [12],

$$D = [\mathrm{A}]_0 \varepsilon k_1 \{\exp(-k_1 t) - \exp(-k_2 t)\}/(k_2 - k_1). \tag{3}$$

Since k_2^{obs} was readily available, k_1^{obs} was obtained using the expression for t_{max}, the time taken to reach maximum absorbance [11],

$$t_{\mathrm{max}} = \{\ln(k_1/k_2)\}/(k_1 - k_2). \tag{4}$$

Finally, the molar absorbance ε was obtained from (3) by use of values of t_{max} and D at the maxima and assuming that $[\mathrm{Fe}]_0 = [\mathrm{A}]_0$. The envelopes of the series of spectra were always accurately reproduced by the values obtained in this way [13]. Typical results are summarized in Table 1, and a mean value of $\varepsilon = 2\,820\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ accepted for $\lambda_{\mathrm{max}} = 614$ nm.

*Dependence of the Observed Rate Constants on [*Fe*]$_T$, [Cys]$_T$, and [*H$^+$*]*

Over the *pH* range 2.5 – 3.9 the observed rate constants were independent of the initial iron(III) concentrations, $[\mathrm{Fe}]_0$, and strictly first-order with respect to the initial cysteine concentrations, $[\mathrm{Cys}]_0$. In other words, neither rate depends upon the speciation but only on the total amounts of iron(III) and cysteine present, i.e. $[\mathrm{Fe}]_\mathrm{T}$ and $[\mathrm{Cys}]_\mathrm{T}$. The basic experimental rate laws at constant $[\mathrm{H}^+]$ can be expressed as (5) and (6).

Formation:

$$\mathrm{d[coloured\ complex]}/\mathrm{d}t = k_1' [\mathrm{Fe}]_\mathrm{T}[\mathrm{Cys}]_\mathrm{T}\,, \tag{5}$$

where

$$k_1' = k_1^{\mathrm{obs}}/[\mathrm{Cys}]_0\,.$$

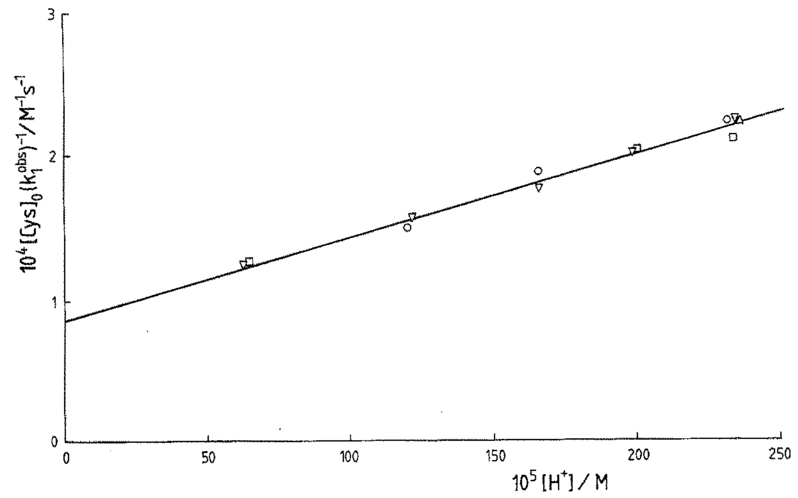


Fig. 3. Dependence of $[\mathrm{Cys}]_0/k_1^{\mathrm{obs}}$ ($= 1/k_1'$) for the formation of FeL^+ on $[\mathrm{H}^+]$ for two $[\mathrm{Cys}]_0$ values: i) $[\mathrm{Cys}]_0 = 0.01$ M: $[\mathrm{Fe}]_0 = 0.001$ M (o), 0.0005 M (△), 0.00025 M (▽) and ii) $[\mathrm{Cys}]_0 = 0.005$ M: $[\mathrm{Fe}]_0 = 0.001$ M (▭)

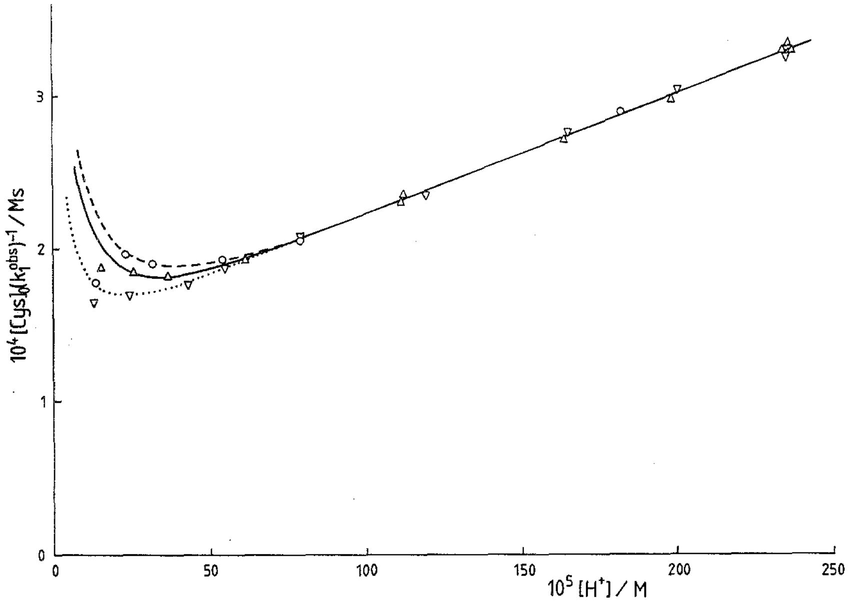


Fig. 4. Dependence of $[\mathrm{Cys}]_0/k_2^{\mathrm{obs}}$ ($= 1/k_2'$) for the decomposition of $\mathrm{Fe}L^+$ on $[\mathrm{H}^+]$. At low $[\mathrm{H}^+]$ values the single line divides into separate curves according to the $[\mathrm{Cys}]_0$ value. The theoretical curves are calculated for $\log K_1^M = 13.70$ (see text) with: $[\mathrm{Cys}]_0 = 0.0125\,\mathrm{M}$ --○--; 0.01 M – △ –; 0.005 M ···▽···

Decomposition:

$$-\mathrm{d}[\text{coloured complex}]/\mathrm{d}t = k_2'\,[\mathrm{Fe}]_\mathrm{T}[\mathrm{Cys}]_\mathrm{T}\,, \tag{6}$$

where

$$k_2' = k_2^{\mathrm{obs}}/[\mathrm{Cys}]_0\,.$$

In Fig. 3 $[\mathrm{Cys}]_0/k_1^{\mathrm{obs}}$ ($1/k_1'$) is plotted against $[\mathrm{H}^+]$. The resulting straight line is expressed in (7)

$$[\mathrm{Cys}]_0/k_1^{\mathrm{obs}} = \alpha[\mathrm{H}^+] + \beta, \tag{7}$$

with $\alpha = (5.80 \pm 0.07)\cdot 10^{-2}$s, $\beta = (8.77 \pm 0.09)\cdot 10^{-5}$M s found.

The equivalent function $[\mathrm{Cys}]_0/k_2^{\mathrm{obs}}$ ($1/k_2'$) is plotted against $[\mathrm{H}^+]$ in Fig. 4. Above $[\mathrm{H}^+] = 5\cdot 10^{-4}$M the results lie on a unique straight line expressed by (8), whereas below this $[\mathrm{H}^+]$-concentration $[\mathrm{Cys}]_0/k_2^{\mathrm{obs}}$ is no longer either independent of $[\mathrm{Cys}]_0$ or linear in $[\mathrm{H}^+]$,

$$[\mathrm{Cys}]_0/k_2^{\mathrm{obs}} = \gamma[\mathrm{H}^+] + \delta. \tag{8}$$

(the construction of the curves illustrating this property in Fig. 4 is explained below). From the linear part of the curve $\gamma = 0.846 \pm 0.009$ s, and $\delta = (1.28 \pm 0.02)\cdot 10^{-3}$ M s can be extracted.

Interpretation of the Rate Law Observed for the Formation of $\mathrm{Fe}L^+$

The empirical constants α and β in equation (7) should be explicable in terms of a plausible reaction mechanism. We begin by postulating that over the *pH* range under investigation all the cysteine present is in the form of the zwitterion (I), H_2L [Eq. (8)], and that the iron(III) exists almost entirely as the aquated species Fe^{3+} and $[Fe(OH)]^{2+}$ [Eq. (10)],

$$[\mathrm{Cys}]_T = [H_2L], \tag{9}$$

$$[\mathrm{Fe}]_T = [\mathrm{Fe(OH)}^{2+}](K^{\mathrm{FeOH}}[H^+] + 1). \tag{10}$$

It is then highly probable that the coloured complex is formed by the initial interaction of $Fe(OH)^{2+}$, with (11) for which the rate expression is (12). By making use of Eqs. (9) and (10), this can be written

$$\mathrm{Fe(OH)}^{2+} + H_2L \xrightarrow{k_1} \mathrm{Fe(OH)}(H_2L)^{2+}\ , \tag{11}$$

$$\mathrm{d}[\mathrm{Fe(OH)}(H_2L)^{2+}]/\mathrm{d}t = k_1[\mathrm{Fe(OH)}^{2+}][H_2L] \tag{12}$$

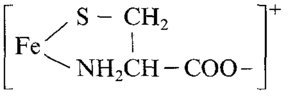
$$= k_1[\mathrm{Fe}]_T[\mathrm{Cys}]_T/(K^{\mathrm{FeOH}}[H^+] + 1), \tag{13}$$

i.e.

$$k_1^{\mathrm{obs}}/[\mathrm{Cys}]_0 = k_1/(K^{\mathrm{FeOH}}[H^+] + 1), \tag{14}$$

as (13), and hence comparison with (5) yields (14), the reciprocal of which is identical with (7) if we put $\alpha = K^{\mathrm{FeOH}}/k_1$ and $\beta = 1/k_1$. Making use of the experimental values of α and β thus gives $k_1 = 1.14 \pm 0.02 \cdot 10^4\,M^{-1}\,s^{-1}$ and $K^{\mathrm{FeOH}} = 6.61 \pm 0.02 \cdot 10^2\,M^{-1}$ ($\log K^{\mathrm{FeOH}} = 2.82 \pm 0.02$). Note that nothing is implied as the actual structure of the coloured complex since (i) the reacting species are arbitrarily defined in Eq. (11) and (ii) there would certainly have been a rapid rearrangement of the initial species, by rapid proton loss, afterwards. Reported values for $\log k^{\mathrm{FeOH}}$ appear to be variable, but a convincing value of 2.8 measured at 25°C and 1.0 M ionic strength has been extracted from the literature [14].

However, there seems little doubt that the species giving rise to the colour is FeL^+ as shown in **(IV)** [14], and that it is formed by the initial reaction of $Fe(OH)^{2+}$ with H_2L **(I)**, followed by rapid proton loss at the sulfur and nitrogen sites and protonation of the iron hydroxyl group,



(IV)

It is interesting that the rate constant, k_1, calculated on this basis is very similar to that obtained [11] for the reaction of $[Fe(OH)]^{2+}$ with SCN^- ($1.0 \cdot 10^4\,M^{-1}\,s^{-1}$).

The presence of chloride ions certainly helps to prevent precipitation in the low *pH* stock solutions of iron(III) (because of the formation of weak chloro-complexes) but these are not able to prevent precipitation in the absence of cystein over the *pH* range studied kinetically. It seems unlikely to us that coordinated chloride ions in the iron-cystine complexes are involved [15].

Interpretation of the Rate Laws Observed for the Decomposition of the FeL^+

Based on the above results we have to assume that the coloured complex FeL^+ of the form **(IV)** decomposes via a reaction which is kinetically controlled (triggered) by protonation of the bound ligand. It appears that firstly the Fe − N bound is broken, followed by protonation of the amino group, which in turn initiates the electron transfer via the sulfur bridge, finally leading to decomposition in Fe(II) and the radical $HL^{\bullet}$. For this Eqs. (15) − (17) should apply:

$$[FeL]^{+} + H^{+} \xrightarrow{k_2} Fe^{2+} + HL^{\bullet}\,, \tag{15}$$

$$2\,HL^{\bullet} \xrightarrow{\text{fast}} \text{cystine}\,, \tag{16}$$

$$-\mathrm{d}[\text{coloured complex}]/\mathrm{d}t = k_2[\mathrm{FeL}^{+}][\mathrm{H}^{+}]. \tag{17}$$

Making use of (9) and (10) and the appropriate equilibrium constants this leads to (18)

$$-\mathrm{d}[\mathrm{Fe}L^{+}]/\mathrm{d}t = k_2 K_1{}^{M} K^{\mathrm{FeOH}}[\mathrm{Fe}]_{\mathrm{T}}[\mathrm{Cys}]_{\mathrm{T}}/\{\beta_2{}^{\mathrm{H}}(K^{\mathrm{FeOH}}[\mathrm{H}^{+}]+1\}. \tag{18}$$

Comparison with (6) then gives Eq. (19), the reciprocal of which is

$$k_2{}^{\mathrm{obs}}/[\mathrm{Cys}]_0 = k^2 K_1{}^{M} K^{\mathrm{FeOH}}\{\beta_2{}^{\mathrm{H}}(K^{\mathrm{FeOH}}[\mathrm{H}^{+}]+1\}^{-1}, \tag{19}$$

identifiable with Eq. (19) when $\gamma = \beta_2{}^{\mathrm{H}}/k_2 K_1{}^{M}$ and $\delta = \beta_2{}^{\mathrm{H}}/k_2 K_1{}^{M} K^{\mathrm{FeOH}}$. From the experimentally determined values of γ and δ, and the value for $\beta_2{}^{\mathrm{H}}$ (obtained from the high *pH* experiments; see section 3.3.1.) therefore, $K^{\mathrm{FeOH}} = 6.61 \cdot 10^{2}\,\mathrm{M}^{-1}$ ($\log K^{\mathrm{FeOH}} = 2.82 \pm 0.02$, identical to the value obtained independently from the formation kinetic data) and $k_2 K_1{}^{M} = (5.40 \pm 0.04) \cdot 10^{18}\,\mathrm{M}^{-2}\,\mathrm{s}^{-1}$, was obtained. The latter is the apparent third-order rate constant for the decomposition reaction, written as $k_2 K_1{}^{M}[\mathrm{Fe}^{3+}][L^{2-}][\mathrm{H}^{+}]$.

For solutions of higher *pH* (i.e. $[\mathrm{H}^{+}] < 5 \cdot 10^{-4}\,\mathrm{M}$) the approximation of neglecting the contribution of $\mathrm{Fe}L^{+}$ to $[\mathrm{Fe}]_{\mathrm{T}}$ is no longer valid. The resulting deviation from both linearity and independence of $[\mathrm{Cys}]_0$ for the variation of $[\mathrm{Cys}]_0/k_2{}^{\mathrm{obs}}$ with $[\mathrm{H}^{+}]$ is therefore to be readily explained by Eqs. (20),

$$\begin{aligned}[\mathrm{Fe}]_{\mathrm{T}} &= [\mathrm{Fe}^{3+}] + [\mathrm{Fe(OH)}^{2+}] + [\mathrm{Fe}L^{+}] \\ &= [\mathrm{Fe(OH)}^{2+}]\,\{K^{\mathrm{FeOH}}[\mathrm{H}^{+}] + 1 + K_1{}^{M} K^{\mathrm{FeOH}}[\mathrm{Cys}]_{\mathrm{T}}/\beta_2{}^{\mathrm{H}}[\mathrm{H}^{+}]\}.\end{aligned} \tag{20}$$

Eq. (21) thus replaces (19) in which $K_1{}^{M}$ in the denominator is the only unknown since $k_2 K_1{}^{M}$ has already been determined from the linear part of the curve (above),

$$k_2{}^{\mathrm{obs}}/[\mathrm{Cys}]_0 = k_2 K_1{}^{M} K^{\mathrm{FeOH}}/\{\beta_2{}^{\mathrm{H}}(K^{\mathrm{FeOH}}[\mathrm{H}^{+}] + 1 + K_1{}^{\mathrm{M}} K^{\mathrm{FeOH}}[\mathrm{Cys}]_{\mathrm{T}}/\beta_2{}^{\mathrm{H}}[\mathrm{H}^{+}])\}. \tag{21}$$

A value of $\log K_1{}^{M} = 13.70 \pm 0.05$, best reproduced the data, was used to draw the curves in Fig. 4. This in turn yields $k_2 = (1.08 \pm 0.03) \cdot 10^{5}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. $K_1{}^{M}$ was also used to calculate the fraction of iron in the form of the complex to obtain the molar absorption from the measured absorption at 614 nm. A value of $\varepsilon = 2\,820\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ was obtained. This value corrects a previous value obtained by the assumption that at maximum absorbance all of the iron could be assumed to be in the form $\mathrm{Fe}L^{+}$ [5].

The observed kinetics presented above leads one to propose from the hydrogen-ion concentration dependence that the iron(III) complex is formed by the loss of only *one* proton. This implies that the complex formed is as shown in (**IV**) and that the internal redox reaction is then triggered by protonation by the amino-group. In basic media, when the iron(III) is coordinated to hydroxide-groups, the complexes are purple and not blue and the decomposition follows a completely different path as is discussed in the following sections.

Addition of oxygen to the reaction mixture in this *pH*-range does not result in observable return of the blue colour reflecting the relative rates of the redox reactions iron(II) (slow) with dioxygen and iron(III) with cysteine (fast).

The *pH*-Range 5.1 – 6.7: Formation and Decomposition of Fe(OH)*L*

Over this *pH* range iron(III) reacts rapidly with cysteine [3, 4] to form a purple colour due to the complex Fe(OH)*L* which slowly fades. The colour is returned on bubbling oxygen through the solution, but disappears again when all the cysteine has been oxidised (this catalytic reaction is presently under investigation).

The shape of the kinetic curves and the constancy of the spectra (Fig. 5) confirmed that we were following the rapid formation followed by the relatively slow decay of a single species, namely the mono-cysteine complex [Fe(OH)*L*].

A plot of the reciprocal of the optical density of the decay part of the curve versus time invariably yielded straight lines, the slope of which were independent of $[\mathrm{Fe}]_0$ originally indicating second order kinetics with respect to $[\mathrm{Fe}]_\mathrm{T}$:

$$-\frac{\mathrm{d}[\mathrm{Fe(OH)}L]}{\mathrm{d}t}=k^{\mathrm{obs}}[\mathrm{Fe}]_\mathrm{T}^{2}. \tag{22}$$

The molar absorbance of Fe(OH)*L* required to calculate k^{obs} from (22) was calculated from the absorbance obtained by extrapolation of the $(\text{absorbance})^{-1}$ vs. time plots to zero time and $[\mathrm{Fe}]_0$. A value of $\varepsilon = 1\,640\ \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ at 503 nm resulted as a constant mean value using the higher *pH*-data. This was used to calculate k^{obs}. Table 2 lists some typical values.

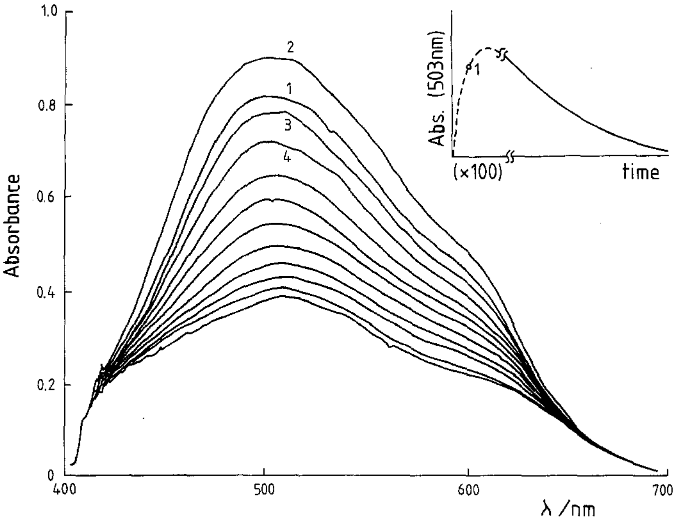


Fig. 5. Kinetic run for the formation and decay of Fe(OH)*L* showing constancy of the spectra obtained (insert shows envelope; *pH* = 6.18 time interval 9 999 ms)

Table 2. Typical values of k^{obs} obtained for the second-order decomposition of Fe(OH)L

pH	$10^5[H^+]$ (M)	$10^4[Fe]_0$ (M)	$10^3[Cys]_0$ (M)	10^3k^{obs} (M)
5.15	7.75	2.50	10.0	27.5
5.18	7.22	1.25	10.0	24.8
5.31	5.32	1.25	5.0	18.7
5.37	4.62	2.50	10.0	16.1
5.39	4.41	1.00	5.0	17.6
5.51	3.33	1.25	5.0	11.8
5.60	2.70	2.50	10.0	9.95
5.64	2.45	1.25	2.0	8.80
5.78	1.77	1.25	5.0	6.20
6.13	0.781	5.00	2.5	2.82
5.22	0.633	2.50	10.0	2.02
6.45	0.371	1.25	10.0	1.41
6.62	0.252	1.25	5.0	1.38
6.83	0.148	2.50	5.0	0.62
6.88	0.133	5.00	10.0	0.60

Dependence of the Observed Rate Constants on [Cys]$_T$ and [H^+*]*

In Fig. 6 all k^{obs} values are plotted against $[H^+]$, from which it is seen that the rate of disappearance of the Fe(OH)L is completely independent of cysteine concentration and first order in $[H^+]$ over the complete range of *pH* considered in this section.

Thus the rate expression is given by (23)

$$-\frac{d[Fe(OH)L]}{dt} = k[Fe]_T^2[H^+], \tag{23}$$

in which $k^{obs} = k[H^+]$ and $k = 3.52 \cdot 10^9\,M^{-2}s^{-1}$ results from the slope of the line.

Determination of the Stability Constant for Fe(OH)L

Using the kinetically determined value ($1\,640\,M^{-1}cm^{-1}$) of the molar absorption coefficient of the purple mono-cysteine complex [Fe(OH)L], the concentration of this species corresponding to the maximum absorption attained in each kinetic run can be calculated. Since the $[H^+]$ for each run was known and the protonation constants for cysteine ($\log K_1^H = 10.34$ and $\log K_2^H = 8.32$) have been determined from the high *pH* kinetic data (see section: *The pH-Range 8.6 – 11.6*), the value of $[L^{2-}]$ could be calculated. A plot of the fraction of iron(III) complexed ($\bar{n}$) vs. $-\log_{10}[L^{2-}]$ is thus a standard $\bar{n}$ vs. *pL* formation curve, where *pK* (= *pL* at $\bar{n} = 0.5$) can be extracted. The results are plotted in Fig. 7. It can be seen that the value of the molar absorption coefficient appears to be accurate. The theoretical formation curve is found to fit the data remarkably well. All the uncomplexed iron(III) can be taken to be $[Fe(OH)]^{2+}$ over this *pH* range [14] so that the equilibrium constant K_1^{MOH} can be obtained [17] from the formation curve. A value of $\log K_1^{MOH} = 10.75 \pm 0.02$ is obtained.

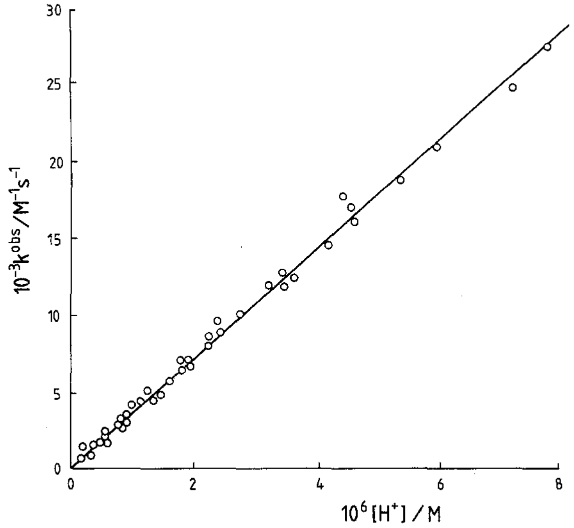


Fig. 6. Plot of observed second-order rate constant k^{obs} for the decay of Fe(OH)L vs. $[H^+]$ in the pH-range 5.15–6.90; $[Fe]_0=(1.0-5.0)\cdot 10^{-4}M$; $[Cys]_0=(2.0-10.0)\cdot 10^{-3}M$

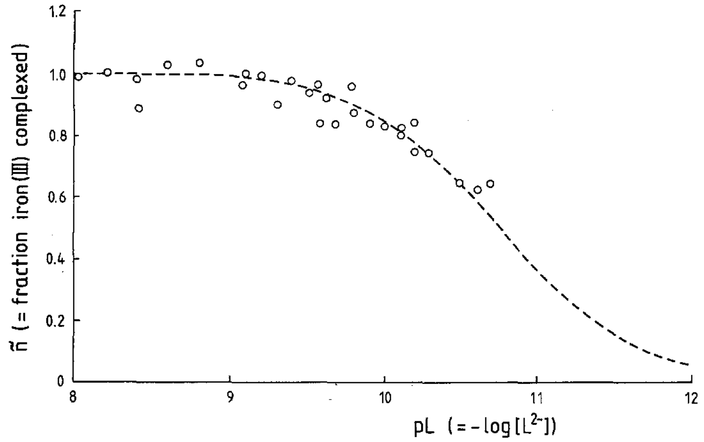


Fig. 7. Formation curve for the formation of Fe(OH)L from Fe(OH)$^{2+}$ and L^{2-} (for the method of computation see Text) (---- defines the theoretical curve giving the best fit to data points)

Formation of the Fe(OH)*L*

Talât-Erben [18, 19] has shown that for the following reaction scheme (which is a model for the reaction studied here when cysteine is present in large excess and the *pH* is constant):

$$A \xrightarrow{k_a} B\,,$$
$$2B \xrightarrow{k_b} C\,. \qquad (24)$$

The complicated mathematical expression for the formation of the intermediate, B, reduces to the relatively simple expression

$$[B]_{max}/[A]_0=\{\tau_{max}/\kappa\}^{1/2} \qquad (25)$$

by putting $\kappa = [A]_0 k_b/k_a$ and $\tau_{max} = \exp(-k_a t_{max})$ for the condition of the absorption maxima [20].

Thus for application of Eq. (25) to the reaction of $Fe(OH)^{2+}$ with the zwitterion, H_2L, a knowledge of $\bar{n} = [B]_{max}/[A]_0$ and $k_b = k^{obs}$ for the decomposition together with t_{max} (the time to reach maximum absorption) enables the calculation of k_a. Expression (25) is rather sensitive to t_{max} which is therefore not often open to accurate evaluation from the kinetic curves (the "sharpness" of the crest varies a lot with concentrations and *pH*). However, Table 3 lists some values. It can be seen that the mean value of $k_a/[Cys]_0 = 1.2 \cdot 10^4\,M^{-1}\,s^{-1}$ is remarkably close to the value of the second-order rate constant for the interaction of $Fe(OH)^{2+}$ with H_2L established in acidic solution ($k_1 = 1.14 \cdot 10^4\,M^{-1}\,s^{-1}$). Obviously it was not possible to examine the $[H^+]$ dependence of k_a over the *pH* range 5.1 − 6.7.

Interpretation of the Rate Law Observed for the Formation of Fe(OH)*L* Complex

There seems little doubt from the above discussion that the monocystein complex $Fe(OH)L$ is formed by the initial reaction of the $Fe(OH)^{2+}$ with H_2L followed by rapid loss of the protons on the sulfur and nitrogen sites to form the chelate. In other words the initial interaction is the same as has been established for the formation of FeL^+ in acidic solution, the *pH* of the solution determining the product. Note that the purple colour is restored by air (oxygen) in the case of the latter product − similarly to the case of the bis-cysteine complex dealt with below − whereas the blue colour of FeL^+ is not so restored.

Interpretation of the Rate Law Observed for the Decomposition of Fe(OH)*L*

For a proper description of the experimental observations we are forced to postulate that a dimer of the complex reacts with a proton yielding products. Since we have no value for the formation constant of such a dimer this in fact leads us to express an apparent termolecular reaction (26),

$$2\,Fe(OH)L + H^+ \xrightarrow{k_2} \text{products.} \qquad (26)$$

Table 3. Values of the time taken to reach maximum absorption (at 503 nm) during a kinetic run and the computed k_1 values (for method of computation see text)

pH	$\bar{n}$	$10^4[Fe]_0$ (M)	$10^3[Cys]_0$ (M)	$10^3 k^{obs\ a}$ ($M^{-1}\,s^{-1}$)	t_{max} (ms)	$10^{-2}k_a$ (s^{-1})	$10^{-4}k_1$ [b] ($M^{-1}\,s^{-1}$)
5.18	0.62	1.25	10.0	24.8	40	1.1	1.1
5.37	0.84	2.50	10.0	16.1	30	1.3	1.3
5.60	0.95	2.50	10.0	9.95	33	1.2	1.2
6.13	1.0	5.00	2.5	2.82	100	0.31	1.3
6.45	1.0	1.25	10.0	1.41	55	1.2	1.2
6.62	1.0	2.50	5.0	0.62	100	0.58	1.2

[a] k^{obs} is identical with k_b in Eq. (24) (see text)

[b] k_1 (= $k_a/[Cys]_0$) is the rate constant for the reaction of $Fe(OH)^{2+}$ with H_2L to form $Fe(OH)L$ (see Text)

The products can be either a single molecule of cystine, (**II**), or two cysteine free-radicals that react fast to subsequently form the cystine (**III**), together with two iron(II) species.

At this *pH* range, the total concentration of cysteine present is in the form of the zwitterion and is given by

$$[\mathrm{Cys}]_\mathrm{T} = \beta_2^{\mathrm{H}}[\mathrm{L}^{2-}][\mathrm{H}^+]^2 \,. \tag{27}$$

The total amount of iron(III), $[\mathrm{Fe}]_\mathrm{T}$, is made up of $\mathrm{Fe(OH)}^{2+}$ and $\mathrm{Fe(OH)}L$ and is given by

$$[\mathrm{Fe}]_\mathrm{T} = [\mathrm{Fe(OH)}^{2+}]\{1 + K_1^{M\mathrm{OH}}[\mathrm{L}^{2-}]\}. \tag{28}$$

By use of the stability constant for the formation of the complex, $K_1^{M\mathrm{OH}}$, and substitution by (27) and (28) we can write

$$-\mathrm{d}[\mathrm{Fe(OH)}L]/\mathrm{d}t = k[\mathrm{Fe(OH)}L]^2[\mathrm{H}^+] \tag{29}$$

$$= k_2 K_1^{M\mathrm{OH}}[\mathrm{Fe(OH)}^{2+}]^2[L^{2-}]^2[\mathrm{H}^+] \tag{30}$$

$$= \frac{k_2(K_1^{M\mathrm{OH}})^2[\mathrm{Fe}]_\mathrm{T}^2[\mathrm{Cys}]_\mathrm{T}^2[\mathrm{H}^+]}{\{\beta_2^{\mathrm{H}}[\mathrm{H}^+]^2 + K_1^{M\mathrm{OH}}[\mathrm{Cys}]_\mathrm{T}\}^2} \,. \tag{31}$$

Under the present conditions $K_1^{M\mathrm{OH}}[\mathrm{Cys}]_\mathrm{T}$ is much larger than $\beta_2^{\mathrm{H}}[\mathrm{H}^+]^2$ so that (31) reduces to (32) which is identical to (23) with $k_2 = k$,

$$-\mathrm{d}[\mathrm{Fe(OH)}L]/\mathrm{d}t = k[\mathrm{Fe}]_\mathrm{T}^2[\mathrm{H}^+]. \tag{32}$$

An alternative mechanism which would equally well fit the data is the reaction of $\mathrm{Fe(OH)}L$ with $\mathrm{Fe}L^+$ (usually referred to as "proton ambiguity") which is expressed by Eqs. (32) – (35), implying that in this case $k_2' = k_2 K_1^{M\mathrm{OH}}/K_1^M K^{\mathrm{FeOH}}$,

$$\mathrm{Fe(OH)}L + \mathrm{Fe}L^+ \xrightarrow{k_2'} \text{products}, \tag{33}$$

$$-\mathrm{d}[\mathrm{Fe(OH)}L]/\mathrm{d}t = k_2'[\mathrm{Fe(OH)}L][\mathrm{Fe}L^+] \tag{34}$$

$$= k_2' K_1^M K^{\mathrm{FeOH}}(K_1^{M\mathrm{OH}})^{-1}[\mathrm{Fe}]_\mathrm{T}^2[\mathrm{H}^+]. \tag{35}$$

The concentration of $\mathrm{Fe}L^+$ over the *pH* range 5 to 7 must be less than $10^{-3}[\mathrm{Fe(OH)}L]$ so that the formation of a dimer of $\mathrm{Fe(OH)}L$ [and therefore the reaction scheme (26)] appears to be more probable than a formation of a bridge between $\mathrm{Fe}L^+$ and $\mathrm{Fe(OH)}L$. Furthermore, the dimer is stabilized by at least one hydroxyl bridge. Accordingly, the concentration of a reactive species $[\mathrm{Fe(OH)}L]_2$, must be less than 1% of the monomer to yield the well defined formation curve (Fig. 7) where the absorption of a dimeric species has been neglected. The reaction in basic solution, however, seems to require the participation of the monomer, as is demonstrated below.

The *pH*-range 8.6 – 11.6: Formation and Decomposition of $[\mathrm{Fe(OH)}L_2]^{2-}$

In strongly alkaline solutions the deep purple bis(cysteine)-complex, $\mathrm{Fe(OH)}L_2^{2-}$ is formed [4]. The kinetics of formation of this species was not examined since the data obtainable by the present method were not nearly complete enough to study what must be a multi-step process. The observed decay, however, is slow enough

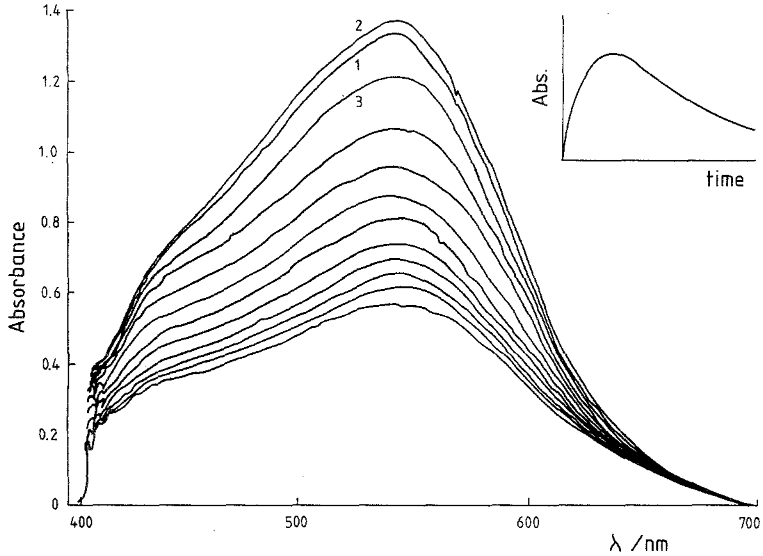


Fig. 8. Kinetic run for the formation and decay of Fe(OH)L_2^{2-} showing constancy of the spectra obtained (insert shows envelope; $pH = 9.58$; time interval = 9 999 ms)

[3, 4] to be followed by the available methods. The spectroscopic kinetic runs clearly confirmed that we were indeed following the initial rapid formation of a single species (a fact not established in previous work [4] partly because to the eye the colours of Fe(OH)L and Fe(OH)L_2^{2-} are indistinguishable) followed by its slow decay (Fig. 8). At a *pH* of 11.6 there was clear spectroscopic evidence for the existence of a further species, most probably the tris(cysteine)-complex, but this was not further investigated.

A plot of the reciprocal of absorbance versus time gave extremely good straight lines indicating accurate second-order kinetics over more than 85% of total reaction [independent of the initial iron(III) content]. Extrapolation of those lines obtained at the absorption maximum (545 nm) and above *pH* 10 (where 100% complex formation could safely be assumed) to zero time enabled the molar absorbance of the complex to be calculated. A value of $\varepsilon = 3\,175\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ was obtained and this compares favorably with the previously reported [3, 4] value of $3\,050\,\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$. The slopes of the straight lines yielded an observed rate constant according to

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2^{2-}]/\mathrm{d}t = k^{\mathrm{obs}}[\mathrm{Fe}]_{\mathrm{T}}^2. \qquad (36)$$

Dependence of the Observed Rate Constants on $[Cys]_T$, $[\mathrm{Fe}]_T$, *and* $[\mathrm{H}^+]$

Some typical results are given in Table 4 and the values of $k^{\mathrm{obs}}[\mathrm{Cys}]_0$ obtained for all the runs carried out are plotted against *pH* in Fig. 9. Above a *pH* of about 9.5 all the results lie on a single line meaning that above this *pH* the rate of disappearance of the bis-cysteine complex, Fe(OH)L_2^{2-}, depends on $[\mathrm{Cys}]_{\mathrm{T}}^{-1}$ and is second order with respect to $[\mathrm{Fe}]_{\mathrm{T}}$. This implies that the basic experimental rate law at constant $[\mathrm{H}^+]$ takes the form of Eq. (37) over the *pH* range 9.5 − 11.6,

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2^{2-}]/\mathrm{d}t = k_1[\mathrm{Fe}]_{\mathrm{T}}^2/[\mathrm{Cys}]_{\mathrm{T}}, \qquad (37)$$

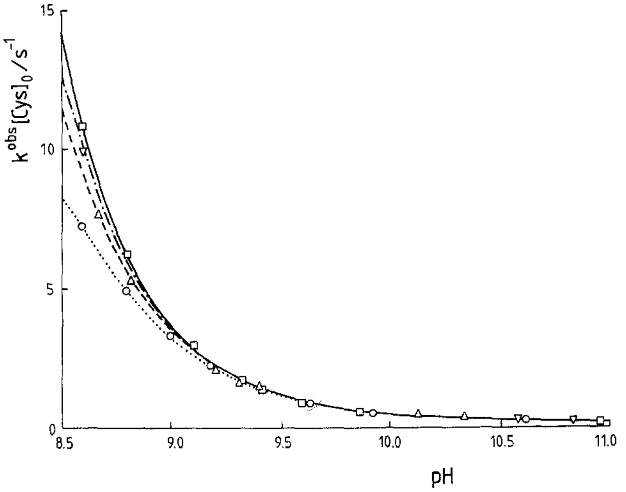


Fig. 9. Dependence of $k^{obs}[\mathrm{Cys}]_0$ for the decay of $\mathrm{Fe(OH)}L^{2-}$ on *pH* (method of calculation of the theoretical curves is given in the text) ($[\mathrm{Cys}]_0 = 0.005$ m (···○···); 0.01 M (---△---); 0.015 M (-·-▽-·-); 0.025 (–□–): $[\mathrm{Fe}]_0 = 5{\cdot}10^4$ to $1.5{\cdot}10^{-3}$ M)

Table 4. Dependence on *pH* and $[\mathrm{Cys}]_0$ of the observed second-order rate constant, k^{obs}, for the decay of $\mathrm{Fe(OH)}L_2{}^{2-}$ (see Fig. 9)

pH	$10^4[\mathrm{Fe}]_0$ (M)	$10^2[\mathrm{Cys}]_0$ (M)	$k^{obs}[\mathrm{Cys}]_0$ (s^{-1})
Separate curves depending on $[\mathrm{Cys}]_0$			
8.62	5.00	1.50	10.1
8.84	2.50	1.00	5.37
8.82	5.00	2.50	6.31
8.82	2.50	5.00	5.01
9.08	2.50	2.50	3.99
9.18	2.50	0.50	2.25
9.23	2.50	0.50	2.17
9.33	5.00	2.50	1.75
9.32	5.00	1.00	1.72
9.41	2.50	1.00	1.45
Single curve independent of $[\mathrm{Cys}]_0$			
9.58	5.00	2.50	0.97
9.91	2.50	0.50	0.49
10.33	2.50	1.00	0.28
10.55	5.00	1.50	0.22
10.57	1.50	1.50	0.22
10.84	2.50	1.50	0.16
11.58	2.50	2.50	0.11

where

$$k_1 = k^{\mathrm{obs}}[\mathrm{Cys}]_0.$$

The shape of the $k^{\mathrm{obs}}[\mathrm{Cys}]_0$ vs. *pH* curve strongly suggested dependence upon a deprotonated form of cysteine (rather than $[\mathrm{Cys}]_\mathrm{T}$), most probably L^{2-}, since this is the species coordinated to the iron(III) in the complexes. Over the *pH* range 9.5 – 11.6, the total cysteine concentration is given by Eqs. (38) and (39) (the presence of metal complexes can be ignored as ligand is always in large excess)

$$[\mathrm{Cys}]_\mathrm{T} = [L^{2-}] + [\mathrm{H}L^{-}] + [\mathrm{H}_2L] \tag{38}$$

$$= [L^{2-}](1 + K_1^{\mathrm{H}}[\mathrm{H}^+] + \beta_2^{\mathrm{H}}[\mathrm{H}^+]^2). \tag{39}$$

As the rate of reaction is actually inversely proportional to $[L^{2-}]$ rather than to $[\mathrm{Cys}]_\mathrm{T}$ Eqs. (37) and (39) lead to Eq. (40), where a rate constant k' defined by (40) is used,

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2^{2-}]/\mathrm{d}t = k'[\mathrm{Fe}]_\mathrm{T}^2/[L^{2-}], \tag{40}$$

$$k' = k^{\mathrm{obs}}[\mathrm{Cys}]_0/(1 + K_1^{\mathrm{H}}[\mathrm{H}^+] + \beta_2^{\mathrm{H}}[\mathrm{H}^+]^2). \tag{41}$$

A simple curve-fitting routine [21] was then employed and yielded the following values of the three constants which were then used to calculate the relevant lines in Fig. 9, namely: $\log K_1^{\mathrm{H}} = 10.34 \pm 0.02$, $\log \beta_2^{\mathrm{H}} = 18.66 \pm 0.03$, $k' = 0.145 \pm 0.002$ s^{-1}.

These protonation constants agree very well with the published values (for example Lenz and Martell [22] report $\log K_1^{\mathrm{H}} = 10.11$ and $\log \beta_2^{\mathrm{H}} = 18.24$) but the variation in the literature is quite broad and so the kinetically determined values above were considered the best to use in the present studies. The apparent rate constant k' was also determined by Leussing et al. [4] who obtained $0.105\ \mathrm{s}^{-1}$ (but see below).

Finally, as will be justified in the next section, the separation of the single line into four separate lines depending on $[\mathrm{Cys}]_0$ was ascribed to the fact that the bis-cysteine complex no longer predominates below *pH* 9.5 and that the presence of the mono-cysteine complex must also be taken into account. The appearance of the spectrum of the mono-cysteine complex towards the end of some of these relatively low *pH* runs confirmed this. The method of calculating $K_2^{M\mathrm{OH}}$ according to this is outlined below so that the lines drawn in Fig. 9 could be based on a value of $5.79 \cdot 10^4\ \mathrm{M}^{-1}$ ($\log K_2^{M\mathrm{OH}} = 4.76$).

Interpretation of the Rate Law Observed Above pH 9.5

The simplest explanation for the second-order dependence on total iron(III) concentration is that two iron-containing species are reacting in the rate-determining step. Similarly, the inverse dependence on $[L^{2-}]$ [Eq. (40)] suggests that one species contains one fewer coordinated cysteine than the other. Thus, in agreement with Leussing et al. [4] we propose that this step involves the interaction of $\mathrm{Fe(OH)}L_2^{2-}$ which implies reaction (42) with the corresponding rate equation (43),

$$\mathrm{Fe(OH)}L + \mathrm{Fe(OH)}L_2^{2-} \xrightarrow{k_1'} \text{products}, \tag{42}$$

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2^{2-}]/\mathrm{d}t = k_1[\mathrm{Fe(OH)}L][\mathrm{Fe(OH)}L_2^{2-}]$$

$$= k_1 K_2^{M\mathrm{OH}}[\mathrm{Fe(OH)}L]^2[L^{2-}]. \tag{43}$$

Over the *pH* range 8.6 – 11.6 all of the iron(III) can be considered as being complexed by cysteine, and hence $[\mathrm{Fe}]_\mathrm{T}$ is given by Eq. (44). Hence Eq. (43) becomes Eq. (45),

$$[\mathrm{Fe}]_\mathrm{T} = [\mathrm{Fe(OH)}L] + [\mathrm{Fe(OH)}L_2{}^{2-}]$$

$$= [\mathrm{Fe(OH)}L](1 + K_2{}^{M\mathrm{OH}}[L_2{}^{2-}]), \qquad (44)$$

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2{}^{2-}]/\mathrm{d}t = k_1 K_2{}^{M\mathrm{OH}}[L_2{}^{2-}][\mathrm{Fe_T}]^2(1 + K_2{}^{M\mathrm{OH}}[L_2{}^{2-}])^2. \qquad (45)$$

Above *pH* 9.5 a further simplification can be made to Eq. (45) since $[\mathrm{Fe(OH)}L_2{}^{2-}]$ is much larger than $[\mathrm{Fe(OH)}L]$ so that (45) simplifies to

$$-\mathrm{d}[\mathrm{Fe(OH)}L_2{}^{2-}]/\mathrm{d}t = k[\mathrm{Fe_T}]^2/K_2{}^{M\mathrm{OH}}[L_2{}^{2-}]. \qquad (46)$$

Comparison of (46) with (40) shows them to be equivalent with

$$k' = k_1/K_2{}^{M\mathrm{OH}}. \qquad (47)$$

Interpretation of the Rate Law Observed Below pH 9.5

Below *pH* 9.5 the plots of $k^{\mathrm{obs}}[\mathrm{Cys}]_0$ vs. *pH* no longer form a coincident set, but lie on curves determined by $[\mathrm{Cys}]_0$. However, over this *pH* range the assumption made above that $[\mathrm{Fe(OH)}L_2{}^{2-}] \gg [\mathrm{Fe(OH)}L]$ is not valid and thus the data must be interpreted on the basis of the full expression (45), which, combined with (47) to obtain Eq. (48) in which the only unknown is $K_2{}^{M\mathrm{OH}}$:

$$k^{\mathrm{obs}} = k'(K_2{}^{M\mathrm{OH}})^2[L_2{}^{2-}]/(1 + K_2{}^{M}[L_2{}^{2-}])^2. \qquad (48)$$

A mean value of $K_2{}^{M\mathrm{OH}} = (5.79 \pm 0.05) \cdot 10^4\,\mathrm{M}^{-1}$ ($\log K_2{}^{M} = 4.76 \pm 0.02$) fitted the data well and was used to construct the lines drawn in Fig. 9. The only previously reported value [4] for this constant is $2.44 \cdot 10^4\,\mathrm{M}^{-1}$ ($\log K_2{}^{M\mathrm{OH}} = 4.39$). Finally, from the values of k' and $K_2{}^{M\mathrm{OH}}$ a value of $k_1 = 8.36 \cdot 10^3\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ was obtained for the reaction of $\mathrm{Fe(OH)}L_2{}^{2-}$ with $\mathrm{Fe(OH)}L$.

A main disagreement between our results and those of Ref. [4] is that we find no evidence for a reaction between two molecules of the bis-cysteine complex of iron(III), $\mathrm{Fe(OH)}L_2{}^{2-}$ (or for the formation of a dimer molecule of the bis-cysteine complex). They base their evidence, however, on the existence of an intercept in the $[L^{2-}]$ vs. k^{obs} graph. If, however, their values of $[L^{2-}]$ are recalculated using the presented kinetically obtained values for the protonation constants of cysteine and the k^{obs} values corrected by employing our value for the absorption coefficient of $\mathrm{Fe(OH)}L_2{}^{2-}$, then the data points lie on a straight line through the origin with a slope ($= k'$) of $0.133\,\mathrm{s}^{-1}$ (instead of $0.105\,\mathrm{s}^{-1}$) which is in excellent agreement with our value of $0.145\,\mathrm{s}^{-1}$.

Despite the fact that at $pH < 7$ the redox reaction involves the interaction of two mono-cysteine complexes, the kinetic results over the *pH* range studied here, namely 8.6 – 11.6, do not allow any significant involvement of this reaction under these conditions.

General Conclusions

The reaction between the two iron(III) – cysteine species at high *pH* (see last section) exhibits several points of interest. Firstly, the most likely mode of interaction is via one, or less likely two, hydroxyl bridges linking the two iron(III) centers. Two electrons could then be transferred into this Fe^{III}-OH-Fe^{III} bridge system from the two cysteine ligands bound to the same iron atom yielding one molecule of cystine (particularly favored if the coordinated sulfur donor atoms in $Fe(OH)L_2^{2-}$ are adjacent in the coordination sphere and indeed might be expected since sulfur is a soft donor) and two iron(II) species, one unoxidized cysteine remaining.

Such a mechanism is closely allied to that proposed [23] for the iron(III)-catalyzed oxidation of cysteine by molecular oxygen in which strong evidence is put forward for the involvement (as rate-determining step) of a reaction between molecular oxygen and the bis-cysteine complex. This reaction thus again proposes the transfer of two electrons from the coordinated cysteine ligands through the iron atom, but this time to the oxygen molecule (yielding peroxide). However, in order to explain the initial coordination of the O_2 to the iron it must be assumed that a large degree of charge transfer is present in the bis-cysteine complex enabling the iron at the center to function in many ways as iron(II). Similarly, in the case of the copper(II)-catalyzed oxidation of ascorbic acid by molecular oxygen where it was found necessary [24] to postulate the interaction of the O_2 with a dimer of the copper(II) complex, the copper centers would have to be assumed to behave as copper(I) towards dioxygen in order to coordinate if such a mechanism is accepted. In fact this non-innocent behaviour of these readily oxidizable ligands such as ascorbic acid, epinephrine, dopa [β-(3,4-dihydroxyphenyl)alanine], and cysteine seems not only to enable the central metal ion in their bis-complexes to function as if it were in a lower oxidation state and thus to coordinate an O_2 molecule, but also confers on them considerable stability. Thus the bis(cysteine)-complex of iron(III) only undergoes an internal redox reaction when either a molecule of the mono(cystine)-complex or a molecule of dioxygen is coordinated to the central metal atom whereupon both electrons required for reaction are transferred via the metal ion.

Finally, a puzzling observation concerning the interaction of O_2 with the iron(III) – cysteine system in acid solution is that both the formation and the decomposition of FeL^+ are apparently slowed by the presence of oxygen (note that oxygen does not restore the colour after initial bleaching in an anaerobic solution). We are currently studying this and related dioxygen – metal-ion – ligand systems in parallel with the kinetics of anaerobic reactions [25].

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