Studies on the Reactions of Ferric Iron with Glutathione and some Related Thiols. Part II. Complex Formation in the pH Range Three to Seven

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Reactions of ferric and ferrous salts with glutathione and related thiols have been studied by Mössbauer spectroscopy and pH titrations. In all cases the final product is an iron(II) complex. Both oxidised and reduced glutathione bind iron(II) in the pH range 3–7 via *the carboxylate groups. Interestingly, glutathione solubilizes iron(II) up to pH 7.*

Introduction

Glutathione (GSH) is considered to be an essential constituent of living cells [1, 2]. The coordination chemistry of glutathione with metal ions has aroused considerable interest [2–9]. It has the possibility of providing eight chelation sites to metals. The binding of these chelating ligands will be dependent on a number of factors; these include pH, valence of metal, size of metal, ionic radius and redox chemistry if any of the metal ion.

The binding of glutathione to copper [2, 8, 9] and iron [5–7] has been found to be particularly complicated by the redox potentials of these metals.

Mössbauer studies of the binding reaction of iron(III) to glutathione have clearly demonstrated the production of iron(II) [5–7]. We have studied the competition for iron between catechol compounds and glutathione [6] over a wide pH range and found reduction of the metal to occur at low pH.

Anaerobic reactions of ferric salts with glutathione and related thiols were studied by Mössbauer spectroscopy and fast reaction kinetic techniques at low pH. In all cases the final product contained iron(II) [7]. This anaerobic study although yielding some information on the nature of the glutathione ligands that bind iron at low pH, drew attention to the difficulties in assigning the ligands coordinating iron in such complexes.

In order to investigate more fully the binding between glutathione (in both its oxidised (GSSG) and reduced forms) with both iron(III) and iron(II) we have studied the solution chemistry of these systems using pH titrations, Mössbauer spectroscopy and magnetic susceptibilities. We report here the results of these studies which enable us to identify the source of proton release upon complex formation. Frozen solution Mössbauer studies performed in parallel substituting other thiol compounds for glutathione are also reported.

Experimental

Materials

Anhydrous glutathione (reduced crystalline, Sigma), anhydrous glutathione [oxidised form, grade II Sigma], s-methyl glutathione (Sigma), anhydrous iron(III) chloride (SLR, Fisons), anhydrous L-cysteine, free base (Sigma), and $FeCl_2 \cdot 4H_2O$ freshly prepared in house were used without further purification.

pH Titrations

Iron (10^{-3} mol dm^{-3}) was used throughout with different ratios of ligand (Table I). Additions of NaOH (1 mol dm^{-3}) were achieved under nitrogen, and monitored by a Philips (pw-9409) digital pH meter.

Determination of Stability Constants

These were calculated from pH titration data using the methods of Albert and Sergeant [10].

Magnetic Moment Measurements were carried out by Evans' method [11] using an EM-360 NMR spectrometer. The iron concentration of the solutions used for the magnetic studies was 7.4 X

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TABLE I. Reduced and Oxidised Glutathione Titrations in the Absence and Presence of Metal Ion and Comparison of the Number of Protons Released up to pH 6 under These Conditions.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Solution	[Ligand] mol dm^{-3}	Starting pH of the Solution	$[H^+]$ mol dm^{-3} before starting the titration	$[OH^-]$ mol dm^{-3} needed to attain pH 6	$[H^+] = [OH^-]$ Total no. of H^+ released up to pH 6[a]	Total number of H^+ released by the ligand[b]	ΔH^+ [c]
GSH	1.0×10^{-3}	3.52	0.3×10^{-3}	1.0×10^{-3}	1	1	–
GSH/$FeCl_3$ (1:1)	1.0×10^{-3}	3.28	0.5×10^{-3}	2.2×10^{-3}	2	1	1
GSH/$FeCl_3$ (1.5:1)	1.5×10^{-3}	3.28	0.5×10^{-3}	2.5×10^{-3}	2.5	1.5	1
GSH/$FeCl_3$ (2:1)	2.0×10^{-3}	3.22	0.6×10^{-3}	3.0×10^{-3}	3	2	1
GSH/$FeCl_3$ (3:1)	3.0×10^{-3}	3.25	0.6×10^{-3}	4.0×10^{-3}	4	3	1
GSH/$FeCl_3$ (6:1)	6.0×10^{-3}	2.85	1.4×10^{-3}	7.04×10^{-3}	7	6	1
GSH/$FeCl_2 \cdot 4H_2O$ (2:1)	2.0×10^{-3}	3.1	0.79×10^{-3}	1.9×10^{-3}	~2	2	0
GS-CH_3 (S-methylglutathione)	2.0×10^{-3}	3.5	0.4×10^{-3}	2.3×10^{-3}	~2	2	–
GS-CH_3/$FeCl_2 \cdot 4H_2O$ (2:1)	1.33×10^{-3}	3.7	0.2×10^{-3}	1.66×10^{-3}	~1.66	1.33	0.33
GSSG (oxidised glutathione)	1.0×10^{-3}	2.94	1.14×10^{-3}	1.95×10^{-3}	~2	2	–
GSSG/$FeCl_2 \cdot 4H_2O$ (0.5:1)	0.5×10^{-3}	3.15	0.71×10^{-3}	0.95×10^{-3}	1	1	0
GSSG/$FeCl_2 \cdot 4H_2O$ (1:1)	1.0×10^{-3}	2.88	1.4×10^{-3}	1.95×10^{-3}	2	2	0

[a]Mole protons released by the mixture or the ligand (*i.e.* GSH releases one mole H^+ per mole GSH). [b]Moles of protons supposed to be released, even in the absence of metal. [c]ΔH^+ = number of protons released by the mixture – number of protons released if it was by itself in solution (Column (6) – Column (7)).

TABLE II. Concentration of Protons in Iron(III)-Chloride and GSH Solutions before and after Mixing.

Compound	Concentration (mol dm^{-3})	$[H^+]$ (mol dm^{-3})
$FeCl_3$	2×10^{-3}	2.63×10^{-3}
	1×10^{-3}	1.047×10^{-3}
GSH	2×10^{-3}	0.63×10^{-3}
	1×10^{-3}	0.27×10^{-3}
GSH + $FeCl_3$	1×10^{-3} (GSH) 1×10^{-3} ($FeCl_3$)	1.12×10^{-3}

10^{-3} mol dm^{-3}. Inert atmosphere was maintained throughout the experiment.

Mössbauer Spectroscopy

Aqueous solutions of glutathione, oxidized glutathione, cysteine and thioglycol were mixed with the iron(III) chloride solution under anaerobic conditions and the pH adjusted, syringes were used to transfer the solutions to a polythene cell and quench frozen in liquid nitrogen. The cell was then transferred to a precooled Harwell MNC-200 cryostat.

Mössbauer spectra were recorded at 80 K using the system described in previous papers [6, 7]. The data were computer fitted.

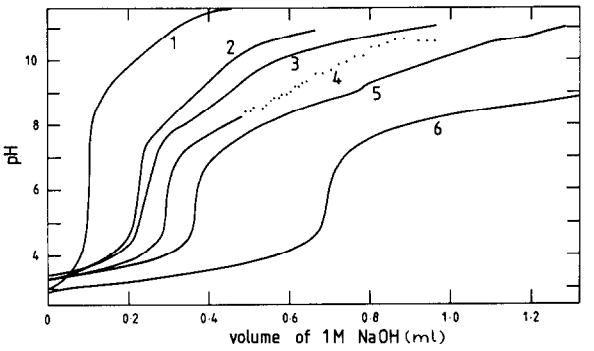


Fig. 1. pH titration curves of GSH (1×10^{-3} mol dm^{-3}) (1); iron–GSH solutions with $FeCl_3$–ligand ratios of 1:1 (2); 1:1.5(3); 1:2(4); 1:3(5); 1:6(6). $[FeCl_3] = 1 \times 10^{-3}$ mol dm^{-3}.

Results and Discussion

Intial studies were carried out to determine the number of protons released when glutathione was mixed with iron(III).

Glutathione (2×10^{-3} mol dm^{-3}) was found to release 0.25 moles H^+ per mole GSH when dissolved in water (pH 7.0). While for iron(III) chloride (2×10^{-3} mol dm^{-3}) the concentration of protons was 2.63×10^{-3} mol dm^{-3}. Allowing for the dilution effect on both solutions, an attempt was made to calculate the number of protons released upon complexation (considering the iron(III) solution the stronger acid). The mixture of iron(III) and glutathione was found to give a similar pH value to that of the iron(III) solution when diluted to 1×10^{-3} mol dm^{-3} (Table II).

This result indicates that no further release of protons occurs upon mixing the ligand and the metal. As GSH on complexation with iron(III) must deprotonate the SH group [7] then this is indicative of the possibility that a proton is being released and absorbed within the system. The most likely reaction is seen in eqn. (1).

$$GSH + [Fe^{III}(H_2O)_5OH]^{2+} \longrightarrow [Fe^{II}(H_2O)_x\dot{G}S]^{2+} + (6 - x)(H_2O) \quad (1)$$

Indeed, the UV peak for iron(III) chloride solution at 300 nm which was assigned for the species $[Fe(H_2O)_5OH]^{2+}$ can be eliminated by adding glutathione to the iron(III) solution [4].

pH Titrations

The anaerobic pH titrations of iron(III)–glutathione at concentrations of 10^{-3} mol dm^{-3} in 1:1, 1.5:1 and 3:1 glutathione to iron proportions exhibited similar behaviour (Fig. 1). In these titrations the solutions were colourless from low pH up to pH's 7.5 to 8, then a green precipitate started to appear. When the GSH proportion was increased from 1 to 3 this precipitate first appeared at slightly higher pH. The colours associated with the pH titrations appear in Table III.

In the iron(II)–oxidized glutathione system (GSSG), (Fig. 2, Table III), a pale yellow colour similar to that of GSH-iron(II) appeared at pH *ca.* 5.9, but the more intense yellow colour of the reduced glutathione systems was not detected; hydrolysis was initiated at pH's higher than those of the iron(II)–GSH system (about pH 10). From iron(II) concentrations 0.05 *M* or higher in a 1:1 and 1:2 metal-ligand ratio, at pH's between 6 to 7, a white or off-white solid precipitated when the solu-

TABLE III. The pH Dependence of Colour of (1) Iron(III)–GSH, (2) Iron(II)–GSH, (3) Iron(II)–GSSG, (4) Iron(II)–L-cysteine, (5) Iron(III)–GSSG. [All Experiments were performed under Nitrogen Atmosphere].[a]

pH	(1)	(2)	(3)	(4)	(5)
1					Yellow
2	Colourless	Colourless	Colourless	Colourless	Reddish-orange
3					Yellow precipitate
4					Brown precipitate
5	Pale-yellow	Pale-yellow			
6					
7	Intense-yellow	Intense-yellow	Pale-yellow	Pale-yellow	
8				Intense-yellow	Reddish-brown $Fe(OH)_3$
9		green precipitate $Fe(OH)_2$	White cloud		
10	Green precipitate $Fe(OH)_2$			Green precipitate $Fe(OH)_2$	
11			Green precipitate $Fe(OH)_2$		
12					

[a]The yellow colours are only observed at concentrations about double that used in the normal titrations.

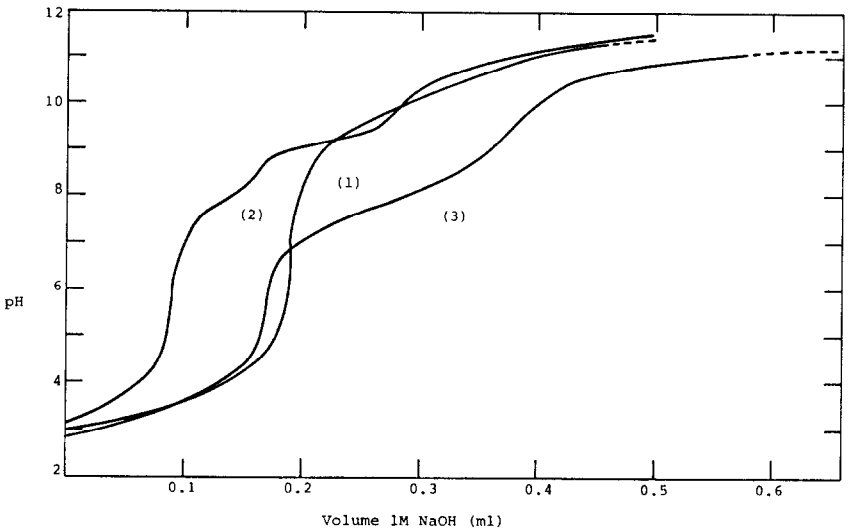


Fig. 2. pH titration curves for GSSG (1×10^{-3} mol dm^{-3}) (1); iron–GSSG with $FeCl_2$–ligand ratios of 2:1 ([GSSG]) = 0.5×10^{-3} mol dm^{-3}) (2); 1:1 ([GSSG]) = 1×10^{-3} mol dm^{-3} (3).

tion was allowed to stand for one hour under nitrogen atmosphere.

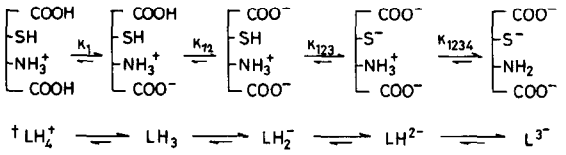
L-Cysteine-iron(II) [12, 13] behaves in a similar manner to Fe(II)-GSH on varying the pH, a precipitate has been reported to occur over a time period.

For the iron(III) chloride–GSSG system, on mixing the reactants a reddish-orange colour appeared (pH 2), then a yellow solid precipitated at pH 3; this at higher pH turns brown until pH 7, then hydrolysis occurs and the red brown $Fe(OH)_3$ precipitates. These solids will be discussed elsewhere.

Calculation of Stability Constants for the Iron-GSH using Titration Data

Stability constants were calculated from the iron-(III)-GSH titration data using a basic programme based on a previously reported method [10, 14].

GSH deprotonation was reported to take place as follows [2, 15]:



When GSH is dissolved in water (1 m*M*) the initial Ph is 3.4 which is above the first pKa of 2.19. This suggests that the GSH species present at pH = 3 is LH_3.

Two sets of stability constants were formulated on the finding that the SH group deprotonates before the NH_3^+, which means the proton on the NH_3^+ group will remain bound in the FeLH complex [2].

Using the above information the following data were obtained from the 1:1, 1:2 and 1:3 titrations of iron(III)–GSH mixtures. (These contain iron-(II), GSH and a half mole GSSG per every mole iron-(II)).

FeLH: $\log K_1 = 9.81$; $[Fe(LH)_2]^{2-}$: $\log K_2 = 8.54$ and $\log \beta_2 = 18.36$

$[FeL]^-$: $\log K_1 = 17.5$; $[Fe(L)_2]^{4-}$: $\log K_2 = 14.6$ and $\log \beta_2 = 32.06$.

Inferences of Ligand Binding from the pH Titrations

From the titration of GSH in the absence of metal up to pH 6 one proton is titrated per GSH (Fig. 1) according to eqn. (2).

(2)

*pKa values at zero ionic strength [15]: $pK_1 = 2.19$; $pK_2 = 3.45$; $pK_{123} = 9.2$; $pK_{1234} = 9.95$.

When the oxidised ligand (GSSG) was titrated to the same pH, two protons were titrated for each GSSG (Fig. 2 and Table I). Once the two protons are removed from the two carboxylate groups (COOH Gly) on the GSSG, then the other titration data can be evaluated for evidence of metal binding by comparing the moles of protons titrated to the known pKa's of the ligands [2] present.

It can be observed from the initial pH values of the mixed solutions of iron(III) chloride and GSH (Table II), that there is no obvious release of more protons upon mixing. On the contrary, the system was found to absorb a proton (see eqn. (1)).

When GSH–iron(III) mixtures were titrated to the first end point (pH 6) an extra proton was released in all cases in addition to that released by the GSH alone when titrated (Table I). To explain where the extra proton originates, the constituents of the solution before and after mixing must be discussed.

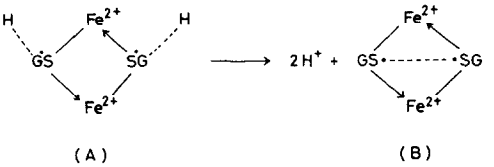
Iron(III) when dissolved in water releases one proton and when reacted with GSH another proton must be released to form GSSG according to the continuation of eqn. (1) *viz*:

$$[Fe^{II}(H_2O)_x\dot{G}S]^{2+} + (6 - x)(H_2O) \longrightarrow [Fe^{II}(H_2O)_6]^{2+} + \tfrac{1}{2}GSSG \qquad (1a)$$

Because of the presence of iron(III) originally there must be another proton in solution which was titrated to the first end point along with the proton on the GSH carboxylate group. This proton must be that from the thiol group, its pKa lowered by metal complexation but somehow still associated with the complex until the first end point is reached.

Thus, though eqn. (1a) shows a separation of GSSG from the iron(II), the data presented here does not support this. We will return to this point later in the text.

The intermediates for eqn. (1a) must include A and B below



(1b)

Between species A and B one proton (the second in total) per GSH is released. This step takes place above pH 3 but below pH 6.

Thus, this scheme is compatible with the release of two protons per GSH in the redox process, the first from the carboxylate group and the second from the SH group. However, the proton from the

TABLE IV. Mössbauer Parameters for Iron–Glutathione Mixtures in Aqueous Solutions at Various pH Values; All Solutions were prepared Anaerobically. Spectra were recorded at 80 K.

Spectrum No.	Solution	pH	δ (mms^{-1})	Δ (mms^{-1})	Γ (mms^{-1})
1	$FeCl_3$/3GSH [$FeCl_3$] = 0.2 *M*	1.0	1.40(1)	3.19(2)	0.24(1)
2	$FeCl_3$/GSH	1.9	1.41(1)	3.13(1)	0.39(1)
3	$FeCl_3$/GSH	2.3	1.41(1)	3.11(1)	0.34(1)
4	$FeCl_3$/3GSH	3.0	1.36(2)	2.98(4)	0.41(3)
5	$FeCl_3$/3GSH	7.0	1.32(4)	3.00(4)	0.37(3)
6	$FeCl_2 \cdot 4H_2O$/3GSH [$FeCl_2$] = 0.2 *M*	2.0	1.41(1)	3.15(2)	0.26(2)
7	$FeCl_2 \cdot 4H_2O$/3GSH	3.0	1.40(1)	3.08(2)	0.26(1)
8	$FeCl_2 \cdot 4H_2O$/3GSH	5.8	1.38(1)	3.02(2)	0.31(2)
9	$FeCl_2 \cdot 4H_2O$/3GSH	6.8	1.26(1)	3.07(2)	0.32(2)
10	$FeCl_2 \cdot 4H_2O$/1.5GSSG [$FeCl_2$] = 0.3 *M*	2.4	1.40(1)	3.12(2)	0.25(1)
11	$FeCl_2 \cdot 4H_2O$/1.5GSSG	3.0	1.38(1)	3.08(1)	0.24(1)
12	$FeCl_2 \cdot 4H_2O$/1.5 GSSG	5.9	1.38(1)	3.00(2)	0.30(2)
13	$FeCl_2 \cdot 4H_2O$/1.5 GSSG	7.1	1.33(2)	2.86(4)	0.53(4)
14	$FeCl_3$/3cysteine [$FeCl_3$] = 0.2 *M*	2.6	1.36(1)	3.27(1)	0.28(1)
15	$FeCl_3$/3 cysteine [$FeCl_3$] = 0.2 *M*	7.1	0.987(1)	3.334(5)	0.447(3)
16	$FeCl_3$/thioglycolic acid [$FeCl_3$] = 0.2 *M*	7.0	0.988(5)	3.151(12)	0.386(11)

SH groups, if released immediately into solution when the GSH radicals combine to form GSSG would have been present and manifested itself as a depression in pH. This did not occur and so this proton must be associated with the iron-glutathione complex until the pH is raised.

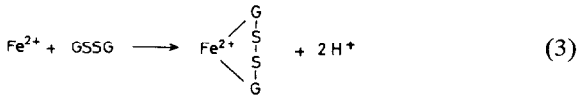
Confirmatory evidence of this explanation comes from the titration data in Table I. The following points are important.

1. The $FeCl_2$–2GSH reaction only releases one proton per GSH (only the carboxylic acid and not that of the SH group).

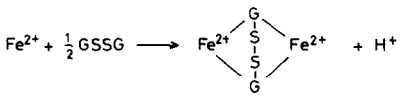
2. The $FeCl_2$–2GS-CH_3 reaction behaves the same as $FeCl_2$–GSH releasing only the carboxylic acid proton (the $GSCH_3$ does not have a sulfhydryl group).

3. The $FeCl_2$–GSSG mixtures only release the protons of the carboxylate groups, *i.e.* the same number of protons released by the GSSG itself when titrated to that end point. It might have been expected that this reaction should have absorbed a proton and then behaved like the Fe^{3+} + GSH (1:1) system if the sulphur in GSSG was bound to Fe(II). The fact that it does not, suggests that the final complexes formed in the ($2Fe^{2+}$-GSSG) mixture and the Fe^{3+}–GSH (1:1) do not contain sulphur bound to iron(II). Indeed, there are no reports of iron(II) bound to the sulphur atoms in oxidised thiols [2, 3].

The experimental evidence for the iron(II) reactions with GSSG and GSH are compatible with the following reactions:



(3)



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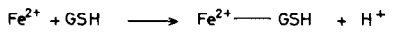

(5)

The protons released in eqns. (3–5) originate from the glycinyl carboxylate groups on GSH or GSSG, contrary to the reaction of iron(III) with GSH which releases two protons as discussed above.

Mössbauer Studies in the pH Range 3 to 7 on Frozen Aqueous Solutions of Iron-Glutathione and Other Thiols

The Mössbauer data are presented in Table IV. All the frozen solutions of iron(III)–GSH, iron(II)–GSH, iron(III)–cysteine and iron(II)–GSSG at pH 3 or below contained high spin iron(II). We have previously reported that the iron(II) is bonded to the thiols or oxidised thiols by their carboxylate groups [7]. It was observed that a change in the quadrupole splitting takes place at pH 3, which is indicative of changes in bonding or coordinating ligands on the metal.

In Table IV the effect of increasing the pH from 1 to 7 on the iron–glutathione system is shown. The Mössbauer data of pH 3.0 and below have been previously discussed [7]. Upon raising the pH to 7.0 for the iron(III) and iron(III)–GSH solutions there are no obvious changes in the Mössbauer data indicating only carboxylate and possible amide nitrogens bound to iron(II).

For the iron(II)–GSSG system the Mössbauer data at 7.0 are different to that of iron(III)–GSH frozen solution (which contained iron(II), GSH and GSSG). This is indicative of the iron(II) in the latter solution bound to GSH at pH 7.

From the titration data for the iron(II)–GSSG system no evidence for iron(II) bound to sulphur was found. It is therefore likely that the Mössbauer data here reflect an iron(II) environment that contains bonds to carboxylates of the GSSG but the iron(II) electronic environment is altered compared to that in the presence of GSH.

The broadening in the line widths which occurs at pH 5.9 may imply the presence of superimposed doublets from similar but not identical iron environments [19].

Other thiols, *i.e.* L-cysteine [16] and thiolglycol [17, 18] were studied to compare their behaviour with that of GSH (spectra numbers 14, 15 and 16, Table IV). At pH 2.6 iron(II)–cysteine frozen solutions gave Mössbauer parameters indicative of only oxygen binding to iron(II) ($FeCl_2 \cdot 9H_2O$) [7]. Upon increasing the pH to 7.1, the isomer shift decreased to 0.987 mms^{-1} and the quadrupole splitting increased to 3.334 mms^{-1}; this indicates a different iron(II) coordination with cysteine such as formation of sulphur bonds with the iron(II) [15].

TABLE V. Magnetic Moments for Iron(III)–GSH and Iron(II)–GSH Mixtures in Aqueous Solutions by Evans' Method at Various pH Values (Temperature 26 °C).

	pH	μ_{eff} (B.M.)[a]
$FeCl_2$/2GSH	3.94	5.39 ± 0.26
$FeCl_2$/2GSH	6.25	5.27 ± 0.14
$FeCl_2$/3GSH	3.0	5.5 ± 0.1
$FeCl_2$/3GSH	4.0	5.5 ± 0.1
$FeCl_2$/3GSH	6.5	5.5 ± 0.1
$FeCl_2$/3GSH	7.4	5.32 ± 0.05
$FeCl_3$/GSH	3.2	5.58 ± 0.25
$FeCl_3$/3GSH	2.57	5.36 ± 0.34
$FeCl_3$/3GSH	2.98	5.15 ± 0.09
$FeCl_3$/3GSH	4.0	5.18 ± 0.06
$FeCl_3$/3GSH	6.8	5.33 ± 0.05

[a] χ was corrected for water and chloride.

The thiolglycol-iron(III) frozen solution at pH 7.1 showed iron(II) with a similar isomer shift to the (cysteine–iron(II) 0.98 mms^{-1} and smaller quadrupole splitting [18]. The crystal structure of $[Fe^{2+}(SCH_2COO)\cdot H_2O]_n$ is known [17] and Mössbauer parameters for this material are similar to those of the thiolglycol–iron(III) frozen solution. This provides evidence in favour of the involvement of a nitrogen ligand along with the sulphur in binding the iron(II) at pH 7 in the iron–cysteine system. It is important to note that up to pH 7.1 GSH did not show comparable parameters to those of cysteine–iron(II), or thiolglycol–iron(II), this may be due to the lower pKa values of the SH group in the latter two compounds and to the smaller molecular size (*i.e.* the shift in the pKa of SH groups caused by iron is greater in the case of cysteine and thiolglycol than in the GSH).

It must be noted that even in the event of rapid freezing equilibria may shift during cooling [19]. If this happens then Mössbauer parameters for the frozen solution reflect the structure not of the initial room temperature solution, but of the solution at the solidification temperature [19].

Magnetic Studies on Aqueous Solutions Containing Iron(III) or Iron(II) with GSH in the pH Range 3 to 7

The values for the magnetic moments (Table V) obtained in both the iron(III)/GSH and the iron(II)/

GSH are of the same order of magnitude within experimental error and both imply the presence of high spin iron(II), in the pH range 2 to 7.

The μ_{eff} value changed slightly from 5.3 B.M. to 5.5 when the pH was increased from 2 to 3. In the pH range 3 to 7 the change in μ_{eff} is negligible. Above pH 7 the solution study was interrupted by precipitation, but one additional measurement was taken before precipitation took place in the iron(III)/GSH mixture (μ_{eff} 5.3 B.M.). This value indicates a small decrease in μ_{eff} above pH 7. The value of 5.4 B.M. is similar to that found for iron(II) high spin bound by oxygen chelating ligands [20]. No evidence is found for the presence of radicals in these systems from the magnetic measurements, though such evidence has previously been found for radicals in catechol systems by this method [21].

Conclusions

Evidence has been presented that illustrates the complexity of the solution chemistry of the iron–glutathione system in the pH range 3 to 7. By comparing the chemistry of other thiols no evidence is found for iron(II) bound to sulphur with GSH in this pH range, nor is evidence found for stable GSH radical species bound to iron(II).

As stated in the text, eqn. (1a) shows a separation of GSSG from the iron(II). The Mössbauer data even at pH 7 does not support this unless excess GSH is present. The fact that the Mössbauer data for an iron(II)/GSSG mixture was different to both iron(II) and iron(III)/GSH mixtures suggests that the iron(II) does not dissociate from GSSG or indeed GSH even on quench freezing. It must therefore be concluded that the carboxylate groups of GSSG and GSH bind iron(II) in the pH range 3 to 7, and that the amide groups may also bind but the sulphurs do not. No evidence was found for deprotonation of the amide groups of GSH in this system unlike the Cu^{2+} and Ni^{2+} GSH systems [3].

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