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Studies of the Reactions of Ferric Iron with Glutathione and some Related Thiols. Part V. Solid Complexes Containing Fe^{II} and Glutathione or Fe^{III} with Oxidized Glutathione

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Abstract

A number of polymeric iron(III) oxidised gluta-thione materials have been prepared. Mössbauer spectroscopic, magnetic susceptibility data and electronic reflectance spectra studies on these iron-(III) materials are discussed.

Two stoichiometries 1:1 and 1:2 have been established for iron(II)–glutathione materials. Spectroscopic studies show that the 1:1 materials contain high spin iron(II) in distorted five and six coordinated environments, whereas the 1:2 materials contain only distorted six-coordinated high spin iron(II). The nature of the coordinating ligands are discussed.

Introduction

In the previous papers in this series [1–4] and in a related paper [5] we have reported the results of systematic studies on iron glutathione (GSH) chemistry. We have demonstrated that when ferric salts react with GSH in all cases the final product contains iron(II) [2]. At low pH kinetic experiments have shown the presence of blue intermediates on the reaction pathway, and we have discussed these in relation to possible binding sites [1].

Studies on the iron catalyzed oxidation of GSH by molecular oxygen using rapid kinetic measurements indicated the production of a transient red species, the rate of production of which was oxygen concentration dependent and required one oxygen per two iron atoms [3]. Mössbauer data indicated

the red complex contained iron(III). A scheme for the iron catalyzed oxidation of GSH by molecular oxygen was presented.

We have also reported a study of the reaction of GSH with protoporphyrin IX iron(III); little evidence for reduction of the Fe(III) in the porphyrin was found [4].

From our solution studies it became apparent that all the potential binding sites of GSH (the two carboxylic acid groups, the amino group, the sulph-hydryl group and the two amide functions) had a role to play in the reaction of the molecule with Fe(III) ions. We therefore concluded that it would be worthwhile to attempt to produce solid complexes from iron with both GSH and GSSG to attempt to elucidate preference of binding ligands to the oxidation state of the iron.

Solid complexes of Fe(II) with GSH are scarce in the literature [6, 7] though some work on frozen solutions has been carried out [1–3, 8]. However little chemical characterisation has been carried out and the nature of the solid complexes are not well understood [6, 8].

Although reports on the characterisation of iron GSH or oxidised GSH (GSSG) complexes are scarce there are many reports of the coordination chemistry of other transition metals with these ligands. Zinc [9, 10], cadmium [10], cobalt [11], manganese [11, 12] and copper [13] complexes of GSH and GSSG have been investigated. In a recent study the crystal structure of the Cu^{2+} complex Na$_4$[Cu$_2$GSSG]·6H$_2$O [13] was reported; each Cu^{2+} is bound to a sulphur, two deprotonated peptide nitrogens, N (Glutamyl) and oxygen of the carboxyl-ate group of the glycine residue, in a square pyrami-dal environment.

In order to further our investigations of the binding of iron to GSH and GSSG we have carried out studies on solid products isolated from these systems, using infrared, UV-visible reflectance and

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Mössbauer spectroscopies. These studies have allowed us to characterise a number of materials containig Fe(II) or Fe(III), and to demonstrate the sensitivity of the Fe(II) complexes to oxidation.

Experimental

Instrumental

Infrared spectra were recorded from mulls using a Perkin-Elmer 257 spectrometer. Electronic reflectance spectra of powders were recorded on a Unicam SP 700 spectrophotometer using magnesium oxide as reference.

Mössbauer spectra were recorded at 80 °K using the system described in previous papers [1, 2]. The data were computer fitted. All isomer shifts are referred to natural iron.

Magnetic susceptibilities of powders were measured by the Gouy method at room temperature. Tubes were packed several times to check for orientation errors.

Analyses were carried out by the Microanalytical Laboratories, Department of Chemistry, University of Manchester, and are given in Table I.

Materials

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

Preparation of Iron(II)-Oxidised Glutathione Solid Complexes

[Fe₂(GSSG)Cl₂]·4H₂O
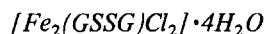
0.05 M $FeCl_3$ solution was mixed with 0.075 M GSSG in a total volume of 10 ml. A reddish-orange colour appears upon mixing indicating a new complex. Addition of NaCl with continuous stirring, precipitated a pale pink polymeric solid, which turns to a red glassy material when filtered and left to dry (probably due to absorption of water of crystallisation).

The same procedure was used for the preparation of $[Fe_{1.67}(GSSG)NO_3 \cdot HNO_3] \cdot 4H_2O$ and $[Fe_{1.22}{}^{III}(GSSG)H_2SO_4] \cdot 15H_2O$ using $NaNO_3$ and Na_2SO_4 respectively. The nitrate complex was a dark red glass when dried, the sulphate was a pink powder.

[Fe₂(GSSG)(OH)Cl]·4H₂O

0.05 M $FeCl_3$ was mixed with 0.075 M oxidised glutathione solution in 10 ml total volume. NaOH (1 M) was added dropwise with continuous stirring, at the same time monitoring the pH. At pH ≃ 3 a yellow solid precipitated, which was filtered and dried over P_2O_5 to a yellow powder.

[Fe₁.₃₃(GSSG)]·6H₂O

Was prepared as a brown solid at pH 7.0, starting with an iron(II)–GSSG mixture and adjusting the pH to 7.0 under a nitrogen atmosphere to avoid precipitation of the above yellow complex at pH 3.0.

TABLE I. Elemental Analysis (%) of the Iron(III)–GSSG and Iron(II)–GSH Complexes.

Formula	% Fe	% C	% H	% N	% S	% Cl
$[Fe_2{}^{III}(GSSG)Cl_2] \cdot 4H_2O$	13.0 (12.94)[a]	27.0 (27.83)	4.5 (4.17)	9.5 (9.74)	7.9 (7.43)	7.9 (8.22)
$[Fe_2{}^{III}(GSSG)(OH)Cl] \cdot 4H_2O$	12.4 (13.2)	28.6 (28.45)	4.5 (4.26)	9.9 (9.95)	6.57 (7.59)	4.1 (4.20)
$[Fe_{1.67}{}^{III}(GSSG)(NO_3 \cdot HNO_3] \cdot 4H_2O$	9.9 (10.36)	27.0 (26.73)	4.0 (4.12)	12.1 (12.446)	7.0 (7.14)	–
$[Fe_{1.33}{}^{III}(GSSG)] \cdot 6H_2O$	9.4 (9.39)	30.2 (30.38)	4.75 (5.05)	10.3 (10.62)	8.1 (8.11)	–
$[Fe_{1.22}{}^{III}(GSSG)H_2SO_4] \cdot 15H_2O$	6.3 (6.25)	22.2 (22.21)	5.36 (5.54)	8.9 (7.77)	9.5 (9.49)	–
$K_2[Fe(GS)_2] \cdot 7H_2O$	7.9 (6.42)	27.6 (27.60)	9.3 (9.65)	4.75 (5.05)	7.8 (7.37)	8.8 (8.98)
$Na_2[Fe(GS)_2] \cdot 7H_2O$	7.2 (6.66)	29.2 (28.65)	9.8 (10.03)	4.9 (5.25)	8.5 (7.65)	3.0 (5.49)
$K_{1.08}Fe_{1.21}(GS)(OH)_{1.5} \cdot 2H_2O$ (yellow material)	14.3 (14.18)	25.4 (25.2)	8.6 (8.82)	4.3 (4.30)	6.4 (6.73)	9.0 (8.86)
as above formula (purple material – see Experimental)	14.5	24.9	8.2	4.6	6.4	8.7

[a]Values in parentheses are calculated.

Open access to air precipitates a brown solid. The brown solid was filtered, washed with water and dried.

All the microanalysis data appear in Table I.

Iron(III)–GSSG Freeze Dried Materials

The following solutions were prepared:
(1) $FeCl_3$/GSSG 1:1 pH 2.2
(2) $FeCl_3$/GSSG 2:1 pH 2.2

These were frozen in liquid nitrogen then freeze dried. The dry samples were then mounted in cells (for Mössbauer spectroscopy).

Preparation of Iron(II)–Reduced Glutathione Solid Complexes

$K_2[Fe(GS)_2] \cdot 7H_2O$

Iron(II) chloride tetrahydrate (6.6 mmol) was mixed with 13.2 mmol reduced-crystalline gluthathione in degassed water in a total volume of 25 ml. When the pH was adjusted to ≈ 6.5 using degassed, concentrated KOH, a deep yellow colour developed. The solution was stirred under a nitrogen atmosphere for 1 h, when an off-white precipitate was formed. This precipitate was filtered under a nitrogen atmosphere, washed with degassed water and left to dry under a stream of nitrogen. The solid was very sensitive to air; if left under aerobic conditions it changed colour to pink if dry, and intense red if damp.

The analytical data are given in Table I.

$Na_2[Fe(GS)_2] \cdot 7H_2O$

This material was prepared as for the K salt above. The analytical data in Table I show that the Na content was low. However $Na[Fe(GS)_2] \cdot 7H_2O$ requires Fe 6.85; C 29.44; H 5.39; N 10.31; S 8.1 and Na 2.82, which fits the data in Table I much better for all but H and N.

Charge balance would be different in the latter material when compared to the K material, and either additional H^+ would be needed, or the high Fe analysis may compensate the charge replacing Na^+ in the formula. We support the latter alternative though no evidence of an additional Fe environment in the Mössbauer data was found. We refer the reader to discussion in ref. 13 on sodium ions where similar problems arose.

$K_{1.08}Fe_{1.21}(GS)(OH)_{1.5} \cdot 2H_2O$

This material was isolated using a similar procedure. 6.6 mmol of $FeCl_2 \cdot 4H_2O$ and 6.6 mmol of GSH were mixed. The colour of this complex upon precipitation is yellow and when filtered and left to dry a dark layer developed on the surface. The bulk constituent was a yellow glassy material. This complex was more stable than the $Fe^{II}(GS)_2$

complexes and a bottled sample remained several months without oxidizing.

When the 1:1 complex was prepared in ethanol/ water solution it was purple in colour upon precipitation, but when dried under a stream of nitrogen it has the same features as that isolated from aqueous solution.

The analysis for both these materials appear in Table I.

Results and Discussion

The chemical analytical data for all the solid complexes are reported in Table I. All the $Fe^{II}GSH$ and $Fe^{III}GSSG$ materials prepared in this work as solids were insoluble. This fact taken together with the analytical data (Table I) lead us to suggest that these materials are polymeric in nature.

Iron(III)–Oxidized Glutathione Solid Complexes

The Mössbauer parameters for the Fe(III)–oxidised glutathione complexes are presented in Table II. The range of QS varies from 0.70 mms^{-1} for the nitrate containing complex to 0.97 mms^{-1} in the hydroxide containing complex. Larger QS values have previously been linked to materials containing coordinated anions [14].

For the complex $[Fe_2(GSSG)(OH)Cl] \cdot 4H_2O$ Mössbauer spectra were collected at various temperatures (Table II and Fig. 1). The asymmetry in these spectra can be explained in one or two ways; either it is due to the polymeric nature of the complex (as explained by Goldanskii [15]), or to paramagnetic spin relaxation [14–16]. A room temperature magnetic susceptibility of 4.96 BM was obtained for $[Fe_2(GSSG)(OH)Cl] \cdot 4H_2O$. This value is lower than expected for high-spin Fe^{III} complexes [17]. The μ_{eff} value of 4.96 BM assumes one iron atom, so if two are assumed a value of 3.52 BM is found for each* (a value of 2.97 BM was obtained for the complex $[Fe(glutathione)(OH)(H_2O)_2](SO_4)_2 \cdot 6H_2O$ [7]). Our value of 3.52 BM corresponds to 3 unpaired electrons per Fe^{III} which can be explained by antiferromagnetic coupling between two iron nuclei through a metal–metal bond or a bridging ligand [7, 18, 19].

Similar values of μ_{eff} are always found to result from two $S = 5/2$ nuclei with varying coupling constants [7, 19]. Temperature variation experiments carried out by Jezowska *et al.* [7] gave the best fit to two high spin Fe^{III} ions with $S = 5/2$ and $J = 130$ cm^{-1}.

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*μ_{eff} was calculated using the equation: $\chi = \chi_g \, 100/y$ at. wt.n; where $y = \%$ metal, $n =$ no. of atoms of metal per molecule. Thus $\mu_{eff} \, (n = 2) = \sqrt{2}\mu_{eff} \, (n = 1)$.

TABLE II. Mössbauer Parameters of Iron(III)—oxidized Glutathione Solid Complexes at 80 °K and Spectra of the Complex [Fe$_2$(GSSG)(OH)Cl]·4H$_2$O, at 180 °K and 298 °K.

No.	Complex		Spectra fitted to two singlets				Calculated doublet		Colour
			δ_1 (mms^{-1})	δ_2 (mms^{-1})	$\Gamma_1{}^a$ (mms^{-1})	$\Gamma_2{}^a$ (mms^{-1})	δ (mms^{-1})	Δ (mms^{-1})	
1	[Fe$_2$(GSSG)Cl$_2$]·4H$_2$O		0.220(8)	0.966(4)	0.251(7)	0.207(3)	0.59(1)	0.75(1)	Red glass
2	[Fe$_2$(GSSG)(OH)Cl]·4H$_2$O		0.101(1)	0.975(2)	0.294(6)	0.302(8)	0.54(1)	0.84(1)	Yellow powder
3	[Fe$_{1.67}$(GSSG)NO$_3$·HNO$_3$]·4H$_2$O		0.185(6)	0.885(7)	0.247(5)	0.214(7)	0.54(1)	0.70(1)	Red glass
4	[Fe$_{1.33}$(GSSG)·6H$_2$O		0.07(2)	1.03(2)	0.36(2)	0.34(3)	0.55(4)	0.97(4)	Brown powder
5	[Fe$_{1.22}$(GSSG)H$_2$SO$_4$]·15H$_2$O		0.191(4)	0.946(4)	0.270(1)	0.251(3)	0.57(1)	0.76(1)	Pink powder
6	Parameters of	298 °K	0.005(4)	0.821(4)	0.242(7)	0.251(8)	0.41(1)	0.82(1)	
	complex (2), at different	180 °K	0.054(5)	0.911(5)	0.261(9)	0.28(1)	0.48(1)	0.86(1)	
	temperatures taken on	80 °K	0.086(4)	0.946(5)	0.257(8)	0.261(9)	0.52(1)	0.86(1)	
	a fresh preparation								

[a]Half width at half height.
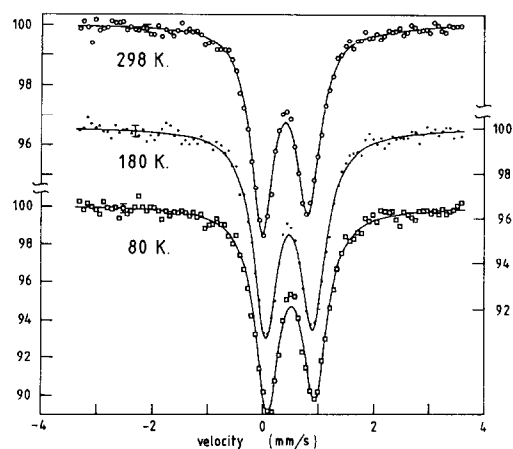
Fig. 1. Temperature dependence of Mössbauer spectra of the [Fe$_2$(GSSG)(OH)Cl]·4H$_2$O complex.

Therefore the Mössbauer parameters and the magnetic susceptibility of [Fe$_2$(GSSG)(OH)Cl]· 4H$_2$O are concomitant with two antiferromagnetically coupled Fe^{III} nuclei.

Reflectance spectra for the iron(III)—GSSG complex (compared to FeCl$_3$·6H$_2$O) are presented in Fig. 2, and Table III. Compounds 1—4 all contain an intense band in the range 38,000 to 27,500 cm^{-1} which also occurs in FeCl$_3$·6H$_2$O from 38,000 to 21,500 cm^{-1}. It is due to ligand to metal charge transfer similar to that found in K$_3$Fe(CN)$_6$ [20]. The band edges that reach into the visible region cause the colours given in Table III. The less intense broad band *ca.* 11,000 cm^{-1} seen in all the complexes and also in FeCl$_3$·6H$_2$O [21] which contains the octahedra [Fe(H$_2$O)$_4$Cl$_2$]$^+$ are most probably due to d—d transitions.

Unfortunately the infrared spectra of the Fe^{III}-GSSG complexes were too crowded to interpret usefully.
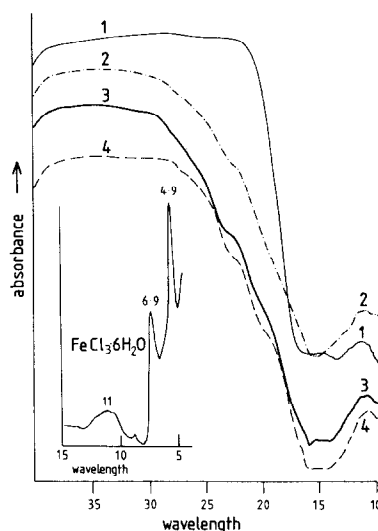

Fig. 2. Electronic reflectance spectra of (1) FeCl$_3$·6H$_2$O; (2) [Fe$_2$(GSSG)(OH)Cl]·4H$_2$O; (3) [Fe$_{1.22}$(GSSG)H$_2$SO$_4$]· 15H$_2$O; (4) [Fc$_2$(GSSG)Cl$_2$]·4H$_2$O powder samples, spread on a white filter paper using white filter paper reference (Table III).

Freeze Dried Samples of Iron(III)—Oxidized Gluta-thione Materials

The 1:1 and 2:1 FeCl$_3$ to GSSG combinations gave Mössbauer parameters that are typical of high spin Fe^{III} (Table IV). The QS of the 1:1 complex was larger than that of the 2:1 and is similar to those of solids 3 and 5 of Table II. The 1:1 complex which was aged in the air (it formed a glasslike material due to adsorption of water) gave rise to a slightly smaller QS (Fig. 3) (like that of complex 2, Table II) which was temperature independent.

The electronic reflectance spectra of these samples are presented in Fig. 4. The 1:1 material gave a broad charge transfer band with a shoulder at 22,500

TABLE III. Electronic Spectra of Fe(III)–GSSG Solid Complexes (Samples were spread on filter paper).

No.	Complex	Colour	Band ($v \times 10^{-3}$ cm^{-1})[a]
1	[Fe$_2$(GSSG)Cl$_2$]·4H$_2$O	Red	38–27.5(br) 22.8(sh) 19(sh) $\approx$ 11(br)
2	[Fe$_{1.67}$(GSSG)NO$_3$·HNO$_3$]· 4H$_2$O	Red	38–27.5(br) 22.8(sh) 19(sh) $\approx$ 11(br)
3	[Fe$_{1.22}$(GSSG)H$_2$SO$_4$]·15H$_2$O	Pink	38–29(br) 23(sh) $\approx$ 12(br)
4	[Fe$_2$(GSSG)(OH)Cl]·4H$_2$O	Yellow	38–29(br) $\approx$ 12(br)
5	FeCl$_3$·6H$_2$O	Yellow	38–21.5(br) $\approx$ 11(br) 6.88 4.90

[a]br = broad; sh = shoulder.

cm^{-1} whilst the 2:1 showed a narrower band and no visible absorption.

Iron(II)–Reduced Glutathione Complexes

The Mössbauer parameters of the iron(II)–reduced glutathione 1:1 and 2:1 complexes are in Table V. The parameters for the 1:1 and 2:1 Fe:GSH complexes are quite different and for clarity each set will be discussed separately.

For the 1:1 Fe^{II}–GSH the Mössbauer spectra of these complexes show two Fe^{II} high spin doublets. In addition the purple K$_{1.08}$Fe$_{1.2}$(GS)(OH)$_{1.5}$·2H$_2$O material also contained around 10% of an Fe^{III} species (Fig. 5). The analytical data for both the yellow and purple materials is similar and rules out the existence of major iron impurities; thus the two Fe^{II} high spin doublets must be due to two non-equivalent electronic environments as reported for the iron(II)–cysteine methyl ester complex [22].
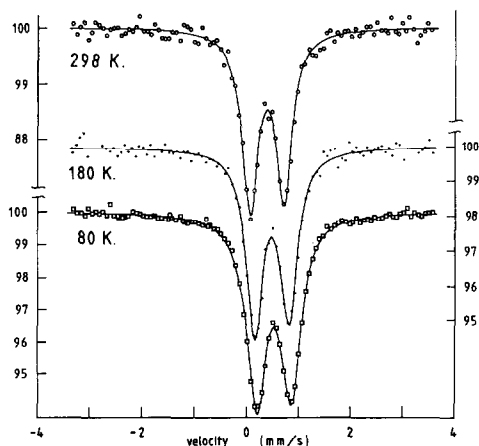
Fig. 3. Temperature dependence of Mössbauer spectra of freeze-dried material of FeCl$_3$–GSSG (1:1) at pH 2.2 (Table IV) (aged red glass).
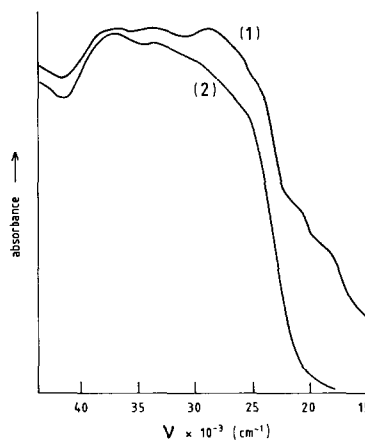
Fig. 4. Electronic reflectance spectra of freeze-dried materials of (1) FeCl$_3$–GSSG (1:1) mixture; (2) FeCl$_3$–GSSG (2:1) mixture, pH 2.2. Conditions as in Fig. 2.

The observed isomer shifts for site (a) of complex 1 (Table V) are consistent with the presence of high-spin iron(II) [23, 24] and are similar to those containing nitrogen [25] or sulphur donor ligands [22, 25–27]. The large QS of 3.79(2) mms^{-1} is similar to values obtained for pentacoordinated

TABLE IV. Mössbauer Parameters of Iron(III)–GSSG Freeze-Dried Materials.

No.	Complex	T (°K)	δ (mms^{-1})	Δ (mms^{-1})	Γ (mms^{-1})
1	FeCl$_3$/GSSG (1:1) (orange) fresh	298	0.32(1)	0.72(1)	0.24(2)
2	2FeCl$_3$/GSSG (2:1) (yellow) fresh	298	0.43(1)	0.57(1)	0.21(1)
3	Sample 1 aged (turned red-glass)	298[a]	0.42(1)	0.65(1)	0.18(1)
		180[a]	0.50(1)	0.67(1)	0.19(1) 0.20(1)
		80[a]	0.53(1)	0.67(1)	0.23(0) 0.23(1)

[a]Slight asymmetric, % absn. area = 47 and 53.

TABLE V. ^{57}Fe Mössbauer Parameters for the Iron(II)–GSH Complexes.

No.	Complex	Site	T (°K)	δ (mms^{-1})	Δ (mms^{-1})	Γ^a (mms^{-1})	% Absn. area
1	$K_{1.08}Fe_{1.21}(GS)(OH)_{1.5} \cdot 2H_2O$ (yellow material)	a	298	0.98(2)	3.69(1)	0.13(1)	39.6(5.6)
		b		1.06(3)	2.63(7)	0.38(5)	60.4(5.0)
		a	80	1.10(2)	3.80(2)	0.17(2)	45.0(3.7)
		b		1.24(2)	2.98(3)	0.25(8)	55.0(5.5)
2	$K_{1.08}Fe_{1.21}(GS)(OH)_{1.5} \cdot 2H_2O$ (purple material)	a	80	1.13(4)	3.79(1)	0.17(1)	34(1)
		b		1.25(2)	2.93(2)	0.30(2)	57(2)
		Fe^{III}		0.50(2)	0.61(3)	0.21(2)	9(1)
3	$Na_2Fe(GS)_2 \cdot 7H_2O$		298	1.16(1)	1.89(2)	0.13(1)	100(3)
4	$K_2Fe(GS)_2 \cdot 7H_2O$ (fresh)^b (off white)		298	1.15(2)	1.99(2)	0.17(1)	100(2)
			298	1.16(2)	1.94(2)	0.18(1)	100(2)
5	$K_2Fe(GS)_2 \cdot 7H_2O$ (aged) (partially oxidised pink)	Fe^{II}	298	0.98(1)	2.39(2)	0.20(2)	
		Fe^{II}		0.47(1)	0.77(1)	0.25(1) 0.30(1)	
		Fe^{III}	180	1.15(1)	2.68(2)	0.22(2)	
		Fe^{III}		0.49(1)	0.86(1)	0.26(1) 0.30(1)	
		Fe^{II}	80	1.28(1)	3.00(1)	0.19(1)	
		Fe^{III}		0.52(1)	0.90(7)	0.28(1) 0.31(1)	
6	$FeCl_3/3GSH$ (white) freeze dried material		298	1.19(1)	2.06(2)	0.34(2)	
7	$FeCl_3/3GSH$ (pink) (sample 6 aged)		180	1.31(1)	2.36(2)	0.33(2)	54(2)
				0.49(1)	0.94(1)	0.20(2)	46(2)
			80	1.38(2)	2.61(3)	0.27(2)	54(2)
				0.49(1)	1.03(2)	0.21(1)	46(2)

^{a}Halfwidth at half height. ^{b}Results from two different samples.
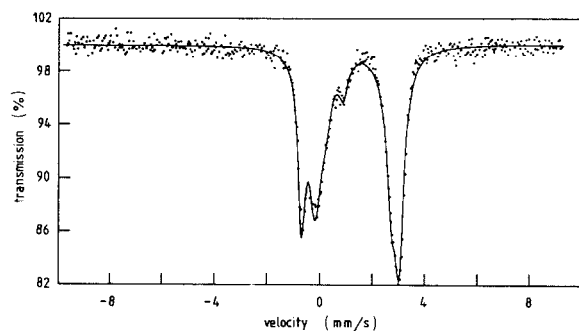
Fig. 5. Mössbauer spectrum at 80 °K of $K_{1.08}[Fe_{1.21}(GS)-(OH)_{1.5}] \cdot 2H_2O$ purple complex, isolated from ethanol/water solvent (number 2, Table V).

iron(II) ions [22, 25–31] (Δ = 3.57 mms^{-1} [22], 3.72–4.30 mms^{-1} [25–31]). Such complexes have been the cause of much controversy in the literature [25, 26, 31, 32] over whether they represent distorted trigonal bipyramidal or distorted square pyramidal structures [25, 31, 32]. We and others have recently reported porphyrin iron(II) Mössbauer data for square pyramidal Fe(II) environments with QS values in the range (4.0–4.3 mms^{-1}) [28–30]

which materials also had isomer shifts in the range 1.0 to 1.10 mms^{-1}. The large QS values for the five coordinate complexes are assumed to be caused by large contributions of like sign from both the nonspherical electron distribution and the ligand field dissymmetry. The contribution to QS from the dissymmetry of the d-electron population will depend on the nature of the ground state. Under a tetragonal distortion the $^5T_2$ state (O_h) is split into $^5B_2$ and ^5E states. The magnitude of the contributions to the QS from a 3d electron in either a $^5B_2$ or ^5E ground state are equal but of opposite signs [25].

Site (a) in the 1:1 complex can be compared with other sulphur bridged dimer complexes of pentacoordinate Fe^{II} ions in distorted trigonal bipyramidal environments [23]. These compounds have thiolate bridges, so the 1:1 complex reported here may also be a dimer. The fact that the QS of our material is higher than those of the $(FeL)_2$ [23] complexes is consistent with a geometry closer to square pyramidal [28–31].

The observed temperature dependence of the isomer shift for complex 1 (Table V) is expected from the second order Doppler effect [23]. The temper-

ature dependence of the QS in this material is small compared to that of complex **5** (Table IV) but still indicative of the presence of thermally accessible excited states [22, 23, 25].

The Mössbauer parameters for site (b) complexes **1** and **2** (Table V) are likely to represent high spin Fe^{II} distorted octahedral environments. The temperature dependence of the QS confirms the availability of low-lying excited states indicating a departure from cubic symmetry [33]. It is important to note that the Mössbauer parameters for site (b) (complexes **1** and **2** of Table IV) at 80 °K are similar to those found for the frozen solutions (Table IV of ref. 3, spectra 5 and 9), but unfortunately the temperature dependence of the latter QS data was not established [3].

For the 1:2 iron(II) glutathione complexes K_2-$Fe(GS)_2 \cdot 7H_2O$ and $Na_2Fe(GS)_2 \cdot 7H_2O$ complexes **3** and **4** (Table V), both the K and Na salts gave similar isomer shifts and quadrupole splittings indicating that the cations do not directly affect the electronic environments. The isomer shifts of $1.15(2)$ mms^{-1} and QS values of these complexes are similar to that found in $FeCl_2 \cdot H_2O$ [34] at room temperature ($\delta = 1.13$ mms^{-1} and $\Delta = 2.03$ mms^{-1}); they are indicative of high-spin iron(II) in a distorted octahedral environment. The most likely ligands for the Fe^{II} in these $M_2Fe(GS)_2 \cdot 7H_2O$ materials are the sulphur, the carboxylate oxygen of the glycine residue and possibly the peptide nitrogens (see infrared discussion below). The Mössbauer parameters of the 2:1 iron(II) glutathione complexes are also similar to those of the six-coordinate iron(II) with oxygen and nitrogen donor ligands (*e.g.* Fe(t-But Sal)$_2$ $\delta = 1.13$ mms^{-1} and $\Delta = 1.76$ mms^{-1}) [35].

The temperature dependence of the QS is marked in these 1:2 iron(II) glutathione complexes (see the partially oxidised complex **5** in Table V, Fig. 6).
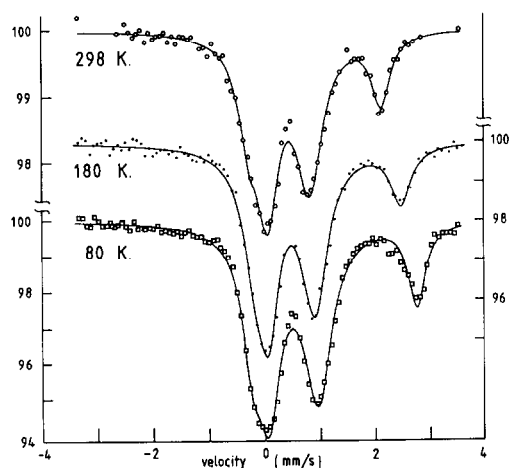
The change indicates a similar behaviour to that exhibited by the $Fe(cysteine)_2$ complex [22]. This behaviour was explained by the presence of low-lying excited states [33].

The electronic reflectance spectra of the Fe^{II} materials were recorded as powders spread on filter papers. As it was difficult to avoid partial oxidation and obtain spectra of pure Fe^{II} samples, the spectra were compared to those of an aged Fe^{II} material (Table VI).

TABLE VI. Electronic Spectra of Fe(II)–GS Complexes in Comparison with other Thiolate–Fe(II) Complexes.

		$\nu \times 10^{-3}$ (cm^{-1})	$\nu \times 10^{-3}$ (cm^{-1})
1	$K_2[Fe(GS)_2] \cdot 7H_2O$ (pink)	35(br)[b] 29–28(br) 19.8(sh)	10(br)
2	$K_{1.08}[Fe_{1.21}(GS)(OH)_{1.5}] \cdot 2H_2O$ (purple)	40 34 30 21.8(sh)	10(br)
3	Aged sample of **2**	35(br)	11(br)
4	(FeL)$_2$[a]	23(sh) 18.5	10
5	(FeL')$_2$[a]	31.3 22.7(sh) 18.5(sh)	10

[a]Reference 27. [b]br = broad; sh = shoulder.

The strong band in the UV-visible (Fig. 7, Table VI) in these complexes is assigned to a charge transfer band, it is similar to iron–sulphur charge transfer bands obtained in other complexes [23, 27].
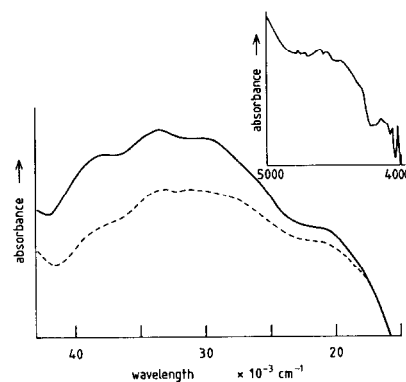

Fig. 6. Temperature dependence of Mössbauer spectra of an aged sample of the complex $K_2[Fe(GS)_2] \cdot 7H_2O$ (number **5**, Table V).

Fig. 7. Electronic reflectance spectra in the range 45,000 cm^{-1} to 15,000 cm^{-1} of $K_2[Fe(GS)_2] \cdot 7H_2O$ partially oxidized sample (- - -); and $K_{1.08}[Fe_{1.21}(GS)(OH)_{1.5}] \cdot 2H_2O$ purple complex isolated from ethanol/water (——). The insert shows the d–d forbidden transition band in $K_{1.08}[Fe(GS)(OH)_{1.5}] \cdot 2H_2O$.

The broad band centred around $\approx 10,000$ cm^{-1} is due to a weak Fe^{II} d—d spin-allowed transition [23, 26]. This band changed shape and position in the aged samples.

Bands in the near-infrared were observed (Fig. 7). The band present at *ca.* 6,000 cm^{-1} in the fresh sample is similar to that found in dithiocarbamate—iron(II) dimers [27]. This band is not present in the aged samples or in the previously discussed Fe^{III} GSSG complexes.

The IR data obtained from nujol and hexachlorobutadiene mulls for the reduced glutathione and its iron complexes are presented in Table VII along with other relevant complexes from the literature [36—40].

The infrared spectrum of GSH is complex and because of this we will centre our discussion on the SH, NH and C=O stretching vibrations in addition to the metal—ligand stretching vibrations.

The strong broad bands at 3285 cm^{-1} for K$_2$-[Fe(GS)$_2$]·7H$_2$O and 3250 cm^{-1} for K$_{1.08}$Fe$_{1.21}$-(GS)(OH)$_{1.5}$·2H$_2$O are comparable to that found in the SnGS complex [38] (3295 cm^{-1}). Co, Cu and Pd [40] complexes with GSH give bands in the 3260—3300 cm^{-1} region. These have been assigned to coordinated amino or amide groups [13, 38—40].

The 1:1 and 1:2 Fe^{II}·GS complexes unlike the uranyl complexes did not show the NH$_3^+$ broad band in the 3100—3000 cm^{-1} region. This is indicative of the presence of the amine group of the glutamyl residue of the GS as NH$_2$ and not as NH$_3^+$.

C—O stretching region: The infrared spectra of GSH contains a strong band at 1715 cm^{-1}. This band, assigned to the —COOH group of the glycine residue [38] is absent in the 1:2 iron—GSH complex and is present as a very weak shoulder in the 1:1 complex at *ca.* 1740 cm^{-1}. In the solid complex Na$_4$[Cu$_2$GSSG]·6H$_2$O prepared at pH 11.0 as in ref. [13] no such band was detected.

Another band in the C=O stretching region at 1665 cm^{-1} in GSH (free ligand) was assigned to the C=O stretching in the peptide bonds [38]. This band shifts to lower frequency in the Fe—GS complexes (the 1:1 complex at 1630 cm^{-1}, and the 1:2 at 1645 cm^{-1}). It must be stated that this band at 1645 cm^{-1} could equally be assigned to the COO$^-$ group [36], *i.e.* COO$^-$ (glutamyl) bound to K$^+$. The difficulty of assigning $\Delta\nu$, the difference in frequency between symmetric and asymmetric vibrations of the COO$^-$ in GSH and its complexes arises from the presence of several bands in this region caused by the different COO$^-$ and peptide C=O bonds.

A sharp strong band at 2525 cm^{-1} in the free ligand (GSH) is the S—H stretch. This band disappears in both the 1:1 and 1:2 Fe^{II}—GS complexes.

TABLE VII. Infrared Spectra of Glutathione and its Iron Complexes in Comparison with other Complexes, (frequency in cm^{-1})[a].

Compound	Ref.	ν(OH)	ν(NH)	ν(C=O)	ν(SH)	ν(M—S)	ν(M—O)	ν(M—N)
GSH (solid)	[39]		3344 3245 3124 3024	1715[b] 1655	2525			
GSH (Nujol and hexa-chlorobutadiene mull)	This work		3350 3255 3130 3030	1715[b] 1665	2525			
GSH (solid)	[38]		3350 3250 3000—3100[d]	1712[b] 1599(s) 1394(s)				
K$_2$[Fe(GS)$_2$]·7H$_2$O	This work	3500(m,sh)	3285(s)	1645(s,br)[c] 1580(s,br)	absent	358(s) 570(s,br)	250(w) 270(w) 290(w)	310(w) 435(s)
K$_{1.08}$[Fe$_{1.21}$(GS)(OH)$_{1.5}$]·2H$_2$O	This work		3250(m,br)[b]	1740(w,sh) 1630(s)	absent			
(n-Bu$_3$Sn)$_2$GS	[39]		3372 3280	1645(s,br)	absent	390		
UO$_2$/GS	[38]	3500	3350 3250 3000—3100(br)[d]	1640[c] 1390				

[a]br = broad; vs = very strong; s = strong; sh = shoulder; w = weak; m = medium.   [b]Assigned for (C=O) stretching in COOH.
[c]Asymmetric stretch higher frequency symmetric stretch lower frequency.   [d]N—H stretching in a hydrogen bonded NH$_3^+$, reference 36.

Comparison with other infrared data [36, 38] allows the assignment of the bands at 358 and 570 cm^{-1} in $K_2Fe(GS)_2 \cdot 7H_2O$ to Fe–S vibrations. Comparison with other amino acid metal complexes [36], permits the bands at 250, 270 and 290 cm^{-1} in this potassium complex to be assigned to Fe–O vibrations.

Freeze Dried Samples of Iron(III)–Reduced Glutathione Materials

The Mössbauer parameters for this material are No's 6 and 7 in Table V. The parameters of the white $FeCl_3/3GSH$ freeze dried material (from a solution at pH 3.0) (Table IV) indicate that only high spin iron(II) is present. This is in agreement with our previous work [3], but the freeze dried material unlike the frozen solution has a QS that is comparable with those for the 1:2 Fe^{II}–GS solid complexes (Table IV, No's 3–5).

When this sample was aged it turned pink. The Mössbauer data (No. 7, Table IV) show evidence of a high spin Fe^{III} site in addition to high spin Fe^{II}. The Fe^{III} site Mössbauer parameters are similar to those of $[Fe_{1.33}^{III}(GSSG)] \cdot 6H_2O$ (Table II). This can be interpreted as evidence that the original Fe^{II} in the solid that changed to Fe^{III} on ageing was not bound to any Cl^-, and this is in keeping with the original parameters' similarity to those of K_2-$Fe(GS)_2 \cdot 7H_2O$.

The smaller QS in the freeze dried materials compared to that of the frozen solution [3] is indicative of the involvement of some H_2O in direct binding to the Fe^{II} in the latter material.

Conclusions

A number of Fe^{II}/GSH and $Fe^{III}/GSSG$ materials have been prepared and partially characterized by chemical analysis and spectroscopic techniques.

Mössbauer data have been interpreted to assign the valence and spin state of the iron electronic environments present. In some cases the Mössbauer data has allowed tentative coordination numbers to be assigned by comparison with the data of other reported complexes of known crystal structures.

On examination of the elemental analysis data it can be seen that the first four of the Fe^{III}–GSSG complexes (Table I) have a similar overall formula *viz*: $[Fe_x^{III}(GSSG)L,L'] \cdot 4H_2O$, where x = 2 if L = L' = L^-$, x = 1.67 if L = L^-$, L' = L^0$, x = 1.33 if L = L' = L^0$.

Unfortunately it was not possible to deconvolute the infrared data of the Fe^{III}–GSSG materials, so direct evidence as to which GSSG ligands bind, is not available.

A similar coordination to that found in the Na_4-$[Cu_2GSSG] \cdot 6H_2O$ [13] would be in keeping with

the limited data presented here for the Fe^{III}–GSSG materials [*viz*. the Mössbauer parameters and magnetic susceptibility data] except that the sixth coordinate position in the Fe^{III} (not occupied in the Cu^{II} compound) would be occupied possibly by a water molecule or the $-COO^-$ group from the next GSSG in a polymeric structure.

Two Fe^{II}–GSH stoichiometries have been established, 1:1 and 1:2 complexes. The 1:1 complex contains two different high spin Fe^{II} coordinations, one five and one six coordinate environment [as indicated by Mössbauer parameters]. From the infrared data there is evidence for Fe–S, Fe–O (from C–O (peptide) and COO^- groups), bonds and some evidence for an Fe–N bond (from the glutamyl residue).

The 1:2 complexes contain only one kind of high spin Fe^{II} in a distorted six-coordinate environment [from Mössbauer data]. There is evidence of oxygen and nitrogen donor ligands [Mössbauer parameters and infrared data], and sulphur, oxygen of the COO^- group of the glycine residue, the nitrogen of the glutamyl, and perhaps the peptide nitrogen [infrared data] bonding to the Fe^{II}.

There is some evidence for Fe–S bonds in both iron(II) complexes from the electronic reflectance spectra.

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