# Model Compounds for Microbial Iron-transport Compounds. Part 1. Solution Chemistry and Mössbauer Study of Iron(II) and Iron(III) Complexes from Phenolic and Catecholic Systems

By **Robert C. Hider, A. Rahim Mohd-Nor,** and **Jack Silver,*** Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, Essex
**Ian E. G. Morrison** and **Lovat V. C. Rees,** Department of Chemistry, Imperial College, London SW7 2AY

The iron complexes found in the systems $FeCl_3$–phenol, $FeCl_3$–catechol, $FeCl_3$-2,3-dihydroxybenzaldehyde, $FeCl_3$–2,3-dihydroxybenzoic acid, and $FeCl_3$–salicylic acid have been investigated by pH and conductance titrations together with Mössbauer spectroscopy. On the basis of further studies using ascorbic acid and salicylaldehyde, a mechanism for the reduction of $Fe^{III}$ to $Fe^{II}$ and the reverse oxidation *via* a phenolic or catecholic radical is discussed. The properties of enterobactin are explored in the light of the chemistry of the model systems. The Mössbauer spectra of frozen aqueous solutions of hydroxybenzene compounds containing iron cations show that those complexes that are green or blue at acid pH contain $Fe^{II}$, whereas those that are purple or red contain $Fe^{III}$. It is proposed that these results make the colour of the complex a good indication of the oxidation state of the iron in phenolic and catecholic systems.

SIDEROPHORES [1] are chelating agents which are manufactured by micro-organisms in order to extract iron from the external environment and to transport it into the organism. The insolubility of iron(II) and iron(III) hydroxides and the low equilibrium concentrations of iron in biological environments are inconsequential compared to the enormous stability and selectivity of the iron(III) siderophore complexes.[2] Enterobactin, one such ionophore, is a sexidentate ligand possessing three catechol functions covalently linked together *via* a cyclic triester of serine. Possible mechanisms for the uptake of iron–enterobactin have been proposed for enteric bacteria such as *Escherichia coli*,[3] but one apparent problem associated with this type of molecule is the difficulty of removing iron from a ligand of high affinity ($K > 10^{45}$ $dm^3$ $mol^{-1}$).[2] We have suggested that an iron(II) enterobactin complex could be a critical intermediate in this process,[4] enterobactin possessing a much lower affinity for iron(II).

The chelating groups in enterobactin are catechols (1,2-dihydroxybenzene) and it is well recognized that iron(III) can form extremely stable complexes with catechol ligands.[5-8] Mentasti *et al.*[9,10] have studied the formation of 1 : 1 (metal to ligand) iron(III) complexes with catechols at pH 1—2, using stopped-flow and spectroscopic techniques. We have demonstrated that the previously reported 1 : 1 (metal : ligand) iron(III) complexes with catechols and phenols are in fact iron(II) complexes.[11] In order to gain a clear understanding of the reason for the preferential stability of these iron(II) complexes at low pH (2.5—4.5) it is necessary to study the pH dependence of the relative stabilities of iron(II) and iron(III) complexes, the mechanism of interchange between the two oxidation states, and how these properties are influenced by the introduction of substituents on the aromatic ring. Such studies should generate a better understanding of the chemistry of catechol-containing siderophores.

Mössbauer spectroscopy provides a valuable tool by which the valence of the iron atom may be determined. Although there have been extensive studies using a variety of physical methods on similar systems to those described here, there have only been four previous reports using Mössbauer spectroscopy.[4,11-13] Mössbauer data on compounds obtained from the iron–catechol system using piperidinium as the cation are reported by Oosterhuis [12] but only $Fe^{III}$ was found. Neilands and co-workers [13] reported Mössbauer data for enterobactin but again only for an iron(III) species.

We have reported [4,11] the existence of iron(II) and iron(III) complexes in enterobactin,[4] phenol, and catechol iron systems,[11] and we now report the results of an extensive study on the following systems: $FeCl_3$–phenol, $FeCl_3$–catechol, $FeCl_3$–2,3-dihydroxybenzaldehyde, $FeCl_3$–salicylic acid, and $FeCl_3$–2,3-dihydroxybenzoic acid.

## EXPERIMENTAL

*Materials.*—Catechol (SLR, Fisons) was recrystallized before use. Anhydrous iron(III) chloride (SLR, Fisons), phenol (AR, East Anglia Chemicals), 2,3-dihydroxybenzaldehyde (Sigma), 2,3-dihydroxybenzoic acid (Aldrich Chemical Co. Ltd.), salicylic acid (BDH), and salicylaldehyde (BDH) were used without further purification. Enterobactin was kindly provided by Professor J. B. Nielands (Berkeley). Solutions were prepared by dissolving weighed amounts of the reagents in deionised water and were stored under an oxygen-free nitrogen atmosphere.

*Characterisation of Complex Ions in Solution.*—The variation method [14] was used to determine the stoicheiometry of complex ions at various values of pH. The ionic strength was kept constant by using 0.5 mol $dm^{-3}$ NaCl.

Optical densities of the mixed metal–ligand solutions were measured at various wavelengths by using a Pye–Unicam digital SP700 spectrophotometer, and visible spectra were determined using a Perkin-Elmer Coleman 575 spectrophotometer. All measurements were taken at 25 °C. The solutions containing iron(III) species were stable and their spectra were time-independent. However the iron(II)-containing species were less stable and solution spectra were taken immediately after mixing.

*pH and Conductometric Titration in Aqueous Solution.*—Iron ($10^{-3}$ mol $dm^{-3}$) was used throughout with different ratios of ligand. Additions of Na[OH] (1 mol $dm^{-3}$) or HCl (1 mol $dm^{-3}$) were achieved under nitrogen. A digital WPA model CD 60 was used for pH measurements and

conductivity measurements were made with a digital Walden Precision Apparatus model CMD 4000. Conductivity units are arbitrary as the cell constant is not known. The values of $C_B/C_M$, where $C_B$ is the concentration of the base (titrant) and $C_M$ is the analytical metal concentration, were corrected for increasing volume during titrations.

*Electrophoresis.*—Buffers in the pH range 3—11 were prepared from acetic acid–pyridine–$NH_3$ mixtures. Whatmann 3MM paper was used throughout and a voltage of 5 000 V was applied across a distance of 30 cm.

*Mössbauer Spectroscopy.*—Aqueous solutions of recrystallised catechol (3.22 mol $dm^{-3}$), iron(III) chloride hexahydrate (1.074 mol $dm^{-3}$), and iron(III) nitrate nonahydrate (1.074 mol $dm^{-3}$) were mixed (catechol, 1 $cm^3$; iron salt 0.5 $cm^3$); the pH was quickly adjusted to the required value using 5 mol $dm^{-3}$ hydrochloric acid or 5 mol $dm^{-3}$ sodium hydroxide. Thus the catechol: $Fe^{III}$ ion ratio was fixed at 6 : 1. All samples from pH 0 to pH 4.5 precipitated a black material; this is only found in the case of concentrated solutions, and is said to be an iron-free polymeric catechol species,[2] although we found them to contain $Fe^{III}$ by Mössbauer spectroscopy (results not reported). The solutions were quickly centrifuged to remove this precipitate, and the supernatant liquid transferred to the Mössbauer sample cell. This consisted of two pieces of PTFE machined to screw into each other, providing a solutions space 2 mm in thickness and 12.5 mm in diameter, with windows 0.5 mm thick. This cell was quick-frozen in liquid nitrogen and transferred to a precooled Ricor MCH5 cryostat; with practice the rise of temperature on transfer was less than 15 K. The temperature of the cryostat during spectra accumulation was controlled to better than 1 K by a Ricor TC4B controller.

Spectra were obtained using an ELSCINT MD3/MVT3 spectrometer and Nuclear Data ND2200 multichannel analyser; the spectrometer was operated in 'triangular' mode, and the mirror-image spectra folded together when computer fitting. This was performed on the Imperial College CDC 6500 computer, using a specially developed program (I. E. G. Morrison, unpublished work) to fit overlapping doublets in the form of an exponential distribution from a maximum value of quadrupole splitting ($\Delta$), while isomer shifts ($\delta$) and line widths ($\Gamma$) were constant. This maximum quadrupole splitting is the value quoted in the Results section.

The source used was *ca.* 4 mCi * of cobalt-57 in copper, obtained from the Radiochemical Centre, Amersham; subsequently a 11 mCi cobalt-57 in rhodium source was used. The spectrometer was calibrated with a 12.5 $\mu$m thick natural iron reference absorber; all isomer shifts are referred to this as zero shift. Differential scanning calorimetry (d.s.c.) results were obtained using a low-temperature module on a Dupont 990 thermal analyser. These d.s.c. results for aqueous solutions all showed, as expected, a glass transition at 180—210 K; thus Mössbauer spectra were obtained for all catechol solutions in the quench-frozen state, and also after annealing at the temperature above this glass transition but below the melting point.

## RESULTS

*Iron–Phenol System.*—The pH titration curves for this system [Figure 1(*a*)] and the conductometric titration data [Figure 1(*b*)] were taken simultaneously on the same 1 : 3 (metal : ligand) solutions. A blue-purple solution ($\lambda_{max.}$ = 560 nm) was present from pH 2.0 to pH 3.4 in agreement with Wesp and Brode [8] and above this pH range the solution was yellow-orange (Table 1). At pH 7.5 an orange precipitate developed. Broumand and Smith [15] have investigated the composition of the blue-purple complex and found it to be 1 : 1 (iron : phenol) in composition. We have previously shown that this compound is an iron(II) phenol

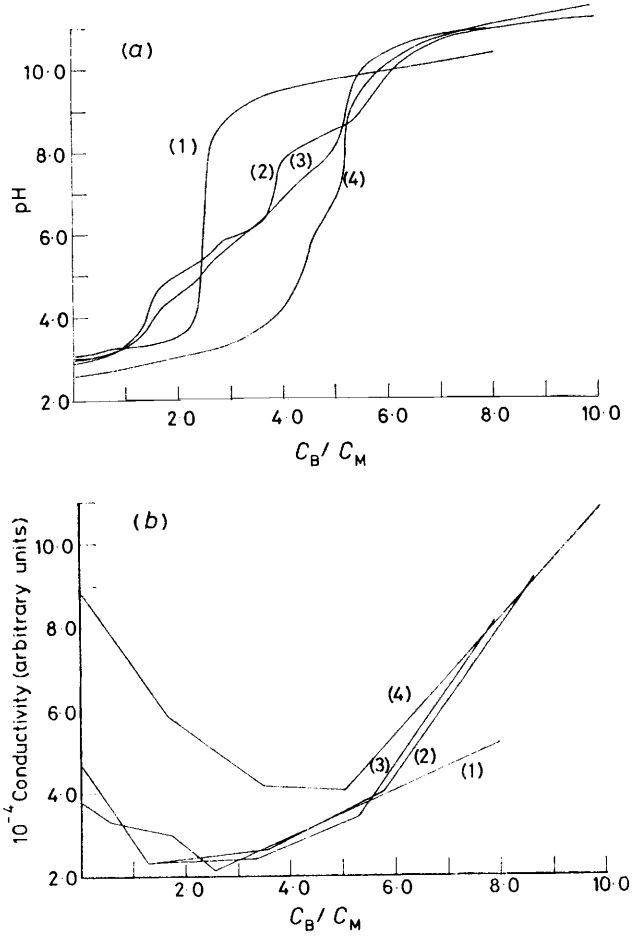



FIGURE 1 (*a*) pH Titration curves, (*b*) conductivity curves for the systems (1) phenol–iron(III), (2) catechol–iron(III), (3) 2,3-dihydroxybenzaldehyde–iron(III), and (4) 2,3-dihydroxybenzoic acid–iron(III) with a metal–ligand ratio of 1 : 3

complex.[11] The sample used for Mössbauer spectroscopy was an aqueous solution of $FeCl_3$ containing a large excess of phenol. The quench-frozen sample gave a Mössbauer spectrum characteristic of high-spin iron(II) (Table 2).

The iron(II) phenol complex is destabilised above pH 3.4, the cation being oxidised to iron(III). Presumably iron(III) becomes progressively co-ordinated to hydroxyl groups above pH 3.4 and eventually precipitates as iron(III) hydroxide. Ernst and Herring [16] give the stability constant of the iron(II) phenol complex as *ca.* $10^8$, although at the time they did not realise that it contained iron(II).

*Iron–Catechol System.*—(*a*) *Starting from* $FeCl_3$. Four distinctly different coloured species were detected in this system. The electronic spectra are shown in Figure 2. The pH and conductometric titrations were carried out at metal : ligand ratios of 1 : 1, 1 : 3, and 1 : 6 (Figure 3). Four

* Throughout this paper: 1 Ci = 3.7 × $10^{10}$ $s^{-1}$; 1 D ≈ 3.336 × $10^{-30}$ C m.

TABLE 1

The pH dependence of colour of (1) $Fe^{III}$–phenol, (2) $Fe^{III}$–catechol, (3) $Fe^{II}$–catechol, (4) $Fe^{III}$–2,3-dihydroxybenzaldehyde, (5) $Fe^{III}$–2,3-dihydroxybenzoic acid, (6) $Fe^{III}$–salicylic acid, and (7) $Fe^{III}$–enterobactin

| pH | (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|---|
| 1 | Colourless | Yellow | | Yellow | Yellow | | Colourless |
| 2 | | | | | | | |
| 3 | Blue | | | Green | Dark blue | Purple | Blue |
| 4 | | Green | Colourless | Green-blue | | | |
| 5 | Yellow-orange | | | | Purple | Red | |
| 6 | | Blue | | | | | |
| 7 | | | | Purple-brown | | | |
| 8 | | | Pink | | | | Red-wine |
| 9 | | Purple | Red | | | | |
| 10 | Orange precipitate | | | Red-brown | Purple-red | Yellow-orange | |
| 11 | | Red-wine | Red-wine | | | | |
| 12 | | | | | | | |

different coloured complexes were observed at each ratio, namely green, blue, purple, and red, although the intensities of the blue, purple, and red species were much reduced in the 1 : 1 solution. The change in slope of the conductometric data coincides with changes in solution colour. These ligand titration curves show similar characteristics to those previously published; 1 : 6 [2] and 1 : 3.[17] However neither research group reported the existence of four catechol–iron complexes.

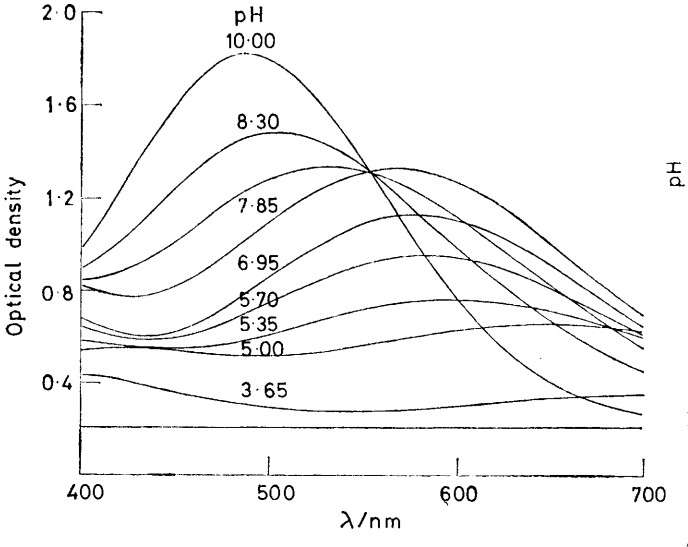



FIGURE 2 Visible absorption spectra of iron–catechol (1 : 3) solutions as a function of pH, [iron] = $2 \times 10^{-4}$ mol $dm^{-3}$

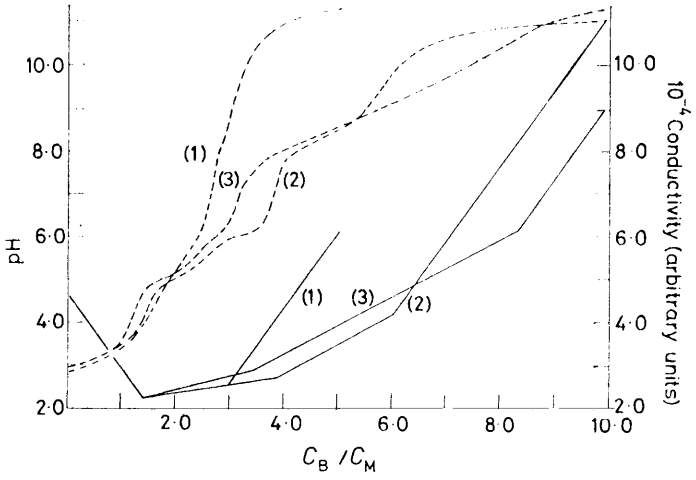



FIGURE 3 pH Titration (— — —) and conductivity curve (——) of iron–catechol solutions with $FeCl_3$–ligand ratios of 1 : 1 (1), 1 : 3 (2), 1 : 6 (3)

The Mössbauer data for these species are reported in Table 2 and Figures 4 and 5 show some of the typical spectra obtained. These data readily establish that two of these

complexes (the green and the blue species) contain $Fe^{II}$ and the remaining two are iron(III) compounds.

From a Job's plot (pH = 4.0 and 3.0), the green compound ($\lambda_{max.}$, 700 nm) was found to be a 1 : 1 iron–catechol complex. Electrophoresis showed it to be positively charged. Thus, in view of the Mössbauer data, the formulation $[Fe^{II}(\dot{O}OC_6H_4)(OH_2)_4]^+$ [(1), Scheme] is most likely. This species is stable over the pH range 2.8—4.5. Mentasti *et al.*[9,10] found that at pH values less than 2.0 iron(II) is produced but at the expense of converting catechol to benzoquinone; however, at higher pH's we find that little or no benzoquinone is produced.

TABLE 2

$^{57}$Fe Mössbauer parameters (mm s$^{-1}$) for solutions of iron(III) chloride–catechol (1 : 6) at different pH values. Spectra are at temperatures from 80 K upwards (quenched, Q) and, after annealing, down again (A). In cases where a magnetic splitting $H_{eff.}$ is found, this is shown (in kOe, 1 Oe = 1 g$^{\frac{1}{2}}$ cm$^{-\frac{1}{2}}$ s$^{-1}$) in the same column as Δ

| | $T$/K | δ | Δ | Γ |
|---|---|---|---|---|
| $FeCl_2$–Phenol | | | | |
| | 80 | 1.38(1) | 3.43(2) | 0.15(2) |
| $FeCl_3$–Catechol | pH 0.0 | | | |
| Characteristic of | 80 Q | 1.372(3) | 3.34(1) | 0.17(2) * |
| $FeCl_2{\cdot}9H_2O$ | 150 Q | 1.343(5) | 3.27(1) | 0.16(2) * |
| | 200 A | 1.314(3) | 3.603(5) | 0.124(4) |
| | 175 A | 1.331(2) | 3.632(5) | 0.119(4) |
| | 150 A | 1.349(2) | 3.657(4) | 0.126(3) |
| | 125 A | 1.357(2) | 3.664(4) | 0.122(3) |
| | 100 A | 1.370(2) | 3.676(4) | 0.122(3) |
| | 80 A | 1.381(2) | 3.688(3) | 0.125(3) |
| | pH 1.5 | | | |
| Mixture of | 80 Q | 1.370(4) | 3.32(1) | 0.18(1) * |
| $FeCl_2{\cdot}9H_2O$ and | 125 Q | 1.352(4) | 3.27(2) | 0.17(2) * |
| $FeCl_2{\cdot}6H_2O$ | 150 Q | 1.343(4) | 3.25(1) | 0.18(2) * |
| | 175 Q | 1.311(7) | 3.03(2) | 0.27(2) * |
| | 200 A | 1.317(2) | 3.618(4) | 0.116(3) |
| | 150 A | 1.337(5) | 3.12(1) | 0.244(9) |
| | | 1.30(1) | 1.72(3) | 0.19(3) |
| | 80 A | 1.370(3) | 3.250(7) | 0.221(6) |
| | | 1.35(2) | 1.74(3) | 0.23(3) |
| | pH 2.9 | | | |
| Characteristic of | 80 Q | 1.378(4) | 3.43(2) | 0.12(2) * |
| $FeCl_2{\cdot}6H_2O$ | 100 Q | 1.368(4) | 3.36(2) | 0.14(3) * |
| | 150 Q | 1.337(6) | 3.20(1) | 0.21(1) |
| | 175 Q | 1.334(9) | 3.07(2) | 0.29(2) |
| | 225 A | 1.247(3) | 1.583(5) | 0.118(5) |
| | 200 A | 1.254(3) | 1.589(5) | 0.119(5) |
| | 175 A | 1.272(2) | 1.617(4) | 0.109(4) |
| | 150 A | 1.280(3) | 1.637(5) | 0.120(4) |
| | 125 A | 1.297(3) | 1.650(6) | 0.127(5) |
| | 100 A | 1.312(3) | 1.682(5) | 0.116(5) |
| | 80 A | 1.317(3) | 1.690(5) | 0.134(5) |
| | pH 3.1 | | | |
| $FeCl_2{\cdot}6H_2O$ | 80 Q | 1.370(7) | 3.40(3) | 0.13(5) * |
| | 200 A | 1.250(8) | 1.61(2) | 0.14(1) |
| | 80 Q | 1.369(4) | 3.39(2) | 0.18(2) * |
| | 200 A | 1.290(5) | 1.660(8) | 0.13(3) |
| | pH 4.0 | | | |
| Mixture of | 80 Q | 1.379(4) | 3.41(2) | 0.14(2) |
| $FeCl_2{\cdot}9H_2O$ and | 100 Q | 1.365(3) | 3.36(2) | 0.16(2) * |
| $FeCl_2{\cdot}6H_2O$ | 125 Q | 1.339(6) | 3.23(1) | 0.21(1) |
| | 150 Q | 1.345(7) | 3.28(3) | 0.17(3) |
| | 175 Q | 1.315(9) | 3.09(2) | 0.23(2) |
| | 200 A | 1.321(4) | 3.597(8) | 0.132(7) |
| | | 1.263(8) | 1.64(3) | 0.13(1) |
| | pH 5.4 | | | |
| Characteristic of | 80 Q | 1.372(7) | 3.20(1) | 0.22(1) |
| blue (1 : 3) iron(II) | 125 Q | 1.333(8) | 3.15(2) | 0.24(1) |
| catechol species | 175 Q | 1.31(2) | 2.97(3) | 0.27(3) |

TABLE 2 (*continued*)

| | pH 6.0 | δ | Δ | Γ |
|---|---|---|---|---|
| Characteristic of | 80 Q | 1.37(1) | 3.20(3) | 0.26(2) |
| blue (1 : 3) iron(II) | | 0.546(4) | 0.833(6) | 0.217(5) |
| catechol species. | 100 Q | 1.35(2) | 3.25(3) | 0.25(3) |
| Also present is a | | 0.537(4) | 0.826(7) | 0.212(6) |
| small amount of an | 125 Q | 1.33(2) | 3.20(3) | 0.27(3) |
| iron(III) species | | 0.531(5) | 0.830(8) | 0.218(7) |
| | 150 Q | 1.33(2) | 3.12(4) | 0.33(4) |
| | | 0.527(5) | 0.821(8) | 0.218(7) |
| | 175 Q | 1.33(2) | 3.08(3) | 0.27(3) |
| | | 0.510(4) | 0.819(8) | 0.210(6) |
| | 225 A | 1.28(2) | 3.03(4) | 0.29(4) |
| | | 0.482(5) | 0.824(9) | 0.225(7) |
| | 200 A | 1.31(3) | 3.03(5) | 0.32(6) |
| | | 0.494(5) | 0.842(9) | 0.212(7) |
| | 150 A | 1.36(2) | 3.13(4) | 0.36(4) |
| | | 0.521(4) | 0.828(8) | 0.211(6) |
| | 125 A | 1.39(2) | 3.19(4) | 0.33(4) |
| | | 0.523(5) | 0.806(8) | 0.206(7) |
| | 100 A | 1.40(2) | 3.19(4) | 0.30(4) |
| | | 0.535(5) | 0.819(8) | 0.222(7) |
| | pH 7.7 | | | |
| Characteristic of | 80 Q | 1.29(1) | 2.98(2) | 0.28(2) |
| purple (2 : 4) | | 0.50(2) | 0.74(2) | 0.26(2) |
| iron(III) catechol | 175 Q | 1.21(2) | 2.88(3) | 0.24(3) |
| species. Also | | 0.47(1) | 0.71(2) | 0.20(2) |
| iron(II) (explan- | 225 A | 1.26(1) | 2.80(3) | 0.32(3) |
| ation in text) | | 0.45(1) | 0.72(2) | 0.20(1) |
| | 80 A | 1.307(6) | 2.99(1) | 0.29(1) |
| | | 0.511(5) | 0.727(8) | 0.214(7) |
| | pH 9.5 | δ | $H_{eff.}$/Δ | Γ |
| Characteristic of | 80 Q | 0.6(1) | 539(6) | 0.6(2) |
| red (1 : 3) iron(III) | | | 0.5(1) | |
| catechol complex | | 1.252(6) | 3.06(1) | 0.21(1) |
| | | 0.48(6) | | 1.1(1) |
| | 175 Q | 1.21(1) | 2.97(2) | 0.18(2) |
| | | 0.2(1) | | 1.0(2) |
| | 225 A | 1.21(2) | 2.90(3) | 0.19(3) |
| | | 0.2(1) | | 1.1(3) |
| | 80 A | 0.5(1) | 539(6) | 0.6(1) |
| | | | 0.5(1) | |
| | | 1.253(6) | 3.02(1) | 0.21(1) |
| | | 0.48(9) | | 1.3(2) |
| | pH 12 | | | |
| | 80 Q | 0.46(4) | 536(6) | 0.41(8) |
| | | | 0.5(1) | |
| | | 1.29(3) | 2.99(5) | 0.15(5) |
| | | 0.4(1) | | 1.0(2) |

* Spectra fitted with an exponentially decreasing intensity of sites with decreasing quadrupole splitting, but constant isomer shift and half-width.

The Mössbauer spectra of the quench-frozen green species show a large quadrupole splitting similar to that for the blue-purple iron(II) complex in the iron–phenol system, which on annealing increases to *ca.* 3.6 mm s$^{-1}$. The temperature dependence of the annealed species is similar to that found by Ruby [18] in $FeCl_2$ solutions and ascribed by him to $FeCl_2{\cdot}9H_2O$ crystals, where the iron is surrounded by six water molecules. A similar behaviour is found using iron(III) nitrate. Thus, a likely explanation for the spectra presented in Figure 4(*b*) is that in concentrated solutions the less soluble chloride hydrate preferentially crystallizes from solution on annealing at low temperatures. Ruby [18] further assigned a splitting of *ca.* 1.6 mm s$^{-1}$ to $FeCl_2{\cdot}6H_2O$ where the iron is surrounded by four water molecules and two chloride anions, in this work some annealed samples displayed both nonahydrate and this hexahydrate spectra, the latter however not being observed in iron(III) nitrate solutions. The crystallisation is found to be perfectly repeatable when the solutions are melted, quench-frozen, and reannealed. Thus the green complex appears to be

also unstable with respect to the chloride hydrate in concentrated solutions at low temperatures.

The blue complex ($\lambda_{max.}$, 590 nm) is the major form in the pH range 4.5—6.5. Job's plots (pH 6) show this compound to be 1 : 3, iron–catechol complex, and failure to migrate under electrophoretic conditions suggests the complex to be uncharged. The Mössbauer parameters for this material correspond to those of an octahedral iron(II) high-spin complex, but with a smaller quadrupole splitting of 3.20 mm $s^{-1}$ compared to the green species and with a different behaviour on annealing. This species may be represented as $[Fe^{II}(\dot{O}OC_6H_5)(O_2C_6H_5)_2]$ [(2), Scheme] although presumably all the aromatic rings would be rendered equivalent by the delocalisation of the unpaired electron.

However, the temperature dependence of the quadrupole splitting of the quenched blue species is quite different to that found for the green species [Figure 4(*b*) and (*c*)], probably indicating stronger bonding due to the change in protonation of the ligand caused by hydrogen-ion concentration changes, and the effect this has on the electron density of the catecholic oxygens. The isomer shift is identical for the blue and green species indicating no change in the co-ordination number.

The purple complex is seen to form over the pH range 6.5 to 9.0. Job's plots at pH 8 show this complex to contain an iron : catechol ratio of 1 : 2. Avdeef *et al.*[2] refer to this complex as a bis complex. However, Anderson *et al.*[19] describe a purple complex which contains one acetate ion,

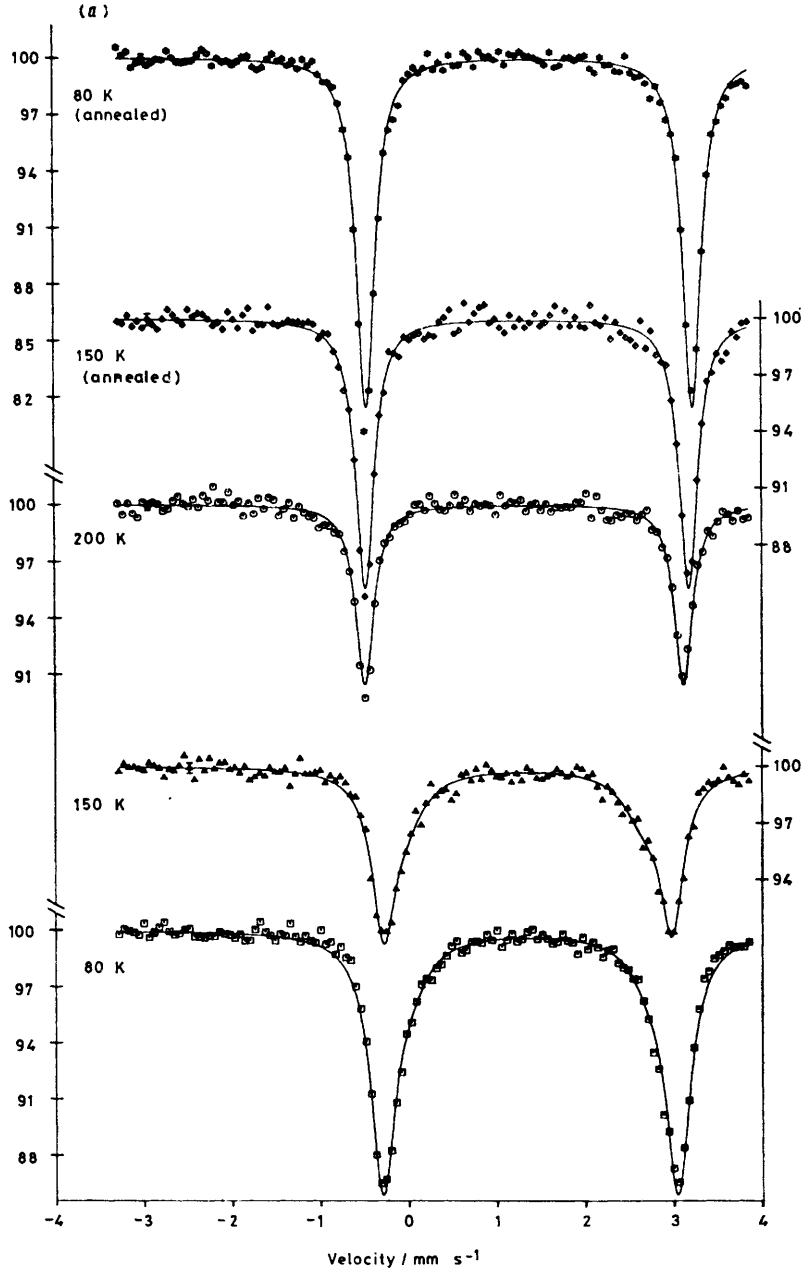



FIGURE 4 Temperature dependence of Mössbauer spectra of solutions of iron(III) chloride–catechol (1 : 6); (*a*) pH 0; (*b*) green complex, pH 3; (*c*) blue complex, pH 6

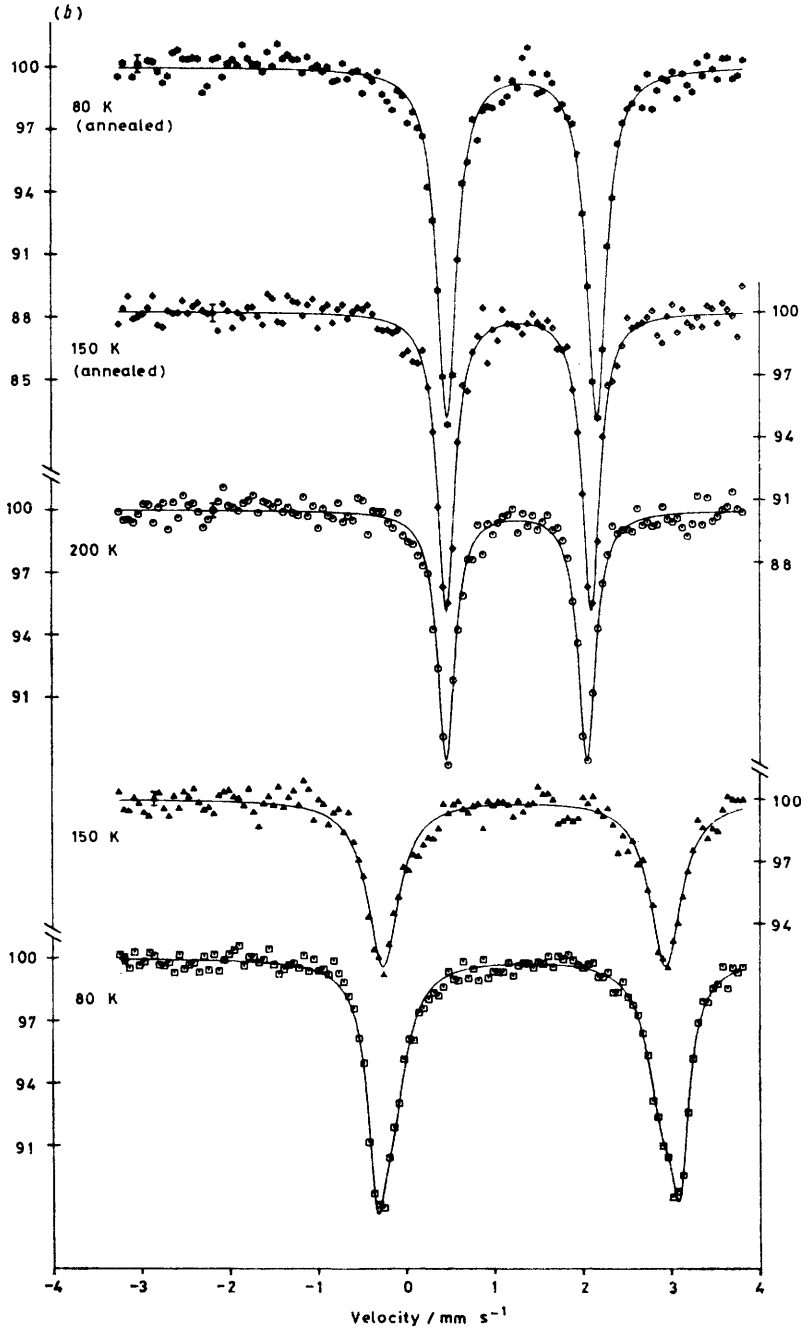



FIGURE 4 (*continued*)

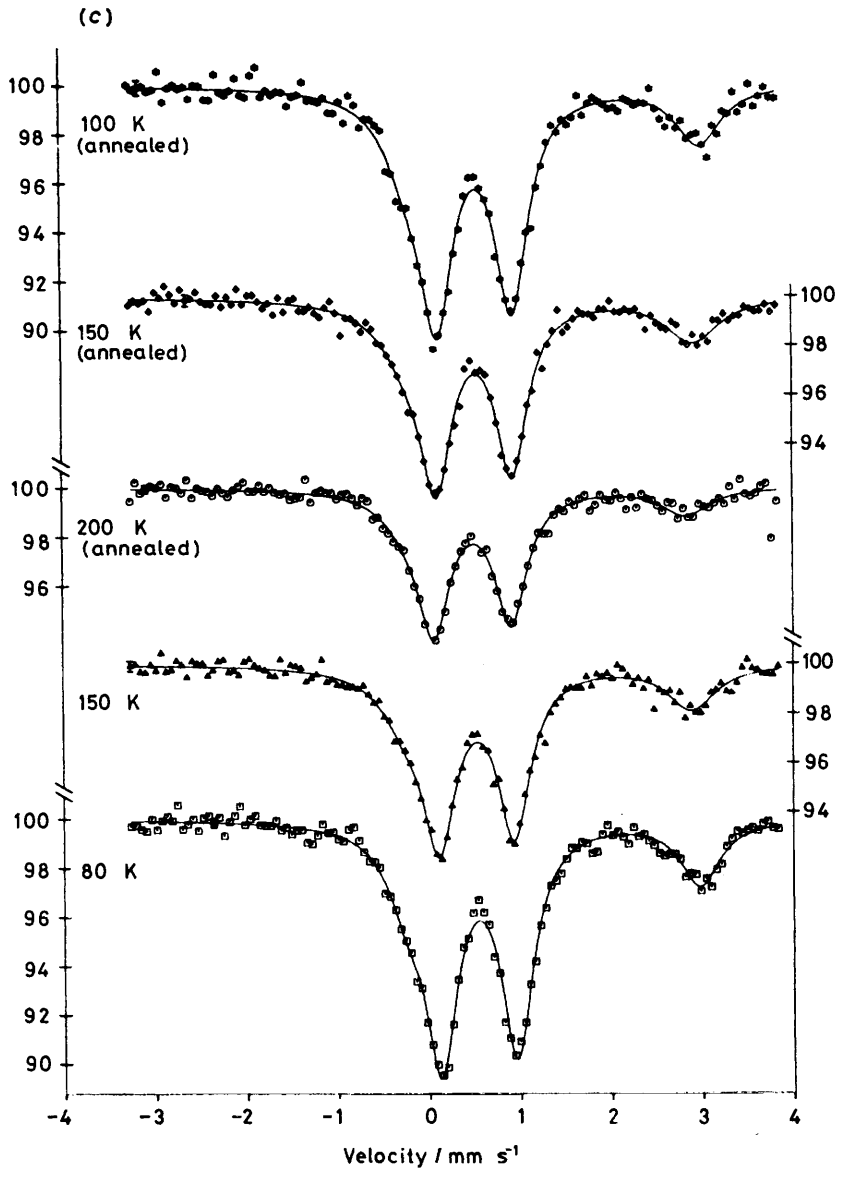



FIGURE 4 (*continued*)

four catechol anions, and two iron(III) ions. We believe that the purple complex described in this work is of a similar nature [(3), Scheme], but contains one bridging hydroxyl group instead of the bridging acetate. The Mössbauer data of this complex show iron(III) (Figure 5, pH 7.7). The relatively narrow line widths of these spectra provide evidence for the existence of either a single iron(III) site or of two similar iron(III) sites, which might be inferred from the structure of the known purple iron(III) catechol complex $[C_5H_{12}N]_3[\{Fe(O_2C_6H_4)_2\}_2(O_2CMe)]$ [19] that contains two equivalent sites.

The red complex ($\lambda_{max.}$, 485 nm) has been well characterised by previous workers [19–21] and possesses an iron : catechol ratio of 1 : 3 [(4), Scheme]. The Mössbauer results for the red species are typical for an octahedral iron(III) high-spin environment and agree well with the strength of the paramagnetic fields reported for $[C_5H_{12}N]_3[Fe(O_2C_6H_4)_3]\cdot H_2O$[19] and iron enterobactin.[13]

The pH dependence of the interconversion of these complexes is displayed in Figure 2. The isosbestic point, obtained over the pH range 7.85—10, corresponds to the transition between the purple and red iron(III) complexes. The blue species ($\lambda_{max.}$, 590 nm) predominates at pH 5.7 and the green species at pH 3.54. Avdeef *et al.*[2] report a similar set of spectra. It is possible to cycle these pH titrations between pH 2.5 and pH 12.0 for at least six cycles without inducing an appreciable loss in the optical density of the four complexes, indicating that all the species observed form and dissociate reversibly. When the pH was changed from 6.0 to 12.0 some unchanged $Fe^{II}$ was always detectable in the Mössbauer spectra. However, over a period of several days total conversion to $Fe^{III}$ was obtained. This time dependence is probably associated with the necessity of using concentrated solutions for the Mössbauer studies. These effects were not apparent in the spectroscopic studies on dilute solutions.

(*b*) *Starting from* $FeCl_2$. The shape of the iron(II)–catechol (1 : 3) pH titration curve (Figure 6) extending from

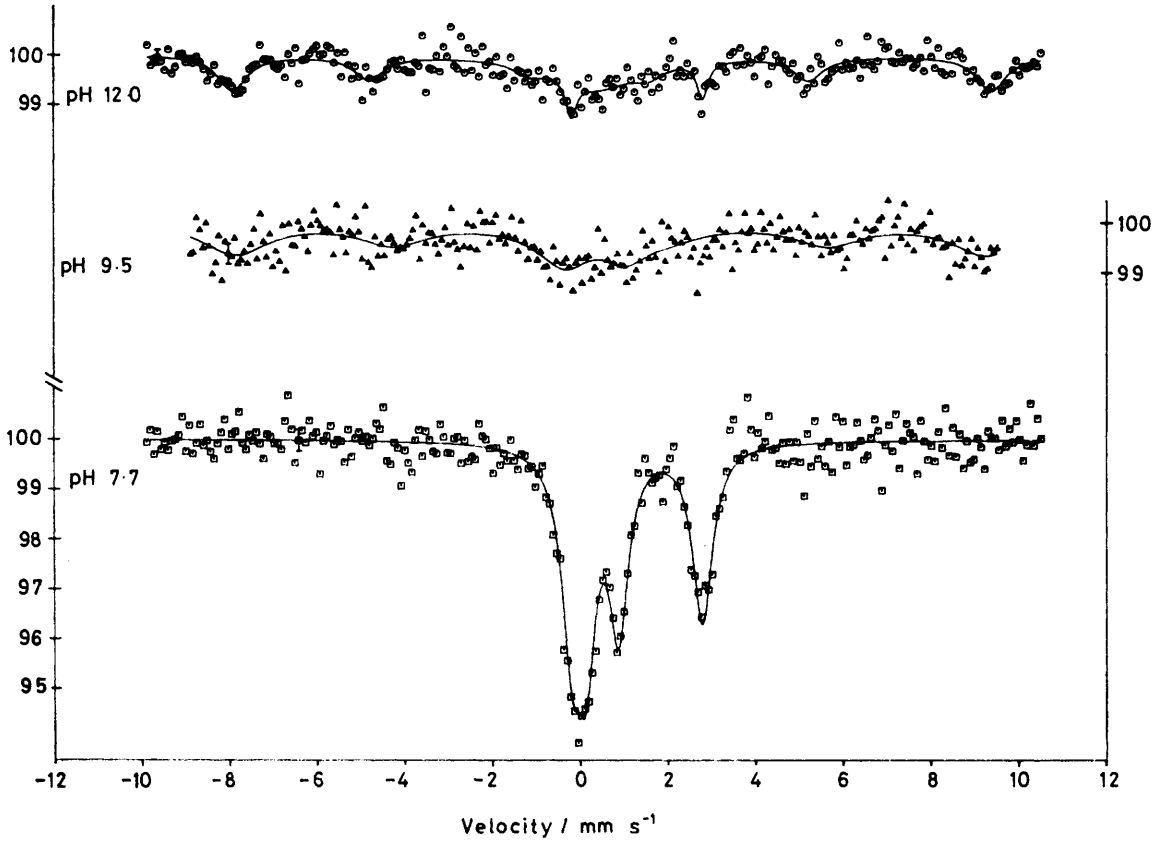



FIGURE 5 pH Dependence of Mössbauer spectra of alkaline solutions of iron(III) chloride–catechol (1 : 6). Spectra run at 80 K

pH 4.25 to pH 11.0 is different to the corresponding iron(III) catechol curve. No colour is observed until *ca.* pH 7.0, when it gradually develops a red tinge on standing, presumably due to trace quantities of oxygen in the system. The reverse curve is similar to that of the curve starting from $FeCl_3$ and subsequent cycles are identical. If these experiments are not run under $N_2$ then there is a very little difference between the initial iron(II) and iron(III) titration curves.

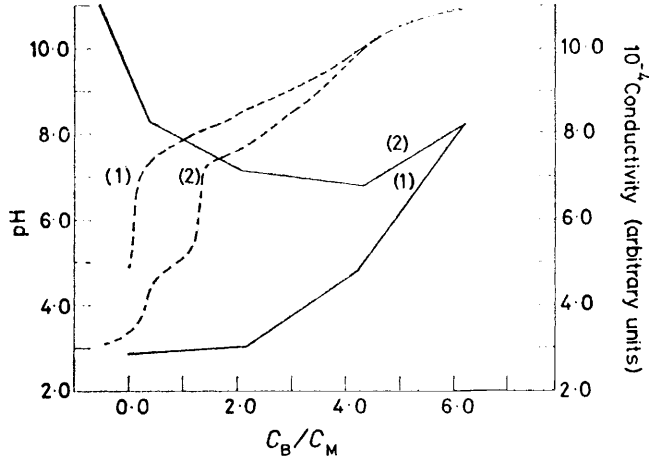



FIGURE 6 pH Titration (— — —) and conductivity curves (——) of iron–catechol (1 : 3) using $FeCl_2$; titration with Na[OH] (1), reverse titration with HCl (2)

*Iron–Catechol–Ascorbic Acid System.*—A solution of iron(III)–catechol–ascorbic acid (1 : 3 : 3) was used throughout the study. The acid-stable green species

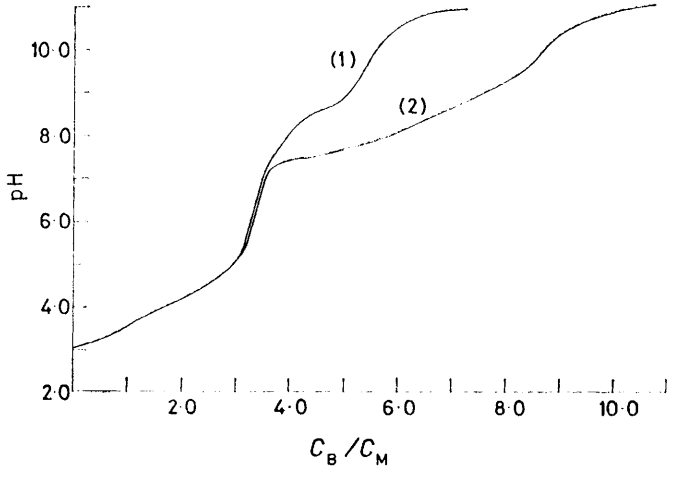
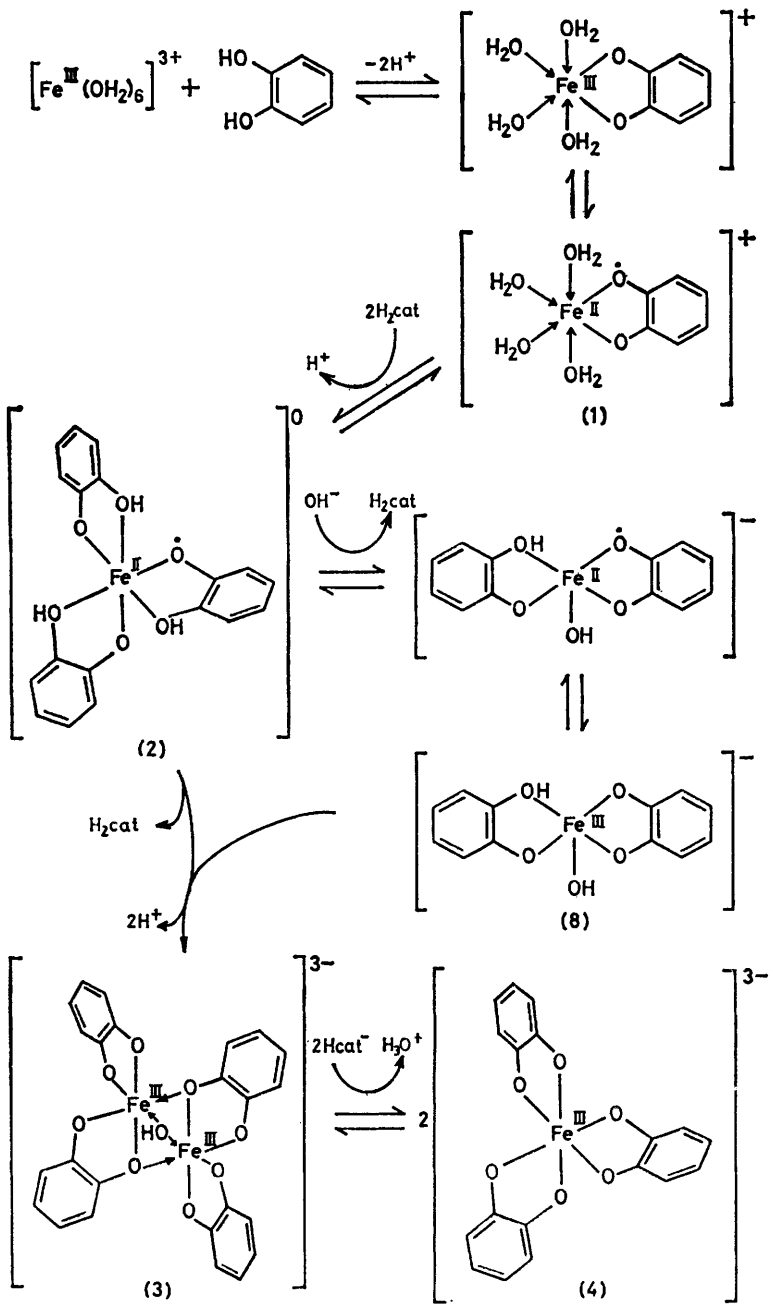



FIGURE 7 pH Titration curves of iron–ascorbic acid (1 : 3) (1) and iron–ascorbic acid–catechol (1 : 3 : 3) (2) using $FeCl_3$

observed in the iron(III)–catechol system is absent in the presence of ascorbic acid, the solution being colourless below pH 4.5. The three other species (blue, purple, red) observed in the iron(III)–catechol solutions are found. This implies that the green iron(II)–catechol species is not a strong complex and that the catechol is easily displaced by ascorbic

acid. In fact ascorbic acid reduces iron more efficiently than catechol below pH 4.5 (Figure 7). No precipitate of *o*-benzoquinone is observed under these conditions. The pH titration data for this system and the corresponding data for iron(III)–ascorbic acid in the absence of catechol are presented in Figure 7. It is clear that below pH 4.5 the curve for the iron–catechol–ascorbic acid system is the same as the iron–ascorbic acid system, whereas above pH 4.5 it is the same as the iron–catechol system [Figure 3(2)].




SCHEME

*Iron–2,3-Dihydroxybenzaldehyde System.*—2,3-Dihydroxybenzaldehyde–$FeCl_3$ produces similar results to the corresponding catechol solutions (Figures 1, 3, and 8). The four species characterised by electrophoresis, electronic spectra, Job's method, conductometric and pH titration are analogous to those of the iron(III) catechol system, with the exception that the relative concentrations of metal–ligand species are modified by a general movement towards lower pH ranges (Table 1). The slight differences observed in the colours, green ($\lambda_{max.}$, 660 nm), blue ($\lambda_{max.}$, 580 nm), purple ($\lambda_{max.}$, 545 nm), and red-brown, when compared with those resulting from the iron–catechol system probably result from the addition of the aldehyde group to the aromatic ring.

Due to the low solubility of 2,3-dihydroxybenzaldehyde in

TABLE 3

$^{57}$Fe Mössbauer parameters (mm s$^{-1}$) for solutions of iron(III) chloride–2,3-dihydroxybenzaldehyde (1 : 6), in methanol–water (2 : 1 v/v) at different pH values

| $T$/K | $\delta$ | $\Delta$ | $\Gamma$ |
|---|---|---|---|
| pH 1 | | | |
| 80 | 1.370(7) | 3.41(1) | 0.15(1) * |
| 150 | 1.44(6) | 3.1(1) | 0.3(1) |
| pH 3 | | | |
| 80 | 1.28(2) | 2.83(4) | 0.13(4) * |
| 150 | 1.16(2) | 2.77(3) | 0.12(3) |
| pH 5 | | | |
| 80 | 0.540(3) | 0.886(6) | 0.243(5) |
| 150 | 0.512(4) | 0.884(7) | 0.243(6) |
| pH 7 | | $H_{eff.}/\Delta$ | |
| 80 | 0.50(4) | 525(6) | 0.55(9) |
| | | 0.42(8) | |
| 150 | 0.29(4) | | 0.8(1) |
| pH 9 | | | |
| 80 | 0.47(2) | 529(3) | 0.46(8) |
| | | 0.41(6) | |
| 150 | 0.2(1) | | 1.0(2) |

* Spectra fitted with an exponentially decreasing intensity of sites with decreasing quadrupole splitting, but constant isomer shift and half-width.

water, the Mössbauer study of this system was carried out in methanol–water solutions (2 : 1 v/v) (Table 3). Although a glass transition is found in this system, it occurs too close to the melting point of these concentrated solutions to allow study of annealed samples. The green solution obtained at a measured pH of 1.0 shows a quench-cooled spectrum with parameters similar to those found using catechol. The blue species shows parameters that identify it as an iron(II) complex, but the quadrupole splitting of 2.83 mm s$^{-1}$ is less than that found for the blue catechol species; this may reflect the distortions caused by the neighbouring aldehyde group when co-ordinated to the iron through the catecholic oxygens. The purple and red species give Mössbauer spectra typical of iron(III) and similar to those of the equivalent species in the catechol system.

No evidence was found for the co-ordination of iron by the aldehyde oxygen (5). The colour of iron(III) salicylaldehyde

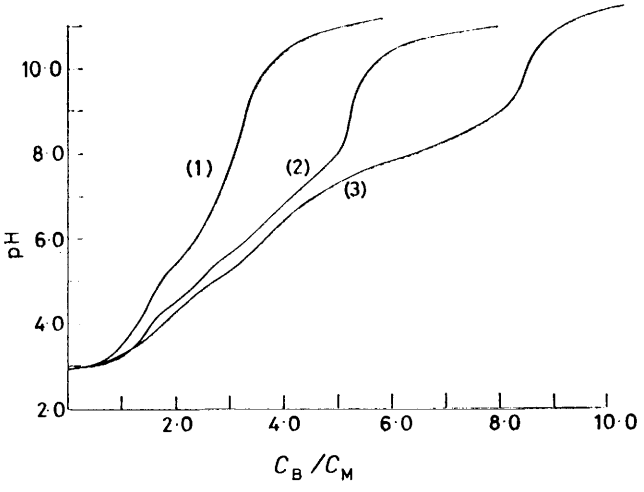
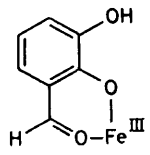
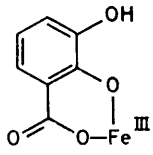



FIGURE 8 pH Titration curves of iron–2,3-dihydroxybenzaldehyde solutions, with $FeCl_3$–ligand ratios of 1 : 1 (1), 1 : 3 (2), 1 : 6 (3)

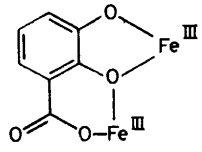
is red-purple ($\lambda_{max.}$, 535 nm) over the pH range 2.0—3.5 and this compound shows no tendency for an iron(II)–iron(III) redox reaction as judged by Mössbauer spectroscopy (unpublished work). No evidence for the existence of trace contributions arising from this possible co-ordination site were found even in the 1 : 6 titrations (Figure 8).




(5)




(6)




(7)

*Iron(III)–Salicylic Acid and Iron(III)–4-Hydroxybenzoic Acid Systems.*—Avdeef *et al.*[2] have discussed the problem associated with the presence of multi iron(III) binding sites on 2,3-dihydroxybenzoic acid. In order to assess the relative efficiency of the salicylato-site at catalysing the $Fe^{II}$–$Fe^{III}$ redox reaction, we have investigated the iron(III)–salicylic acid system by Mössbauer spectroscopy. In the pH range 2—3.5, salicylic acid forms a purple complex with iron ($\lambda_{max.}$, 530 nm) possessing a 1 : 1 stoicheiometry. The Mössbauer spectrum of iron–salicylic acid shows only a trace of iron(II) at a measured pH 0.5 in methanol–water (2 : 1, v/v). A broad diffuse absorbance centred at 0.5 mm s$^{-1}$ which is similar to the highly relaxed absorption found for $FeCl_3$ in methanol–water (Table 4) is also observed. This contrasts with the results obtained for other substituted phenols [11] and can be attributed to the higher p$K_a$ values of the ligands. At pH 1.5 salicylic acid displays a barely detectable iron(II) species, the major constituent being an iron(III) complex possessing a splitting of 0.70(1) increasing to 0.77(3) at pH 2.9.

Iron(III)–4-hydroxybenzoic acid behaves in a similar manner to salicylic acid at both pH values except that the iron(III) parameters at pH 1.5 show a smaller quadrupole splitting (Table 4).

These two compounds are only able to reduce iron(III) at lower pH values than required for phenol itself.

*Iron–2,3-Dihydroxybenzoic Acid System.*—This ligand shows differences to the previously described systems in that only three distinct species have been identified. These complexes are coloured blue ($\lambda_{max.}$, 575 nm), purple ($\lambda_{max.}$, 540 nm), and red ($\lambda_{max.}$, 480 nm). As with the related aldehyde, the pH ranges corresponding to the optional stability for each species move towards more acid values, when compared with those of iron(III) catechol (Table 1). Differences between the colours of these compounds and the corresponding catechol complexes can be explained by similar arguments as those proposed for the iron–2,3-dihydroxybenzaldehyde system. The acid-stable blue

TABLE 4

$^{57}$Fe Mössbauer parameters (mm s$^{-1}$) for solutions of iron(III) chloride–2- and 4-hydroxy- and 2,3-dihydroxy-benzoic acid (1 : 6) in methanol–water (2 : 1 v/v) at different pH values

| | T/K | δ | Δ | Γ |
|---|---|---|---|---|
| (*a*) $FeCl_3$–2-Hydroxybenzoic acid (salicyclic acid) | | | | |
| | pH 0.5 | | | |
| | 80 | 1.39(3) | 3.45(6) | 0.21(6) |
| | pH 1.5 | | | |
| | 80 | 1.6(1) | 3.1(2) | 0.3(1) |
| | | 0.522(4) | 0.70(1) | 0.176(5) |
| | pH 2.9 | | | |
| | 80 | 0.52(3) | 0.77(3) | 0.25(4) |
| (*b*) $FeCl_3$–4-Hydroxybenzoic acid | | | | |
| | pH 0.5 | | | |
| | 80 | 1.44(3) | 3.44(6) | 0.17(5) |
| | pH 1.5 | | | |
| | 80 | 1.6(1) | 2.9(1) | 0.4(2) |
| | | 0.532(1) | 0.456(3) | 0.165(2) |
| (*c*) $FeCl_3$–2,3-Dihydroxybenzoic acid | | | | |
| | pH 1 | | | |
| | 80 | 1.381(4) | 3.46(2) | 0.11(2) * |
| (minor constituent) | | 0.56(6) | 1.0(1) | |
| | pH 3 | | | |
| | 80 | 1.43(3) | 3.27(5) | 0.18(4) |
| | | 0.551(4) | 0.883(7) | 0.28(1) |
| | pH 4.5 | | | |
| | 80 | 0.543(3) | 0.919(6) | 0.283(6) |
| | pH 7 | | | |
| | 80 | 0.53(1) | 0.94(2) | 0.25(1) |
| | pH 10 | | $H_{eff.}/\Delta$ | |
| | 80 | 0.45(3) | 533(3) | 0.41(5) |
| | | | 0.4(1) | |

* Spectrum fitted with an exponentially decreasing intensity of sites with decreasing quadrupole splitting, but constant isomer shift and half-width.

species is a 1 : 1 complex as shown by Job's plots and Mössbauer spectroscopy confirms iron(II) to be present over the pH range 2—3.5 (Table 4). No evidence was obtained for salicylic acid type co-ordination (6) to iron(II). The titration curve of dihydroxybenzoic acid is quite complicated (Figure 9) but above pH 9.0 the red complex predominates. This compound has a 1 : 3 (iron : ligand) stoicheiometry and contains iron(III) (Table 4). The purple complex which exists over the pH range 3.5—8.5 proved difficult to

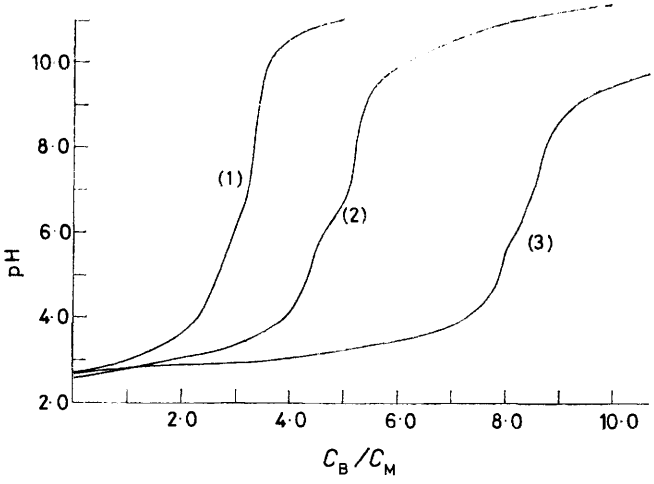



FIGURE 9 pH Titration curves of iron–2,3-dihydroxybenzoic acid solutions, with $FeCl_3$–ligand ratios of 1 : 1 (1), 1 : 3 (2), 1 : 6 (3)

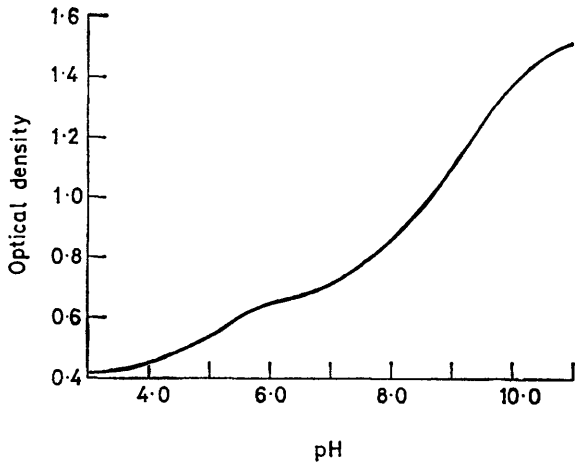



FIGURE 10 pH Titration curve of enterobactin–iron (1 : 1) using $FeCl_3$ in methanol–water (1 : 1, v/v). Optical density measured at 450 nm

characterise. The metal : ligand stoicheiometry of the complex varied with pH indicating the presence of more than one species. At pH 5.0, for instance, the iron : ligand ratio was determined as 2 : 3. At this pH, no evidence for the presence of iron(II) could be obtained using Mössbauer spectroscopy (Table 4). While running spectra of the Job's method solutions it became apparent that at iron : ligand ratios greater than 1 : 2 the spectra changed, the largest proportional change occurring close to the $\lambda_{max.}$ of the red iron(III) salicylic acid complex (480 nm).[22] Consequently, Job plots with iron : dihydroxybenzoic acid ratios greater than 1 : 2 were run with corresponding iron : salicylic acid ratios as a control. Under these conditions the spectral discontinuities were minimised and an iron : dihydroxybenzoic acid ratio of 1 : 2 was obtained. One interpretation of these data is that dihydroxybenzoic acid forms a 2 : 4 complex corresponding to (3) over the pH range 3.5—8.5 and that in the presence of excess of iron(III) further co-ordination occurs as indicated in (7). In dilute solutions, below pH 3.5, there is no evidence for such co-ordination when the catecholato-co-ordinated iron is in oxidation state (II). However above pH 3.5 evidence for such co-ordination is obtained with the oxidation state (III) (Table 4).

*Iron–Enterobactin.*—The spectrophotometric titration study of iron–enterobactin (1 : 1) starting with iron(III) chloride was performed in 50% methanol–water in order to prevent precipitation of the neutral species. Two transitions were observed in the titration curve over the pH range 3—11 (Figure 10). One change ($pK_a$ 5.0) corresponded to the conversion of the blue complex to the well characterised wine-red iron–enterobactin complex.[4,13] The other transition is associated with the formation of the blue complex from iron(III) and the ligand. This blue colour, which is indicative of iron(II), gives a low-temperature Mössbauer spectrum,[4] that from the work in this paper corresponds to $FeCl_2 \cdot 9H_2O$, thus indicating the relative weak affinity of enterobactin for $Fe^{II}$.

This blue complex is not formed by treatment of enterobactin with iron(II) salts in the strict absence of oxygen.

## DISCUSSION

*Conclusions on Mössbauer Spectroscopy.*—The iron(III) catechol systems studied here show that the phenolic

groups facilitate reduction of $Fe^{III}$ to $Fe^{II}$ in acid pH ranges. However, if the phenolic group is positioned next to a carboxylic acid function as in salicylic acid it fails to facilitate iron(III) reduction. This finding confirms the previous suggestion that the blue acid-stable species found in the iron(III)-2,3-dihydroxybenzoic acid system consists of iron bound to the two hydroxy-groups and not the carboxylate function. The fact that all green and bluish species found in these systems have been shown to contain $Fe^{II}$, and purplish or red species contain $Fe^{III}$, renders the traditional $FeCl_3$ test for phenols more informative to the authors' knowledge. The only exceptions to this colour assignment are associated with the presence of the nitrate group.[11]

*General Conclusions.*—Clear analogies between the titration curves and the stoicheiometries of the iron complexes of catechol, 2,3-dihydroxybenzaldehyde, and 2,3-dihydroxybenzoic acid are reported in this study. The chemistry of the systems, typified by catechol, is presented in the Scheme. From the titration data of iron(III) and iron(II) with catechol it would appear that the acid-stable green and blue iron(II) compounds do not consist of simple catechol complexes but rather a semiquinone ligand generated by the reduction of iron(III), otherwise the green and blue iron(II) complexes would have formed as a result of the direct addition of iron(II). Equation (1) indicates a mechanism for the formation of the green complex. Due to protonation, this complex is unstable below pH 2.0, yielding a semiquinone radical [equation (2)]. This radical can undergo disproportionation to catechol and *o*-benzoquinone [equation (3)]. In contrast, above pH 2.0 the green iron(II) benzosemiquinone complex (1) is stable. Mentasti *et al.*[9,10] did not isolate

$$Fe^{3+} + H_2cat \rightleftharpoons [Fe^{II}(cat\cdot)]^+ + 2H^+ \quad (1)$$

$$[Fe^{II}(cat\cdot)]^+ + H^+ \rightleftharpoons Fe^{2+} + Hcat^{\cdot} \quad (2)$$

$$2Hcat^{\cdot} \rightleftharpoons H_2cat + o\text{-benzoquinone} \quad (3)$$

the green complex, although they detected its spectrum by stopped-flow methods ($\lambda_{max.}$, 700 nm). This is because they worked in the pH range 1—2. It is important to note that the presence of water is necessary for the reaction corresponding to equation (1) to occur. If catechol is added to iron(III) dichloride in ethyl ether or dry methanol, a purple complex is formed, which is converted to the green compound only on the addition of water. The chemistry associated with this reaction is presently under study.

A mechanism for the pH-dependent interconversion of the major iron–catechol species reported in this study is presented in the Scheme. As the hydrogen-ion concentration is reduced, complex (1) is converted to complex (2) which corresponds to iron(II) enterobactin.[4] The Mössbauer results of de Vries *et al.*[23,24] and Fitzsimmons *et al.*[25] for iron(II) chelates of salicylaldehyde and Schiff bases possess smaller quadrupole splittings 2.3—2.7 mm $s^{-1}$. The larger quadrupole splitting found in our systems may be due to the fact that $Fe^{II}$ is bound to a radical [(2), Scheme].

Over the pH range 6—9 we propose that complex (3) is the predominant species and that its formation is due to an increasing concentration of hydroxide anion, co-ordination of which favours iron(III). One possible route to the proposed bridge complex (3) is *via* the five-co-ordinate complex (8). Continued increase in pH increases the concentration of the catechol monoanion ($pK_a = 9.2$) which presumably favours complex (4).

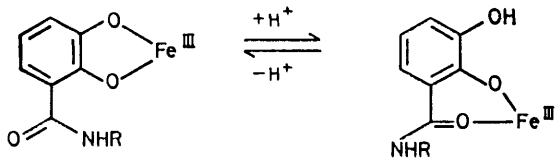
Our Scheme is rather different to that proposed by Avdeef *et al.*[2] where they suggest that three iron catechol complexes exist over the pH range 4—10, namely the mono-, bis-, and tris-catecholato-iron(III) complexes.

The pH at which the iron(II)–iron(III) redox reactions occur is strongly dependent on the nature of the catechol ligand involved. The transitions occur at pH's 7.0, 5.5, and 3.5 for catechol, 2,3-dihydroxybenzaldehyde, and 2,3-dihydroxybenzoic acid respectively (Table 1). If it is assumed that a similar phenomenon occurs for 2,3-dihydroxy-*N*,*N*-dimethylbenzamide, and the spectra of the iron complexes of this compound [26] suggest this to be the case, then the data of Harris *et al.*[26] can be interpreted to indicate that the redox reaction for this compound occurs in the region of pH 3.5. The larger influence of the carboxylic acid and amide functions over the aldehyde group is probably related to the relative electron withdrawing effect of these substituents. That amides induce a larger polarising influence than do aldehydes is shown by the dipoles of *N*-methylbenzamide and benzaldehyde which are 4.0 and 2.8 D respectively. The carbonyl function at position 1 will preferentially delocalise charge on the oxygen atom at position 2. This in turn might be expected to render the redox reaction less favourable and thus stabilise the iron(III) state. It is possibly significant that enterobactin possesses amide functions on each catecholato-ligand, thus preferentially stabilising iron(III) in solutions near neutrality.

No evidence was found for the existence of the salicylato-mode of co-ordination with dihydroxybenzaldehyde (this study) and apparently dihydroxy-*N*,*N*-dimethylbenzamide.[13] However we did find evidence for traces of such co-ordination with dihydroxybenzoic acid, although it was only observed in the presence of a high molar ratio of iron, the catecholato-co-ordination type predominating at all pH values. No evidence for salicylato-complexes was observed in the pH ranges where iron(II) was the co-ordinated cation. Avdeef *et al.*[2] have suggested that the acid-stable species is a 1 : 1 complex which exists completely in the salicylic acid mode of co-ordination. We found no evidence to support this suggestion.

The data presented in this paper shed new light on the recent results concerning the structure of the iron enterobactin.[26] It was suggested by Harris *et al.*[26] that in acid solution protonation of enterobactin occurs in such a manner as to shift catechol ligands in a stepwise manner to a salicylato-mode of co-ordination (9). Thus addition of three protons was predicted to yield two intermediate complexes before the final product, a neutral iron(III) enterobactin molecule. In support of

this suggestion, spectral changes in the visible and i.r. regions were sited, together with the fact that iron enterobactin precipitates from aqueous solution at *ca.* pH 3.5. If the titration of iron(III) enterobactin is conducted in aqueous methanol, in order to avoid precipitation of the neutral complex, then a different phenomenon to that predicted by Harris *et al.*[26] is obtained. A transition occurs (Figure 10) involving a single spectral change, indicating that there are no intermediates in the conversion of iron(III) enterobactin (ent) (10) to iron(II) enterobactin (11). This result is in agreement with the aqueous titration data of Salama *et al.*[17] and clearly indicates the difference in behaviour between enterobactin and monocatechol ligands. The sexidentate ligand enterobactin apparently forms a much simpler system with iron than the analogous monocatecholato-complexes, only forming two major complexes [(11) and (10)] corresponding to the iron–catechol complexes [(2) and (4)].[4] The structure proposed for (11) is slightly different to that proposed earlier,[4] the correction being based on the failure of enterobactin to form a blue complex with iron(II). The suggestion[26] that an intermediate in the enterobactin titration with a $\lambda_{max.}$ of 510 nm corresponds to that of a sexidentate siderophore isolated by Tait[27] is fortuitous, as recent work by Neilands *et al.*[28] suggests that the compound isolated by Tait does not co-ordinate in a bis(catecholato)-, mono-(salicylato)-mode[26] but rather a bis(catecholato)-, mono-oxazolino-mode, with one of the ligands being nitrogen.[28] Such co-ordination is not possible with enterobactin.




(9)

$$[Fe^{III}(ent)]^{3-} + 3H^+ \rightleftharpoons [Fe^{II}(H_3ent')]^0$$

(10) (11)

It would appear that cyclic tris(catecholato)-ligands have several advantages, as siderophores, over the corresponding mono(catecholato)-compounds. In addition to a pronounced chelate effect, enterobactin fails to form intermediate complex types and thus is less susceptible to polymer formation.[29] The fact that enterobactin possesses an amide carbonyl function at position 1 favours the iron(III) complex at quite low pH values unlike simple catechol complexes. Nevertheless, the reduction of iron by the catechol ligands is probably physiologically important as it offers a simple means of removing the tightly bound metal without disruption of the ligand. Thus edta (ethylenediaminetetra-acetate) is readily able to remove iron from enterobactin at pH 5.0,[26] a value where iron is largely in the reduced state (Table 1, Figure 10). An analogous environment could be readily created by an enzyme and thus it is conceivable that *E. coli* removes iron from enterobactin in the iron(II) state. Such a mechanism requires subsequent reduction of the enterobactin radical, if the siderophore is to be re-utilised. This reduction could be readily achieved by ascorbic acid or glutathione.

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