# Model Compounds for Microbial Iron-transport Compounds. Part 3.† Solution Chemistry and Mössbauer Study of Iron(II) and Iron(III) Complexes from 2,3-Dihydroxypyridine and 2-Mercapto-3-pyridinol

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The iron complexes found in solutions containing $FeCl_3$ and 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol have been studied by pH and conductance titrations together with Mössbauer spectroscopy. Some of these complexes are identified as $Fe^{II}$ species not unlike $Fe^{II}$ catechol and $Fe^{II}$ enterobactin complexes. Black precipitates formed in these systems give evidence for the presence of radicals and such radicals are offered as an explanation for the presence of $Fe^{II}$. It is proposed, in view of the findings of this paper that 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol are unsuitable as analytical agents for iron analysis.

THE compounds 2,3-dihydroxypyridine (dhp) and 2-mercapto-3-pyridinol (mhp) have been shown to be useful analytical reagents for spectrophotometric and chelatometric determinations of iron. Both compounds form coloured complexes with iron(III) which are relatively poorly characterized.[1-8] In the course of a systematic study of the interaction of iron(III) with phenol and catechol derivatives [9,10] we have studied the relative stabilities of the resulting iron complexes as a function of pH. Four distinct coloured complexes were observed with catechol, a green iron(II) catechol 1 : 1 complex, a blue iron(II) catechol (1 : 3), a purple iron(III) catechol (2 : 4), and a red iron(III) catechol (1 : 3). Similar results were also found with 2,3-dihydroxybenzaldehyde and 2,3-dihydroxybenzoic acid.[10] In these systems, the acid-stable blue and green complexes were found to contain iron(II) whereas the purple and red complexes were shown to be iron(III).[10] The ability of iron(III)–phenol solutions to undergo redox reactions is well documented and has led to a widely adopted synthetic method, the oxidative coupling of phenols.[11]

A mechanism for the pH dependence of the valence state of iron in the presence of hydroxy derivatives of benzene has been presented.[10] In the course of these studies it was found that at high concentrations, black precipitates were formed which contained little iron. Also it was observed that if the starting material was iron(II) and not iron(III) then the previously observed iron(II) complexes were not detected; furthermore, the oxygen sensitivity of these redox reactions necessitates the carrying out of such work under a nitrogen atmosphere.[10] As part of our continuing study of iron complexes with hydroxy derivatives of benzene, we considered it important to monitor the chelating capabilities of similar co-ordinating centres of heterocyclic analogues of benzene. It was anticipated that the presence of a heterocyclic nitrogen atom would influence the co-ordinating characteristics of the resulting iron complexes.[5]

We report here the results of a study of the chelating properties of 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol with iron(II) and iron(III) under nitrogen.

† Part 2 is ref. 15.

## EXPERIMENTAL

*Materials.*—2,3-Dihydroxypyridine (dhp) and 2-mercapto-3-pyridinol (mhp) (Aldrich Chemical Co. Ltd.) were recrystallized before use. Anhydrous $Fe^{III}Cl_3$ (SLR, Fisons), was used without further purification, $Fe^{II}Cl_2 \cdot 4H_2O$ was freshly prepared before use. 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 [12] 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 determined using a Perkin-Elmer Coleman 575 spectrophotometer. All spectra were recorded using a cell designed for the purpose of working under a nitrogen atmosphere. All measurements were taken at 25 °C. The solutions were unstable with respect to time, depositing black precipitates which complicated interpretation of the results.

*Determination of Stability Constants.*—These were calculated from pH titration and Job's plot data using the methods of Albert and Sergeant [13] and Likussar.[14]

*pH and Conductiometric Titration in Aqueous Solution.*—Iron ($10^{-3}$ mol $dm^{-3}$) was used throughout with different ratios of ligand. Additions of NaOH (1 mol $dm^{-3}$) or HCl (1 mol $dm^{-3}$) were achieved under nitrogen. A digital WPA model CD60 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 and $C_M$ is the analytical metal concentration, were corrected for increasing volume during titrations.

*Electrophoresis.*—Buffers in the pH range 3—11.2 were prepared from acetic acid–pyridine–$NH_3$ mixtures and glycine–NaOH. Whatmann 3MM paper was used throughout and a voltage of 5 000 V applied across a distance of 30 cm.

*Mössbauer Spectroscopy.*—Aqueous solutions of recrystallized mhp (1.61 mol $dm^{-3}$) and iron(III) chloride

hexahydrate (1.074 mol $dm^{-3}$) were mixed (mhp 1 $cm^3$, iron salt 0.5 $cm^3$); the pH was quickly adjusted to the required value using 5 mol $dm^{-3}$ NaOH or 5 mol $dm^{-3}$ HCl. Thus the mhp : iron ratio was fixed at 3 : 1.

Iron(III) chloride containing $^{57}Fe$ was prepared by heating elemental $^{57}Fe$ (1 mg) in a stream of dry chlorine, and dhp (13 mg) added to achieve a 3 : 1 ligand : iron ratio. The solutions were adjusted to the correct starting pH as before but were freeze-dried after immersion in liquid nitrogen. The mhp solutions were transferred to liquid cells and the dhp freeze-dried solutions mounted in solid cells. Both types of solution were frozen in liquid nitrogen and transferred to a precooled Harwell MNC 200 cryostat. The Mössbauer spectra were obtained at 80 K.[15] The spectrometer was calibrated with a 25 μm thick natural iron reference absorber. All isomer shifts are referred to this as zero shift.

## RESULTS

*Iron–dhp System.*—(*a*) *Starting from* $FeCl_3$. Three distinctly different-coloured species were detected in this system (Table 1). The electronic spectra are shown in Figure 1. The pH and conductiometric titrations were carried out at metal : ligand ratios of 1 : 1 and 1 : 3 (Figure 2). Three different coloured complexes were observed at each ratio, namely blue, purple, and red, although the intensities of all three species were much reduced in the 1 : 1 solution. The change in slope of the conductiometric data coincides with changes in solution colour. Above pH 4.4 a black precipitate occurs which progressively increases with increasing pH and becomes particularly marked in the range pH 7—10. If oxygen is introduced then the black precipitate becomes more dominant. Table 2, which summarises the results of other workers,[1-3,5-8] shows a broad agreement on the colours of the species and the pH ranges over which they occur. However, no mention has previously been made of the formation of a black precipitate.

From a Job's plot at pH 2.0 the blue species ($\lambda_{max.}$ 600 nm) was found to be a 1 : 1 iron–dhp complex with a stability constant of $2.5 \times 10^8$; when a solution of this complex is acidified to the pH range 0.0—2.0 and chemically analysed for the redox state of iron using potassium ferricyanide the iron is found to be predominantly in the +2 oxidation state. Indeed this result was confirmed by Mössbauer spectroscopy (Figure 3, Table 3). The Mössbauer data for dhp at pH 0.0 are different from the frozen solution data reported for the corresponding catechol system at pH 0.0 [10] and clearly result from an iron(II) environment containing

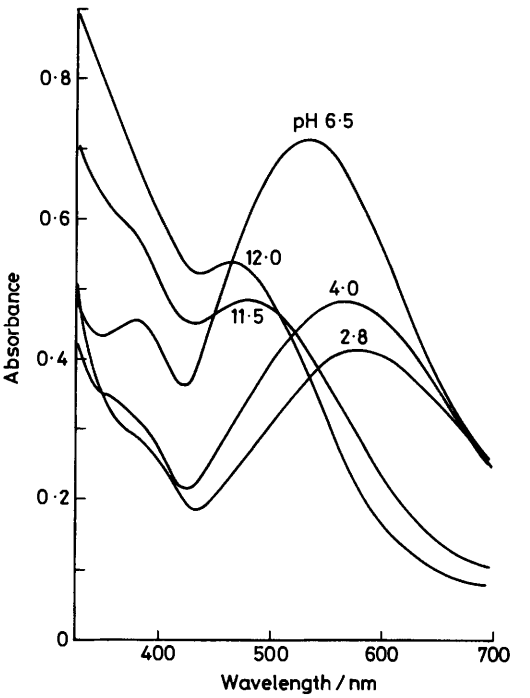



FIGURE 1 Visible absorption spectra of iron–2,3-dihydroxypyridine (1 : 3) solutions as a function of pH, [iron] = $10^{-3}$ mol $dm^{-3}$. A precipitate develops over the pH range 7—10 and consequently no spectra are given

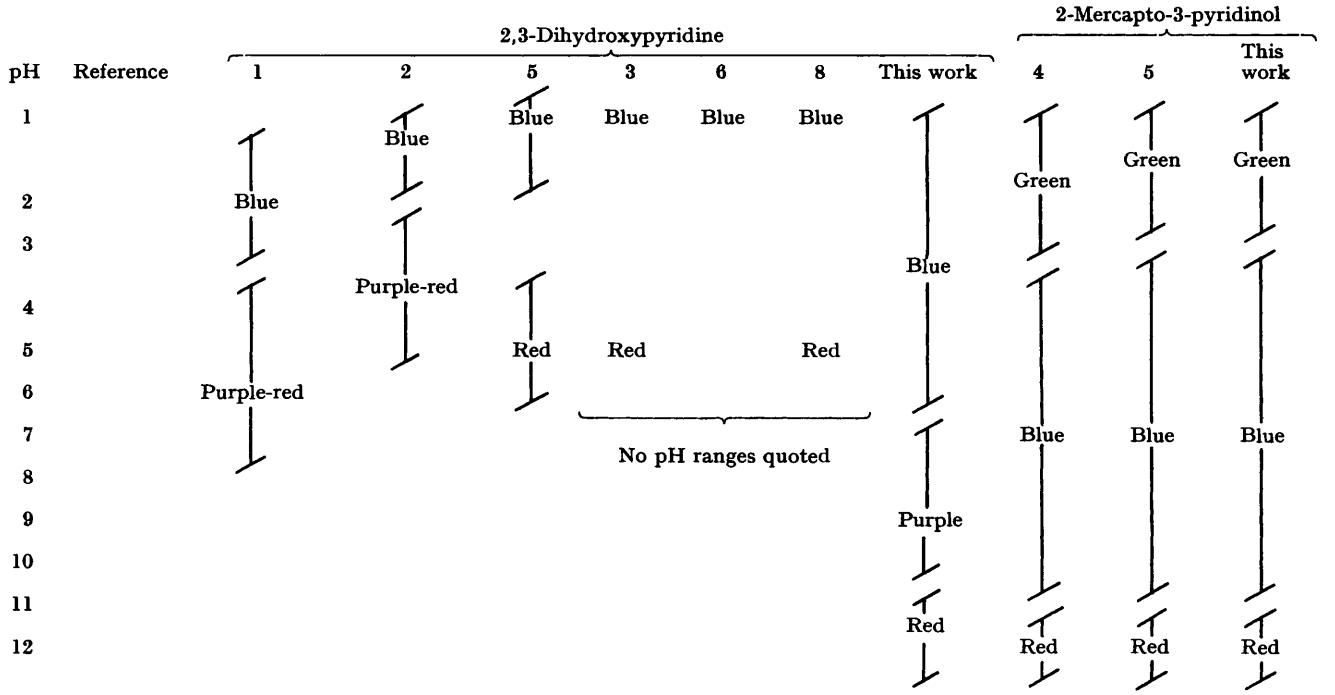
TABLE 1

The pH dependence of colour of 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol with $Fe^{II}$ and $Fe^{III}$, and catechol with $Fe^{III}$

| pH | 2,3-Dihydroxypyridine | | 2-Mercapto-3-pyridinol | | Catechol |
|---|---|---|---|---|---|
| | $Fe^{II}$ | $Fe^{III}$ | $Fe^{II}$ | $Fe^{III}$ | $Fe^{III}$ |
| 1 | | | | | |
| 2 | | | | Green | |
| 3 | Pink | | | | Green |
| 4 | | Blue | Light blue | | |
| 5 | | | | | |
| 6 | | | | | Blue |
| 7 | | | | Blue | |
| 8 | Pale blue | Purple | Green | | |
| 9 | | | | | Purple |
| 10 | | | Blue | | |
| 11 | Green precipitate | | | | |
| 12 | | Red | Purple | Purple | Red-wine |
| | Red/brown | | | | |

TABLE 2

The pH dependence of colour of 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol with $Fe^{III}$ as reported from various laboratories




dhp. Kushwaha *et al.*[3] suggested a tentative structure for the blue complex based on the assumption that it was an iron(III) species, as did Curtis and Atkinson,[7] although the latter workers did give a composition which included a chloride ion, namely Fe·dhp·Cl, the results reported in this work enabled us to reinterpret their data and to suggest formula (1) by analogy to our previous work centred on catechols.[10]

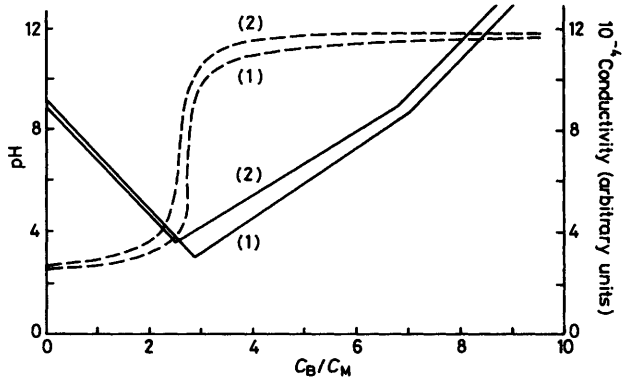



FIGURE 2 pH Titration (– – –) and conductivity (——) curves for 2,3-dihydroxypyridine–iron(III) at ligand : metal ratios of 3 : 1 (1) and 1 : 1 (2)

No attempt was made to run a Job's plot with the purple species due to the presence of a black precipitate, which is particularly marked in the pH range over which this complex is formed. Other workers have assumed that this is the red species (or a mixture of the blue and red, Table 2). Katyal *et al.*[1] obtained a 1 : 2 (Fe : dhp) ratio above pH 5.0 but did not specify the precise pH. The absorption coefficient of this purple species in the 500—600 nm range is higher than either that of the red or the blue species (Figure 1) and therefore is not a simple mixture of these two complexes. The Mössbauer data for a freeze-dried sample of the purple species show a broad iron(III) singlet (Table 3) with no detectable iron(II) indicating that the blue iron(II) is absent. Clearly, the purple species is not a mixture of the blue and red species.

The red complex, which exists above pH 11.0 yielded an unambiguous Job's plot which identified the complex as a 1 : 2, iron : dhp species. The net charge of the complex is negative as shown by electrophoresis and thus we assign structure (2) to this species, which is different to that proposed by Kushwaha *et al.*[3] Although we were unable to obtain a Mössbauer spectrum from freeze-dried samples at 80 K (probably because of a low *f*-factor) we would anticipate that, by analogy with iron-catechol at high pH, the complex remains in the iron(III) state.

(*b*) *Starting from* $FeCl_2$. The shape of the iron(II)–dhp (1 : 3) pH titration curve (Figure 4) is different from the corresponding iron(III)–dhp curve. Below pH 5.5 a rose-pink species is observed and over the pH range 5.5—9.0, a pale blue species persists. Above pH 9.0 a green precipitate of iron(II) hydroxide develops. On introducing oxygen, the colours were found to follow those of the iron(III)–dhp system and iron(II) hydroxide was not observed. This system is extremely oxygen sensitive and even in the presence of trace amounts, the rose-pink species changes to blue.

In view of the lability of this material, no further analysis was undertaken.

*Iron–mhp System.*—(*a*) *Starting from* $FeCl_3$. The pH titration and conductivity curves (Figure 5) indicate that three different species occur in this system which correspond to the three different observed colours, namely green, blue, and purple (Tables 1 and 2).

Job's plots for all three species together with the ranges

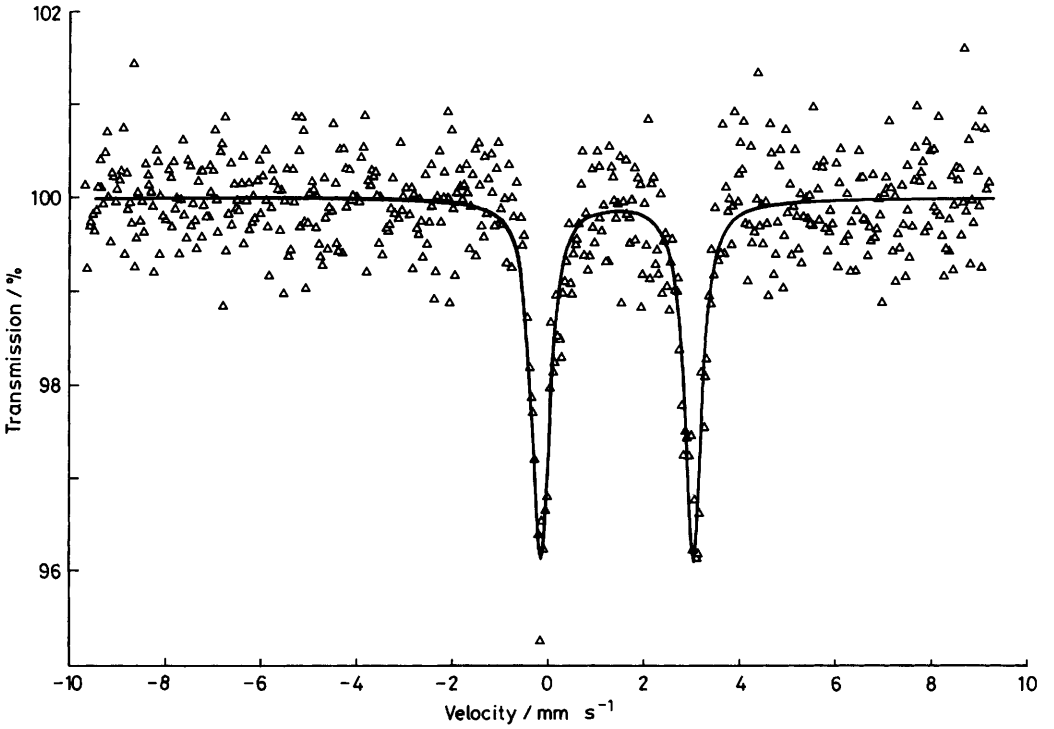



FIGURE 3 Mössbauer spectrum of a freeze-dried 2,3-dihydroxypyridine $^{57}$Fe sample taken at 80 K, which is not necessarily identical to that present in solution. It is unlikely that the freeze drying step would change the valence state of iron

TABLE 3

Mössbauer parameters for 2,3-dihydroxypyridine, $^{57}$Fe samples freeze-dried from solutions of given pH, and for 2-mercapto-3-hydroxypyridine, iron as frozen solution samples at given pH

| | pH | δ(Chemical shift) | Δ(Quadrupole splitting) | Γ(Half-width) |
|---|---|---|---|---|
| 2,3-Dihydroxypyridine | 0.1 | 1.41(1) | 3.19(1) | 0.21(1) |
| | 1.0 | 1.40(6) | 3.26(11) | 0.11(9) |
| | 6.0 | 0.61(1) | 0.0 | 0.27(1) |
| 2-Mercapto-3-pyridinol | 2.8 | 1.43(1) | 3.14(1) | 0.25(1) |
| | 6.0 | 1.46(2) | 3.21(4) | 0.25(3) |
| | | 0.57(2) | 0.70(1) | 0.26(1) |

over which the complexes are stable agree with the results found by other workers.[4,5] Thus we found that the green species is a 1 : 1 iron : mhp complex with a stability constant of $2.0 \times 10^7$. The net charge of this complex is positive as shown by electrophoresis, and when chemically analysed for the redox state using ferricyanide anions, iron(II) was found to predominate. Consequently, we suggest structure (3) as the likely formula for this complex by analogy with the corresponding complex with 2,3-dihydroxypyridine and in contrast to the previous suggestions which inferred the presence of iron(III). A frozen solution Mössbauer spectrum at pH 2.8 shows the presence of high-spin iron(II) (Table 3). With the blue complex, Mössbauer spectra of frozen solutions at pH 5.0 showed the presence of both iron(II) and iron(III), although iron(II) predominates. The presence of iron(III) is probably due to the extreme sensitivity of this system towards oxygen during handling procedures. This blue complex possesses a 2 : 1 stoicheiometry as judged by Job's plots and as it failed to move on electrophoresis (pH 5.0) we propose structure (4) which possesses no net charge.

The purple species was also found to possess a 2 : 1 stoicheiometry in agreement with the formulation assigned by Katyal *et al.*[4] with iron in the +3 state, structure (5).

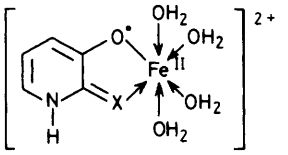
As with dhp, on starting from $FeCl_2$, entirely different phenomena were observed to those in the iron(III)–mhp system (Figure 5, Table 1).

## DISCUSSION

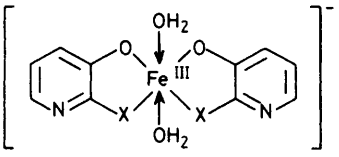
The most obvious difference between the behaviour of catechol and the two compounds dhp and mhp studied in this work is their p$K_a$ values. With both dhp and mhp, only one proton was detected potentiometrically thus giving support to the lactam structure initially proposed by Spinner and White [16] and subsequently supported by other structures (6).[4,7,17] Thus the major co-ordination species (7a) of dhp and mhp are different to those of catechol, (8a) and (8b). That dhp and mhp form complexes with iron at lower pH values than catechol (Table 1) is readily explained by their lower p$K_a$ values. Although the green colour of the 1 : 1 iron(II) mhp complex (3) is similar to that of the corresponding catechol species, with dhp the analogous complex (1) possesses a blue colour, closer to that observed with 2,3-dihydroxybenzoic acid.[10]

The major difference between the two systems des-

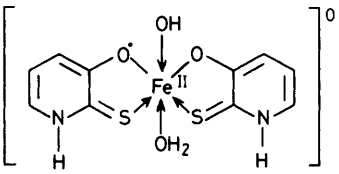
cribed in this work and that of catechols [10] is the apparent non-occurrence of a 3 : 1 ligand: iron complex with both dhp and mhp. This phenomenon must be related to the relative inability of dhp and mhp to lose a second proton



(1) X = O
(3) X = S



(2) X = O
(5) X = S



(4)

presumably because of the relative stability of the pyridone structures (6) and (7a). Studies with iron(III) catechol in water–methanol mixtures add weight to this concept in that, as the proportion of methanol is increased, the nature of the complexes change.[18] In 90% methanol, for instance, it is not possible to form the 3 : 1 red complex, presumably due to changes in the p$K_a$ values of catechol which are 13.1 and 15.5 in methanol.[19]

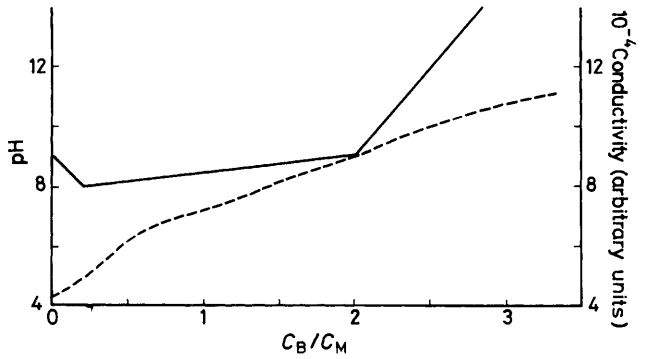



FIGURE 4 pH Titration (– – –) and conductivity (——) curves for 2,3-dihydroxypyridine : $FeCl_2$ (3 : 1)

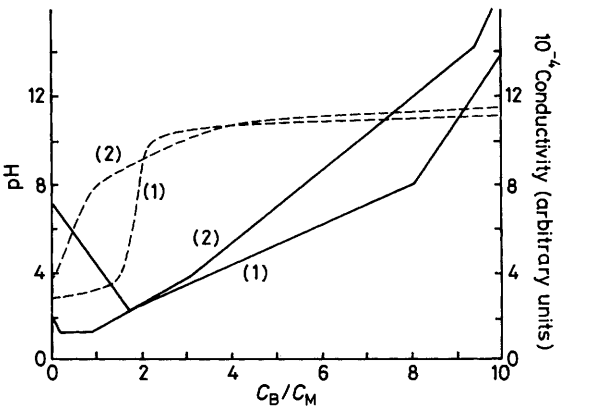
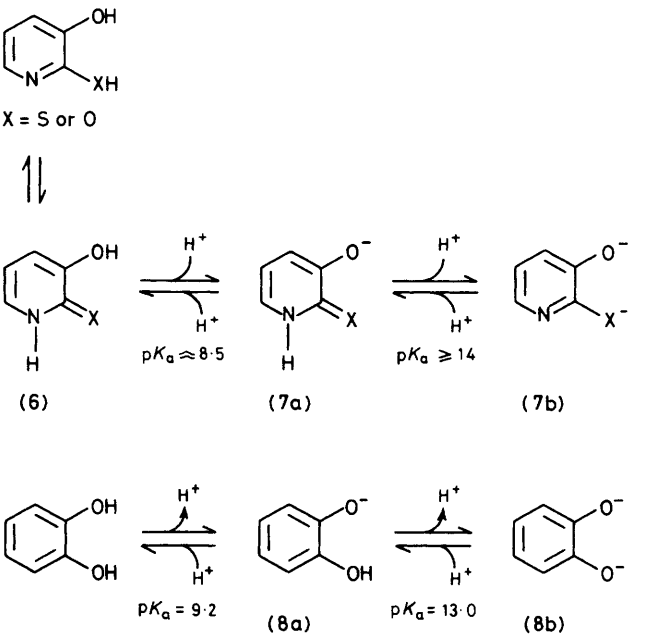



FIGURE 5 pH Titration (– – –) and conductivity (——) curves for 2-mercapto-3-pyridinol in the presence of either iron(III) chloride (1) or iron(II) chloride (2) at ligand : metal ratios of 3 : 1

As reported in previous work [9,10] iron complexes possessing either phenolic or catecholic ligands which are either green or blue are generally iron(II) and those that are purple or red are iron(III). This general observation holds true for the two ligands studied in this work, and the presence of a nitrogen atom in the ring or a sulphur ligating atom seems not to perturb this general observation.




Explanation of the chemistry offered previously and here necessitates the formation of radicals and the presence of the black precipitates may be taken as evidence for the radicals (*i.e.* the association of some radicals to form polymeric species). Both 2,3-dihydroxypyridine and 2-mercapto-3-pyridinol have been widely used for the analysis of iron(III) by chelatometric, gravimetric, and spectrophotometric methods.[1–8] In view of the findings reported in this paper concerning the ligand-

facilitated redox reaction and subsequent polymerisation of the ligand, it would appear that the compounds are unsuitable for use as iron analytical agents.

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