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Solution Chemistry and Mössbauer Study of Iron(II) and Iron(III) Complexes from Gallocyanine

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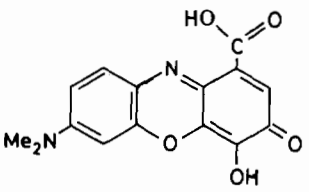
The iron solution chemistry of the $FeCl_3$–gallocyanine system has been investigated by pH titration, UV visible spectroscopy and Mössbauer spectroscopy. Iron reduction was found in the pH range 2–5 and photo-reduction of the iron(III) present was also noted. Due to the instability of the species present in solution, the use of gallocyanine as a spectrophotometric indicator in iron systems is not encouraged. The iron–gallocyanine system was proposed as a potential model of the photosensitive anti-cancer drug, Bleomycin.

Introduction

Gallocyanine (Dimethylaminohydroxyphenoxazone carboxylic acid), *1*



(*1*)

has been used widely as a dyestuff, in the staining of DNA and RNA [1–13] and in the photometric determination of indium [14], gallium [14], thorium [15], hafnium [16], zirconium [17, 18] and vanadium [19].

Its chemistry with iron has received relatively little investigation, although a method for determining microgram quantities of gallocyanine by spectrophotometric titration with iron(II) has been proposed [20]. It has also found use as an indicator in the direct EDTA titration of copper(II) and iron(II) [21].

As part of a continuing investigation into the chemistry of iron(II) with phenol and catechol derivatives [22–24] and the related iron/pyridinol systems [25], as model compounds for microbial iron transport, it was important to define the chelating capabilities of a similar centre in a heterocyclic analogue possessing two potential iron binding sites.

We report here the results of a study of the chelating properties of gallocyanine with iron(II) under nitrogen and the photostability of these complexes.

Experimental

Materials

Gallocyanine (Aldrich Chemical Co. Ltd.) was recrystallized from water 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 in deionized water and were stored under an oxygen-free nitrogen atmosphere.

Visible Spectroscopy

Optical densities of the mixed metal–ligand solutions and visible spectra were determined using a Beckmann DU-7 spectrophotometer. All measurements were taken at 25 °C. The solutions were unstable with respect to time depositing black precipitates, which complicated interpretation of the results.

The variation method [26] was used to determine the stoichiometry of complex ions at high pH. The ionic strength was kept constant by using 0.5 mol dm^{-3} NaCl.

pH Titration in Aqueous Solution

Iron (10^{-3} mol dm^{-3}) was used throughout with additions of NaOH (1 mol dm^{-3}) or HCl (1 mol dm^{-3}) effected under nitrogen. The pH was monitored using a Philips (pw-9409) digital pH meter.

Mössbauer Spectroscopy

Aqueous solutions of gallocyanine (1–3 mol dm^{-3}) were mixed with varying proportions of iron(III) chloride (1–2 mol dm^{-3}) and the pH quickly

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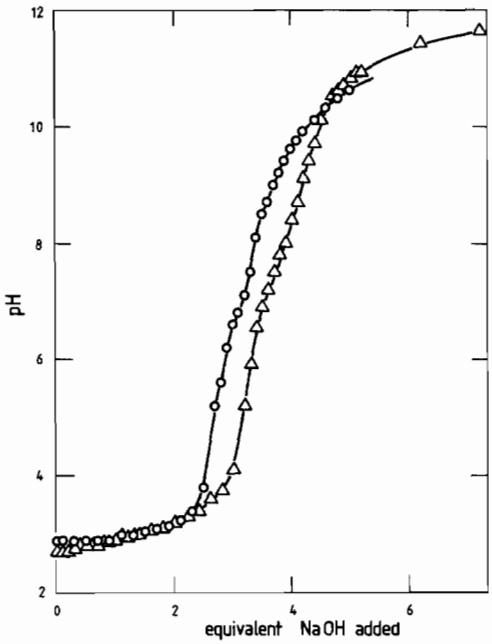


Fig. 1. pH titration curves for gallocyanine 1 × 10^{-3} mol dm^{-3} (△) and gallocyanine: $FeCl_3$, (1:1), 1 × 10^{-3} mol dm^{-3} each (○).

adjusted to that required using 5 mol dm^{-3} HCl or 5 mol dm^{-3} NaOH. The solutions were transferred to liquid-cells, quench frozen in liquid nitrogen and transferred to a pre-cooled Harwell MNC 200 cryostat.

Samples from photolysis were filtered, the supernatant was dried in air and both supernatant and filtrate transferred to solid-cells.

Mössbauer spectra were recorded and analysed by computer at 80 K fitting as described previously [23].

Photolysis

Photoirradiation of solutions was effected by means of a Quantum Yield Photoreactor (Model 2001, Applied photophysics) with a 250-W medium-pressure mercury lamp. Photolyses were performed in quartz cuvettes (1 cm path-length), thermostatted at an appropriate temperature (usually 20–25 °C). Photolysis apparatus components were mounted on an optical rail allowing photolysis to be carried out at various source-sample distances, usually 15–25 cm, under nitrogen.

Results

The pH titration of gallocyanine showed three species: a red-mauve species was present up to pH 4.1, a blue species (with copious precipitation) to pH 8.70 and a red-mauve species to pH 13.00, in agreement with earlier work [27].

Similar behaviour with respect to colours and their pH ranges was observed on titration at 1:1 iron:ligand ratio (Fig. 1). The presence of the metal ion obviously perturbed the pK_a values of gallocyanine as additions of three equivalents of alkali to the ligand alone changed the solution pH to 4.1, whereas the pH achieved in the presence of one equivalent of iron (initially present as Fe^{3+}) was 6.60.

Precipitations in the ligand only solution occurred at ≳pH 4.1, whereas in the 1:1 ligand:metal solution precipitation was noted from pH 2.90.

An apparently similar situation prevailed on titration of a 1:1:1 iron:copper:gallocyanine solution (Fe(III) and Cu(II) were the initial oxidation states when the solutions were first prepared). Both metals bind simultaneously to gallocyanine as no precipitates of ferric hydroxide or cupric hydroxide were noted at any pH.

The pH dependence of the UV/visible spectra of gallocyanine:iron solutions was found to be complex. In the presence of iron, changes occurred for the peaks in the region 460–540 nm over the pH range 5–9. The low pH red-mauve species had λ_{max} = 565 nm, similar to that [28] for the low pH complex of gallocyanine methyl ester with gallium. The ligand is itself red from pH 1 to 3.7. A broad maximum was noted for the blue species at pH 5.00 and no stable isosbestic point was observable for this and the species present at pH 4.5, indicating the presence of more than two complexes. No further analysis was undertaken because of the precipitation occurring in this region, even in the presence of nitrogen. A species with variable λ_{max} = 515 nm was observed in the pH region 7.5–12.0. Again isosbestic points were not sharp in this pH region. A Job's plot for this system at pH 11.34 gives evidence of the presence of both 3:1 and 1:2 ligand–metal complexes. The 3:1 complex was confirmed by adding iron to the ligand using the Mole ratio method [29], but precipitation of $Fe(OH)_3$ occurred in the 1:2 complex region.

The pH values of the solutions prepared from ferric chloride alone (1 × 10^{-3} mol dm^{-3}), gallocyanine alone (1 × 10^{-3} mol dm^{-3}), a mixture of ferric chloride–gallocyanine (each 1 × 10^{-3} mol dm^{-3}) were 2.98, 3.43 and 2.90 respectively. Dissolution of gallocyanine releases effectively 1 mol equivalent of protons. Dissolution of $FeCl_3$ in water also releases ~1 mol equivalent of protons. However, it is clear from the above that the gallocyanine:iron complex releases only one mol equivalent of protons (and not the two equivalents which would have been released had gallocyanine and the iron dissolved independently in each others' presence). The most reasonable explanation for this is that the iron is replacing a proton in the gallocyanine, although not necessarily at the same site. This is

TABLE I. ^{57}Fe Mössbauer Parameters (mm sec^{-1}) for Solutions of Iron(III) Chloride–Gallocyanine at Different pH Values, at 80 K and at the Indicated Metal:Ligand Ratios.

	pH	δ	Δ	Γ	% Absorption Area
Red Mauve species	1.0	0.31(3)	0.0	0.23(2)	54
		0.35(4)	0.56(4)	0.18(1)	46
Purple species (Gallocyanine:iron)	2.0				
(1:1)		1.36(6)	3.31(1)	0.21(9)	36
		0.50(1)	0.48(8)	0.65(5)	64
(2:1)		1.36(1)	3.26(2)	0.19(2)	28
		0.49(2)	0.0	0.73(5)	72
(1:2)		1.35(1)	3.25(2)	0.25(2)	25
		0.49(1)	0.0	0.69(2)	75
	3.0				
(3:1)		1.31(2)	3.28(3)	0.26(3)	31
		0.48(2)	0.64(3)	0.43(4)	69
Blue species	5.0				
(3:1)		0.45(1)	0.81(2)	0.26(1)	100
Red species	11.0				
(3:1)		0.42(8)	0.61(1)	0.24(1)	100

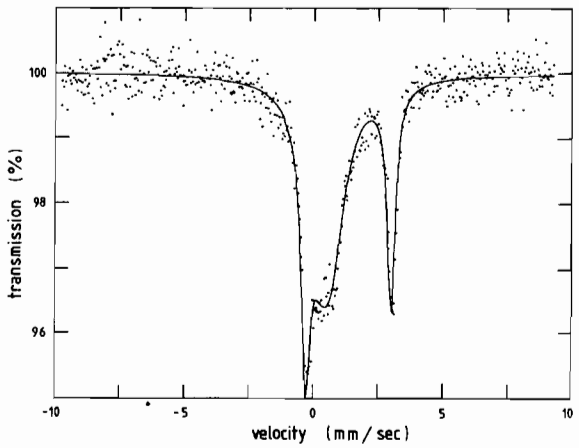


Fig. 2. Mössbauer spectrum at 80 K of gallocyanine:$FeCl_3$ (1:1), frozen solution at pH 2.00.

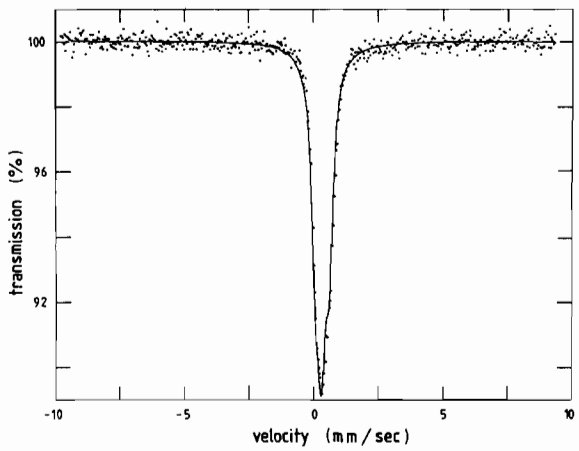


Fig. 3. Mössbauer spectrum at 80 K of gallocyanine:$FeCl_3$ (1:1), frozen solution at pH 1.00.

consistent with the loss of the carboxyl proton on complexation with iron(III), to give a 2:1 metal: ligand species, (*e.g.* as in site A of *2*).

Mössbauer spectroscopy of frozen solution was performed to further illuminate this system. Mössbauer spectra of frozen solutions at pH 2.00 and pH 3.00 indicated two iron sites, Fig. 2, Table I. The iron(II) site for an iron–ligand ratio of 1:1, (δ = 1.35 mm s^{-1}, Δ = 3.26 mm s^{-1}) is similar to that reported for the blue catechol 1:3, metal:ligand species [23] and is probably an octahedral iron-(II) environment at site B (see *2*), with H_2O molecules in the 'vacant' co-ordination positions of the iron. Occupation of this site appears not to be greatly dependent on the ratio of metal:ligand, as indicated from the various ligand to metal ratios studied (Table I). The putative iron(III) site on gallocyanine (see A of *2*) is not distinguishable by Mössbauer spectroscopy as frozen $FeCl_3$ solutions give similar iron(III) parameters [32].

Although the relative intensities of the resonance lines are strongly dependent on the f-factor for each site, the iron(II) site represents approximately 25% of the total iron as one would expect similar f-factors in frozen solutions at 80 K for sites involving the same molecule. For other metal:ligand ratios, (*viz.* 1:2, 2:1), similar iron(II) sites were detected and data are collected in Table I. From the fact that the excess metal to gallocyanine 2:1 frozen solution only shows 25% iron(II) then some iron(III) does not bind to gallocyanine at this pH. Also as iron-(II) is found, then the phenolic site reacts with

TABLE II. ^{57}Fe Mössbauer Parameters (mm sec^{-1}) at 80 K, for Photoirradiated (t = 15 min), Iron(III) Chloride–Gallocyanine Solutions at pH 3.0; (a) at a metal:ligand ratio of 1:2; (b) at a metal:ligand ratio of 1:3.

	δ	Δ	Γ	% Absorption Area
(a)				
supernatant	1.31(1)	3.13(2)	0.19(1)	78
(dried in air)	0.26(8)	0.26(2)	0.45(2)	22
precipitate	0.43(1)	1.26(2)	0.22(1)	100
(b)				
supernatant	1.31(2)	3.04(5)	0.17(4)	72
(dried in air)	0.61(5)	0.00	0.26(7)	10
	0.21(3)	0.00	0.29(3)	18
precipitate	0.49(6)	1.28(2)	0.19(9)	100

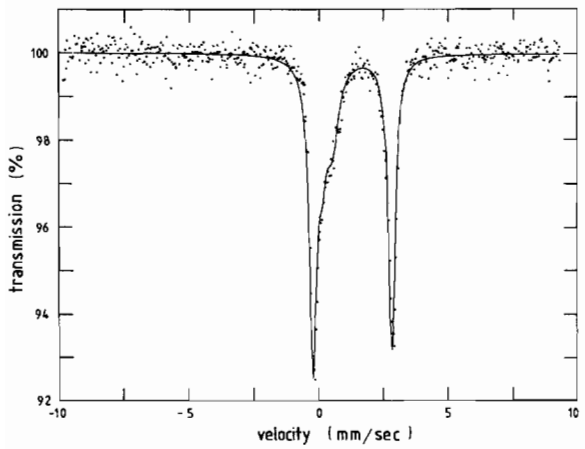


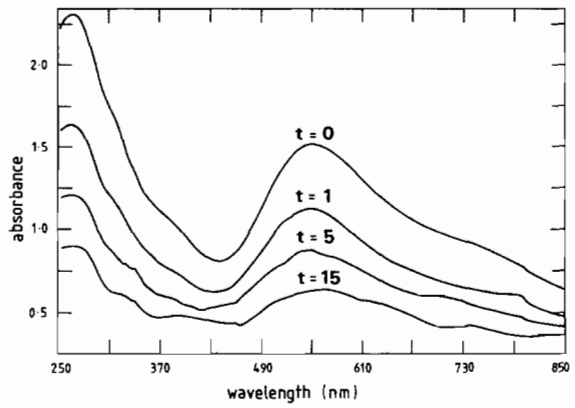
Fig. 4. Mössbauer spectrum at 80 K of the supernatant (dried) from photoirradiation of a gallocyanine:$FeCl_3$ solution at 2:1 ligand:metal ratio.

iron(III) to form iron(II) as found in other systems [22–25].

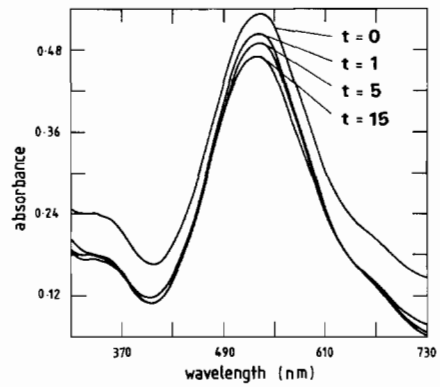
The second site δ = 0.50 mm s^{-1}, Δ = 0.48 mm s^{-1} is similar to the minor constituent reported for 2,3-dihydroxybenzoic acid [22] which can be interpreted as evidence of some iron(III) binding in the salicylato-mode of co-ordination and is a typical iron(III) environment as found for iron: catechol [23] and iron:pyridinol systems [25].

The low pH Mössbauer spectrum of gallocyanine: iron (red-mauve species pH 1.00) (Fig. 3), gives evidence for two iron(III) sites, though our fitting (Table I) will not be unique. These iron(III) sites have lower chemical shifts, ≅0.3 mm s^{-1}, than those reported previously [22–25, 30] and may indicate low spin, S = ½, iron(III) environments [31]. There is no evidence for iron(II) at this pH.

The gallocyanine molecule would be expected to be fully protonated at pH 1.00 thereby allowing



(a)



(b)

Fig. 5. (a) Electronic absorption spectra for gallocyanine: $FeCl_3$ (3:1), 3 × 10^{-3} mol dm^{-3}:1 × 10^{-3} mol dm^{-3} before (t = 0) and after photoirradiation for the indicated time in minutes at pH 3.00. (b) Electronic absorption spectra for gallocyanine, 3 × 10^{-3} mol dm^{-3}, before (t = 0) and after photoirradiation for the indicated time (in minutes) at pH 3.00.

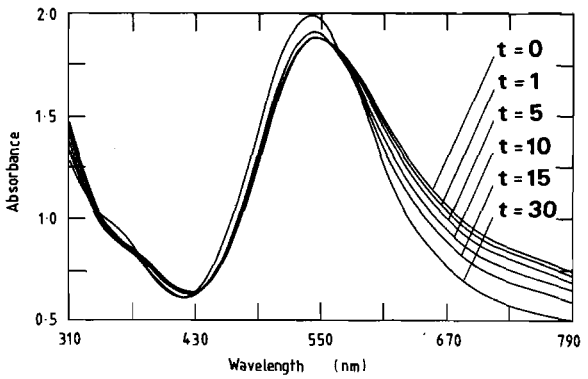


Fig. 6. Electronic absorption spectra of gallocyanine:iron solution (3:1), 3×10^{-3} mol dm^{-3}: 1×10^{-3} mol dm^{-3}, respectively at pH 3.00 at the indicated intervals of time in minutes.

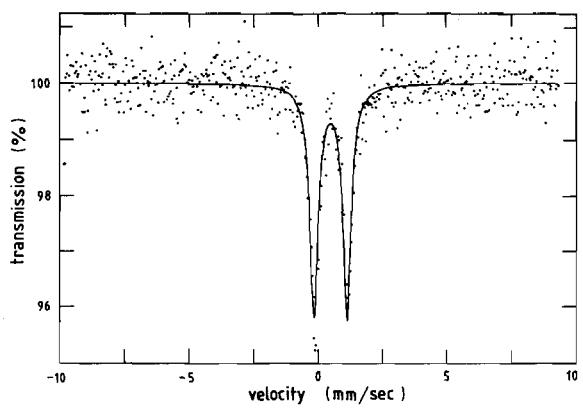


Fig. 7. Mössbauer spectrum at 80 K of the precipitate from photoirradiation of a gallocyanine:$FeCl_3$ solution at 2:1 ligand:metal ratio. This precipitate by analysis showed the presence of only a small amount of iron, accounting for the poor statistics of this spectrum.

the carboxyl group to exert an electron withdrawing effect. This would reduce the electron density available for radical reduction of the iron, and seriously reduce the iron binding properties of the ligand, at least as far as the oxygen functions are concerned.

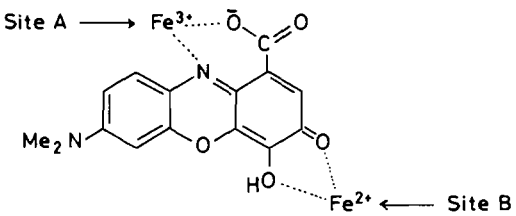
The Mössbauer spectra of the species present at pH 5.00 and pH 11.00 in 3:1, ligand:metal, frozen solutions show parameters consistent with high spin iron(III) environments. A second iron site is not apparent in these spectra although the line widths could indicate two similar iron(III) sites as in the purple catechol complex [23].

Effects of Light

Photoirradiation of the purple species at pH 2.00 (Fig. 2) led to slight precipitation (20% w/w), and the Mössbauer spectrum of the evaporated supernatant resulting from photoirradiation (15 minutes), (Fig. 4), showed a marked decrease in the amount of iron(III) (Table II). Photoirradiation was accompanied by a decrease in the absorbance at 565 nm (Fig. 5a). The rate and extent of this decrease was significantly greater than that found for the ligand alone (Fig. 5b), and also greater than the slight decrease found for gallocyanine:iron solutions at this pH but in the absence of photoirradiation (Fig. 6). Varying the metal:ligand ratio between 1:2 and 1:3 also appeared to have little effect on the iron(II) behaviour as the Mössbauer spectra for this site in precipitates from both systems were very similar (Table II).

The precipitate produced from the photolysis contained little iron (analysis showed 3% iron and 44% carbon) and its Mössbauer parameters (Fig. 7, Table II) are indicative of iron(III) in a strongly asymmetric environment. No pH change was noted on photoirradiation, the pH being 3.00 before and after irradiation.

Discussion



(2)

Clearly gallocyanine bonds to iron much as do the corresponding catechols and pyridinols, although the larger conjugated ring structure of the gallocyanine molecule is accompanied by a corresponding shift in absorbance to the red end of the spectrum of the species formed. Whereas catechol forms green, blue, purple and red complexes, the complexes of gallocyanine with iron are red or red-mauve. There appears to be little evidence for a distinct iron(II) complex at low pH, although there is some evidence of iron(II) binding to gallocyanine.

Reduction to iron(II) also occurs in the gallocyanine:iron system probably involving a radical mechanism much as in the catechol systems [22–24]. Indeed this may help to explain the photolysis results as a comparison of the percentage absorption of the sites before and after photolysis indicates that the iron(III) site is being photo-reduced to iron(II) and a light-induced radical could account for this.

The presence of two binding sites for iron complicates the equilibria, so that specific stoichiometries for the species formed in solution cannot always be assigned readily. The species formed is also dependent on the ratio of iron to ligand. Some species in

solution contain more than one valence state and this coupled with the polymerization of the ligand (in the form of black precipitates) makes gallocyanine an unsuitable reagent for iron analysis. Indeed Kotouček *et al.* [28] have suggested using gallocyanine methyl ester because of its greater solubility and higher photostability.

The iron-binding properties of gallocyanine, and especially the photoproperties, including photoreduction of the metal, are reminiscent of the complex chemistry of the useful anticancer drug Bleomycin which is markedly photolabile both in the absence [33] and presence of various metal ions including iron [33, 34], copper [33] and cobalt [35]. This gallocyanine:iron system may be regarded as a suitable bioinorganic model of the difficult antibiotic system for which the photochemistry [36] and metal complex chemistry [37] has been discussed.

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