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# Is there a redox reaction between Cu(II) and gallic acid?

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**Abstract**
Interactions between transition metal ions and polyphenols can result in complexation, redox or polymerization, but the relative importance of these reactions is unclear. The present paper reports results from the reaction of gallic acid (GA) with Cu(II) using electron paramagnetic resonance (EPR) and UV/visible spectroscopy for various relative concentrations and pH values. Reduction of Cu(II) by GA does not occur under strongly acidic or strongly alkaline conditions. Di- or polymerization reactions between Cu(II) and carboxylate groups of GA dominate the results at acidic pH, whereas mononuclear complexes increase in importance at higher pH and GA concentrations. There was no evidence for any redox reaction between Cu(II) and GA and free radical formation from GA at high pH was shown to be the consequence of auto-oxidation, which was inhibited by Cu(II). Serious questions are thus raised about the existence of the frequently assumed redox reactions between Cu(II) and polyphenols.

**Keywords:** *Gallic acid, copper, free radical, EPR spectroscopy, UV/VIS photometry*

**Abbreviations:** *GA, gallic acid; EPR, electron paramagnetic resonance; hfc, hyperfine coupling; MA, modulation amplitude; MF, modulation frequency; MP, microwave power; DI, double integral.*

## Introduction

Interactions between transition metal ions and phenolic compounds are widespread in nature and can involve complexation of metal ions by the phenols or their oxidation products, polymerization and redox reactions. Although such reactions have been studied extensively over several decades, there are still important gaps in our understanding of the factors responsible for determining the specific course of reactions in many natural systems.

Gallic acid (3,4,5-trihydroxybenzoic acid) (GA) is an important biological and industrial molecule, in which the presence of hydroxyl and carboxylic acid groups in the same molecule allows the formation of numerous esters, salts and polymers including digallic acid. GA is regarded as a natural antioxidant molecule and GA derivatives (e.g. n-propylgallate) are common food additives [1]. Copper is an important industrial metal and an essential micronutrient for life; its chelating ability and positive redox potential allow participation in biologic electron transport reactions, but it is also an environmental pollutant [2,3].

Although polymerization and complexation reactions between Cu(II) and GA have been reported [4,5], it is frequently assumed that redox is the major reaction process, especially in the biological literature [6–10]. In redox reactions between Cu(II) and polyphenol molecules, Cu(II) is reduced to Cu(I) and the hydroquinone ($H_2Q$) is oxidized to the semiquinone (HQ·).

$$Cu(II) + H_2Q \rightarrow Cu(I) + HQ\cdot \quad (1)$$

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In a second oxidation step, the semiquinone (HQ·) is oxidized to the quinone (Q) also by Cu(II) [11].

$$Cu(II) + HQ\cdot \rightarrow Cu(I) + Q \quad (2)$$

In contrast to the reactions described above, Satoh and Sakagami [9] reported that Cu depressed GA-derived free radical production. However, their system was complicated by working in solutions controlled by Tris-HCl buffer, which is known to exert a strong influence on the chelation chemistry of Cu(II) [12] and their free radical electron paramagnetic resonance (EPR) spectra did not correspond to that of the semiquinone from oxidized gallic acid [13].

Understanding this system is further complicated by the fact that production of the semiquinone and quinone also occurs as a result of auto-oxidation reactions in the absence of any transition metal ions [13–15].

$$H_2Q + O_2 \rightarrow HQ\cdot + O_2^{\bullet -} \quad (3)$$

$$HQ\cdot + O_2 \rightarrow Q + O_2^{\bullet -} \quad (4)$$

Whilst reaction (3) is slow, except under strongly alkaline conditions, reaction (4) is an alternative pathway for oxidation of the semiquinone radical, HQ·. Reactions (3) and (4) generate $O_2^{\bullet -}$, which in turn leads to the production of other reactive oxygen species [8]. Furthermore, the Cu(II) speciation is complicated by hydrolytic polymerization, which occurs at pH values $> 6.0$ in the absence of polyphenol [16], and results in a loss of EPR spectral intensity analogous to that which would occur on reduction of Cu(II) to Cu(I).

In the present paper we report the results of a comprehensive investigation of the gallic acid/copper system using EPR spectroscopy as a tool to provide further insight into the reaction chemistry between Cu(II) and GA over a wide range of experimental conditions. In addition, since quinones are intensely coloured with absorption maxima in the range 380–450 nm [17–19], UV/VIS photometry was used for the identification of quinone formation.

## Experimental

### *Materials*

Gallic acid (GA, $\geq$ 98%) was purchased from Fluka (Sigma–Aldrich Handels GmbH, Vienna, Austria) and copper sulphate anhydrous ($CuSO_4$) was bought from Merck (VWR International GmbH, Vienna, Austria).

### *Sample preparation*

Except where stated otherwise, all solutions were prepared using Millipore water containing ambient $O_2$ levels. Two independent sets of measurements were performed. In the 1st experiment, the effects of variable GA concentrations were investigated for a constant concentration (2 mM) of Cu(II) over the pH range 1–12; Cu:GA ratios were 1:0, 1:1, 1:2, 1:5 and 1:10 and solution pH values were adjusted using either $HClO_4$ or NaOH and measured using a WTW Inolab Level 2 pH meter with a SenTix 41 pH electrode. Separate sets of measurements were performed using either pure water or 1:1 water/methanol (the latter for consistency with published stability constant data for the Cu/GA system). The data presented all correspond to one set of measurements, but the qualitative nature of the results was confirmed in several separate measurements that were made at selected pH values and Cu:GA ratios.

In the 2nd experiment, measurements were made with alkaline solutions (pH 13) containing 5 mM GA and Cu(II) concentrations in the range of 0.1–7.5 mM. The mixtures were shaken for a few seconds before transferring to a quartz flat cell and recording the EPR spectra of the semiquinone radical, which commenced 3 minutes after adding the NaOH. As with the data for the 1st experiment, the reported results correspond to one experiment, although many related measurements were made when optimizing the conditions to use for these measurements.

When the pH of the Cu/GA solutions was increased above ~4.5, a precipitate was observed to form. In a separate experiment, this was produced on a larger scale at pH 5.5. It was separated by centrifugation, washed with water at pH 5.5 and dried in a vacuum dessicator. This solid was then used for additional EPR measurements after dissolution under $N_2$ at pH 11.5 and again after adding additional GA.

Solutions (1 ml) for UV/VIS photometry contained 5 mM GA and various Cu(II) concentrations in deionized water in the range of 0–10 mM. These measurements were made at pH 11.0, because of interference with the quinone peak at 425 nm by a broad peak with a maximum at 490 nm at higher pH values. In order to support the assignment of the peak at 425 nm to the quinone, a similar solution of 5 mM GA was prepared initially under a $N_2$ atmosphere and its UV/visible spectra then recorded as the solution was slowly auto-oxidized.

For samples where the Cu-intensity was measured, the pH was determined before (and in some experiments also after) the EPR measurement. Within the ~10 min required for the EPR experiments, the pH was relatively stable and changes of only ~0.1–0.2 pH units were observed. In some preliminary experiments where the GA semiquinone radical was determined, the pH was measured after the EPR measurements. At these alkaline pH values, there is a strong sensitivity of the GA auto-oxidation reaction to pH and, as a consequence, variations in signal intensity occur for very small pH changes. Thus, errors in reproducibility of spectral intensity, which were of the order of 10% in replicate measurements,

were probably the consequence of small differences in the actual pH at which they were made.

Measurements of the stability of the free radical signal were performed with GA:Cu concentration ratios of 1:0, 1:0.02, 1:0.06 and 1:0.5 using 10 mM GA and a pump-flow system. Separate solutions containing the Cu/GA mixture and 0.5 M NaOH were pumped into a flat cell and the spectrum recorded until its intensity stabilized. The pump was then switched off and spectra recorded continuously over 30 minute periods.

The measurements performed at high pH were made with the objective of examining in detail the influence of Cu(II) on the production of the semiquinone free radical, whose EPR signal was weak to non-existent at lower pH values. Although such pH values are not directly relevant to living systems, these extreme conditions were used to demonstrate what is chemically possible in the reaction between Cu(II) and gallic acid, because of a need to explore the possibility of a redox reaction over as wide a range of conditions as could be reasonably obtained.

### *EPR spectroscopy*

EPR spectra were acquired as first derivatives of the microwave absorption with a Bruker EMX CW spectrometer, operating at X-band frequencies (9 GHz) and using a high sensitivity cavity. Microwaves were generated by a Gunn diode and the microwave frequency was recorded continuously with an in-line frequency counter. The Cu(II) spectra were measured using 20 mW microwave power (MP), 100 kHz modulation frequency (MF) and 1 mT modulation amplitude (MA), whereas the free radical spectra were detected using 2 mW MP, 20 kHz MF and 5 μT MA. g-values were determined by reference to the signal of DPPH (g = 2.0036), which was used as an external standard. All parameters quoted are accurate to ±1 in the last significant figure. Signal intensities were determined by double integration (DI) using the Bruker WINEPR software. For determination of the Cu(II) intensity, the DI of the whole Cu(II) spectrum was carried out, followed by subtraction of the DI of the intensity of the free radical signal in the measurements at very high pH. In the case of the free radical intensity, the DI was performed on the 1st peak of the triplet signal.

### *Qualitative nature of the EPR spectra*

The chemistry of copper is based almost entirely on the Cu(I) and Cu(II) ions, which have 10 and nine 3*d* electrons, respectively; thus Cu(I) is diamagnetic and Cu(II) is paramagnetic. Many Cu(II) complexes have structures based on the square planar or tetragonally-distorted octahedral geometries, in which the unpaired electron is located in the $3d_{x2-y2}$ orbital. Such complexes are amenable to characterization by EPR spectroscopy [20,21]. The signals show little tendency to saturate and the relatively broad linewidths mean that spectra can be recorded with quite high values for the MP and MA. Hyperfine structure arises from interactions between the unpaired electron and the $^{63}$Cu and $^{65}$Cu nuclei (I = 3/2), whose magnetic moments [22] and hence hyperfine coupling (hfc) constants (*A*-values) differ by ~7%. The peaks from the individual isotopes are, however, often not resolved. Both *g*- and *A*-tensors are sensitive to the symmetry at the Cu site in a complex and to the degree of covalency of the bonding between the Cu and ligands. Furthermore, in fluid solutions of Cu(II) complexes, spectra generally have anisotropic linewidths because of incomplete averaging of the rigid limit spectral parameters by molecular motion. Also, if Cu(II) forms di- or poly-meric complexes (e.g. with simple carboxylic acids [23,24]), the spectra may be lost completely, either as a result of the formation of Cu–Cu bonds or through anti-ferromagnetic exchange interactions. Thus, the absence of an EPR spectrum does not necessarily indicate that the copper is in the Cu(I) form.

The EPR spectrum of the semiquinone formed by oxidation of GA consists of a triplet signal with a 1:2:1 intensity ratio from two equivalent $^{1}$H atoms [13]. The signal saturates readily and spectra need to be recorded with low MP values for quantitative work. Furthermore, the narrow linewidths mean that low values of both the MF and MA are needed for optimum signal quality.

## Results

### *Total Cu(II) EPR signal intensity*

Plots of the total Cu(II) EPR signal intensity as a function of pH for various Cu:GA ratios in 1:1 aqueous methanol are shown in Figure 1; similar results were observed when water was used as the solvent. In the absence of GA, the intensity of the Cu(II) signal decreased to zero between pH 5.5 and 6.0, and it remained at zero to pH 12. In the presence of GA, the decrease in Cu(II) signal intensity occurred around pH 4.0, i.e. ~1.7 pH units lower than in the absence of GA. There was little effect of GA concentration on the EPR spectra at these acidic pH values, although with the 1:10 Cu:GA ratio a small fraction of the original signal intensity remained in the pH range 4–7. Although this amounted to ≤2% of the intensity at low pH values, the relatively sharp lines allowed its EPR spectrum to be clearly observed (see following paragraph). Under alkaline conditions, the intensity of the signal from the 1:10 Cu:GA solutions increased with increasing pH and at high pH it approached that observed under acidic conditions.

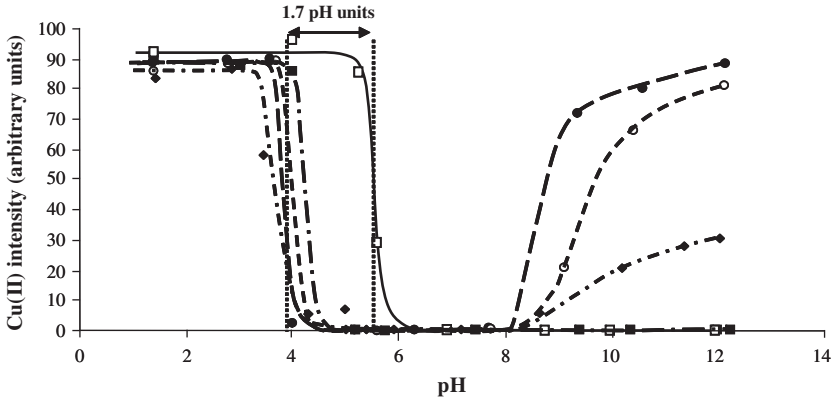


Figure 1. Variation of the overall Cu(II) signal intensity as a function of pH for 4 mM Cu(II) in the presence of various concentrations of GA in 1:1 $H_2O$:methanol (□ Cu:GA = 1:0, ■ Cu:GA = 1:1, ♦ Cu:GA = 1:2, ○ Cu:GA = 1:5, • Cu:GA = 1:10).

Smaller increases were observed with the solutions having 1:5 and 1:2 Cu:GA ratio and little signal intensity was observed below pH 11 for lower GA concentrations. These results can all be understood in terms of the $pK_a$ values for gallic acid, which have been reported as 4.72, 8.81 and 10.15 for GA in 20% aqueous methanol [25–27].

### *Cu(II) EPR spectra*

Considerable qualitative variations in the Cu(II) signals were observed as a function of pH, and to a lesser extent of the Cu:GA ratio; representative spectra from the solutions with the 1:10 Cu:GA ratio are shown in Figure 2. At least two, and probably three, different Cu(II)-GA complexes were observed along with a signal similar to that from the uncomplexed Cu(II) ion. The signals from the Cu(II) complexes all showed strong linewidth anisotropy, and only the two highest field peaks were well resolved. Because of this, only approximate *g*- and *A*-values could be calculated, and the discrimination of the separate species was made primarily on the basis of the positions of these two highest field peaks.

In the low pH-range (≤4), broad almost featureless spectra were observed for all solutions ($g \approx 2.19$); these correspond to the hydrated Cu(II) ion $[Cu(H_2O)_6]^{2+}$ [28,29] and dominated the spectra at these low pH values (Figures 2A–C). A weak signal with parameters $g \approx 2.15$, $A(Cu) \approx 6.4$ mT[1] (Complex A, Figures 2C–E) was detected in the pH range 4–6, and the spectrum of a second complex ($g \approx 2.11$, $A(Cu) \approx 7.4$ mT) (Complex B, Figures 2F–I) was seen at pH > ~6. The intensity of this latter signal increased with increasing pH and dominated the spectra at higher pH values. Small changes in the shape of the Cu(II) spectra were observed at very high pH and the highest field copper hf peak was shifted to higher field (Figure 2J), indicating the formation of a third complex (Complex C) with slightly different spectral parameters from those

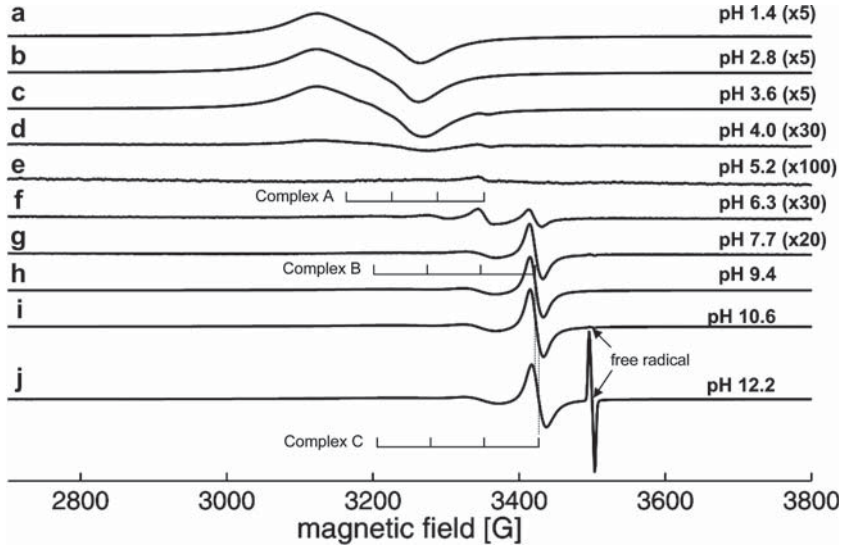


Figure 2. Representative room temperature EPR spectra from solutions of Cu/GA in a 1:10 ratio in 1:1 aqueous methanol at room temperature. (A) pH 1.4, (B) pH 2.8, (C) pH 3.6, (D) pH 4.0, (E) pH 5.2, (F) pH 6.3, (G) pH 7.7, (H) pH 9.4, (I) pH 10.6 and (J) pH 12.2. Note the free radical signal appears here as a single peak because of the large modulation amplitude that was used to record the Cu(II) signals.

of Complex B. Qualitatively similar spectra were obtained with the solutions containing lower concentrations of GA, but, as indicated in Figure 1, their total intensities decreased with decreasing concentration of GA in the alkaline pH range.

When the sample precipitate at pH 5.5 was dissolved in weak alkali at pH 11.5 under $N_2$, an EPR spectrum identical to that of Complex B was observed. Addition of GA to this solution (also under $N_2$) resulted in an appreciable increase in intensity of this spectrum; thus this result is consistent with that reported in Figure 1.

### *GA semiquinone spectra*

GA is readily auto-oxidized at alkaline pH, and formation of its semiquinone free radical (HQ·) can be detected in the pH-range of ~9–13 by its characteristic triplet EPR signal (Figure 3A) from interaction with two equivalent $^1H$ atoms (A = 0.108 mT). The intensity of the HQ· EPR signal at pH values < 9 was too weak to detect using the acquisition conditions optimized for quantitative measurements (i.e. low microwave power and modulation amplitude), and except for very high pH values, Cu(II) exhibits limited solubility at pH > 6 [16], a factor which might explain the relatively slow equilibration of solutions in the alkaline pH range. Thus, all measurements of the influence of Cu(II) concentration on the HQ· signal intensity were performed at a constant pH of 13. The Cu(II) signals were also measured as a function of the concentrations of added Cu(II) on the same solutions as those used for the HQ· measurements. These were identical to those of Complex C reported for the highest pH values in the previous section.

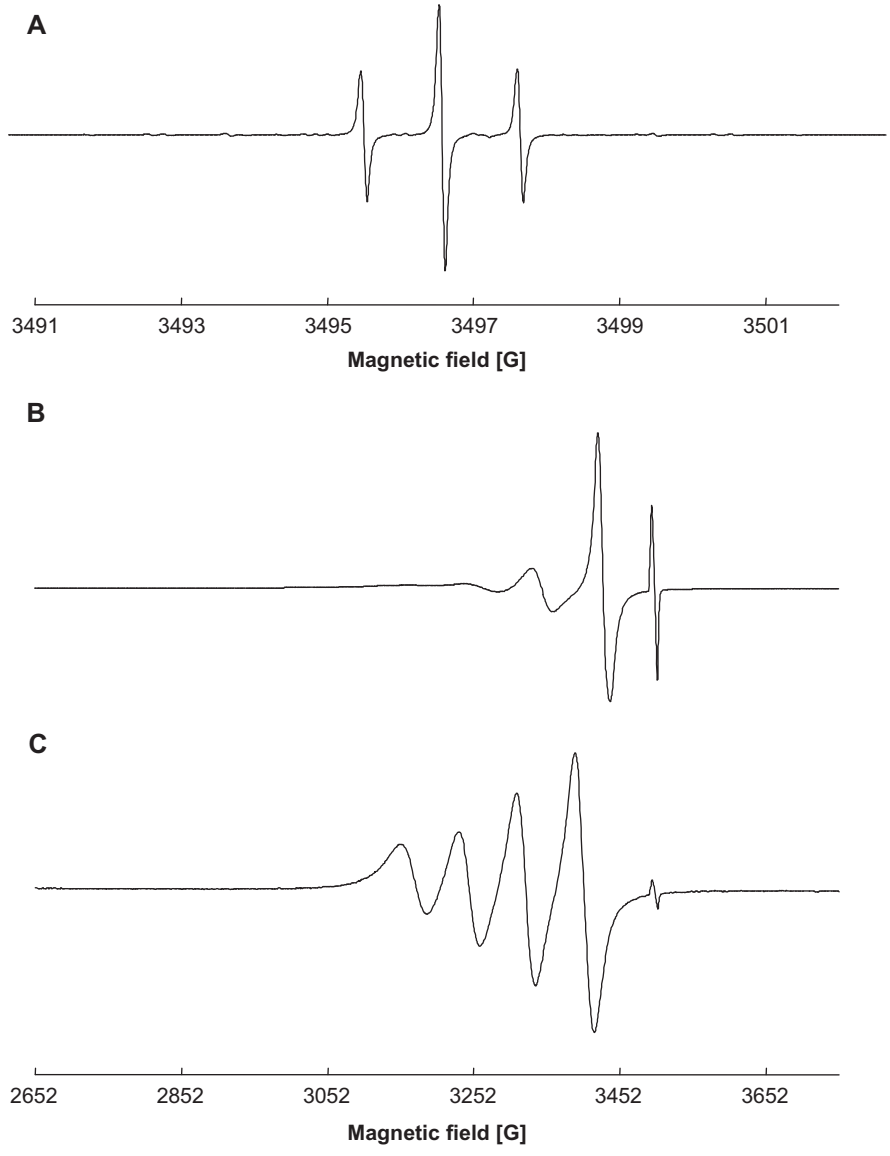


Figure 3. EPR spectra of (1:1) aqueous methanol solutions at pH 13 of (A) the GA semiquinone free radical, (B) the Cu(II)-GA complex in a solution of 5 mM GA and 2.5 mM Cu(II) and (C) the $Cu(OH)_4^{2-}$ ion in a solution of 5 mM GA and 7.5 mM Cu(II). Note that because of the high modulation amplitude used to record the spectra in (B) and (C), the semiquinone signal appears as a single peak in (B) and (C).

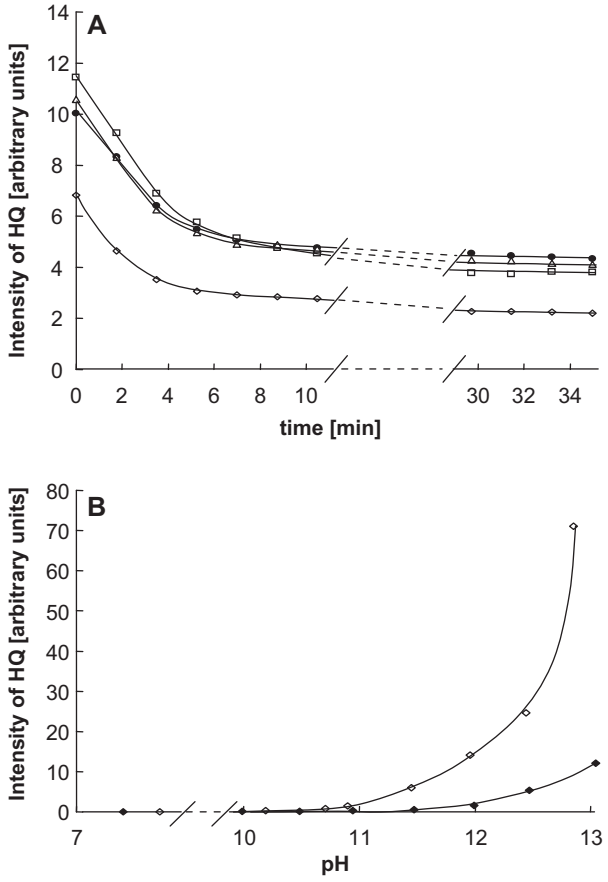


Figure 4. (A) Variation with time of the intensity of the HQ· EPR signal from aerated solutions of GA in the absence or presence of various Cu(II) concentrations. (-•- GA, -□- GA:Cu 1:0.02, -Δ- GA:Cu 1:0.06, -◊- GA:Cu 1:0.5), (B) Variation with pH of the intensities of the HQ· EPR signal from aerated solutions of 20 mM GA in the absence or presence of 2 mM Cu(II); spectra recorded 10 min after mixing the solutions. (◊ = GA without Cu(II), ♦ = GA in the presence of Cu(II).)

The HQ· signal has moderate stability and, since $O_2$ is involved in both its formation and decay, its intensity is strongly dependent on the experimental conditions. However, the shape of the intensity decay curve is similar in both the absence and presence of Cu(II) (Figure 4A). Attempts were made to fit the decay curves to 1st and 2nd order kinetic decay models. The data collected after ~7 min fit well to a 1st order model, but the data for the first ~5 min indicate a 2nd order reaction. The change probably corresponds to the using up of the $O_2$ that was present in the initial solution. The presence of a small amount of Cu had only a minor effect on the free radical decay kinetics, but the largest Cu concentration had a destabilizing effect in the 2nd order region.

As a further complication in this system, the intensity of the HQ· signal is strongly pH-dependent, in both the presence and absence of Cu(II) (Figure 4B). Thus it is essential to control both pH and time in order for measurements of signal intensities to be meaningful, and this sensitivity to the experimental conditions is probably the main reason for the variability of ~10% in replicate measurements (see Experimental section).

Variations in the intensities of the free radical and Cu(II) EPR signals as a function of Cu(II) concentration at pH 13 are shown in Figure 5. At low Cu(II) concentrations, there was a progressive (linear) decrease in the intensity of the HQ· free radical signal with increasing Cu(II) concentration, and the slope of the line showing the decrease in the HQ· signal intensity was proportional to –2-times the Cu(II) concentration. The Cu(II) signal increased with increasing Cu(II) concentration up to 2.5 mM Cu(II) (Cu:GA = 0.5:1), after which it decreased in the Cu(II) concentration range 2.5–5.0 mM (Cu:GA = 1:1 at 5 mM Cu(II)) and finally increased again at higher Cu(II) concentrations. There were also qualitative changes in the nature of the Cu(II) EPR spectra as a function of the Cu(II) concentration. For Cu:GA ratios < 1:1 the Cu(II) spectra (Figure 3B) were similar to that of Complex C (Figure 2J), whereas those observed for Cu:GA ratios > 1:1 (Figure 3C) correspond to the $Cu(OH)_4^{2-}$ species ($g$ = 2.136, $A$ = 7.9 mT) [28–30], which was also seen with Cu(II) solutions at pH 13 when GA was absent (spectrum not shown).

The UV-VIS spectra of GA at pH 11 are dominated by absorptions at 225 nm and 290 nm which are characteristic of GA [31], but the quinone peak at 425 nm is also clearly visible (Figure 6). However, its intensity decreased in the presence of Cu(II). The interpretation of the 425 nm peak as corresponding to the quinone was confirmed by measurements at pH 12 of GA whilst it was undergoing slow autooxidation. The initial spectrum recorded under a $N_2$ atmosphere (Figure 6) showed only a very weak peak at 425 nm, but its intensity increased progressively with subsequent exposure to oxygen (not shown).

## Discussion

Gallic acid is stable as the neutral molecule and mono-, di- and tri-anions, their relative concentrations depending on the pH of the solution; removal of the proton from the final -OH group is, however, only achieved at pH > 13. The neutral molecule and mono-anion are the major forms at acidic pH values [25–27,32,33], and the similarity between the EPR spectra of uncomplexed Cu(II) and those from Cu/GA solutions for pH values < 4 indicates that complexation does not occur with the neutral molecule at very low pH. Furthermore, the similarity in intensities of the Cu(II) signals seen in the presence and absence of GA shows that there was little if any reduction of Cu(II) to Cu(I) by GA at pH < 4.

In the Cu/GA solutions in the pH range 4.0–4.5 there was a major decrease in the intensity of the Cu(II) signal. This could be consistent with reduction

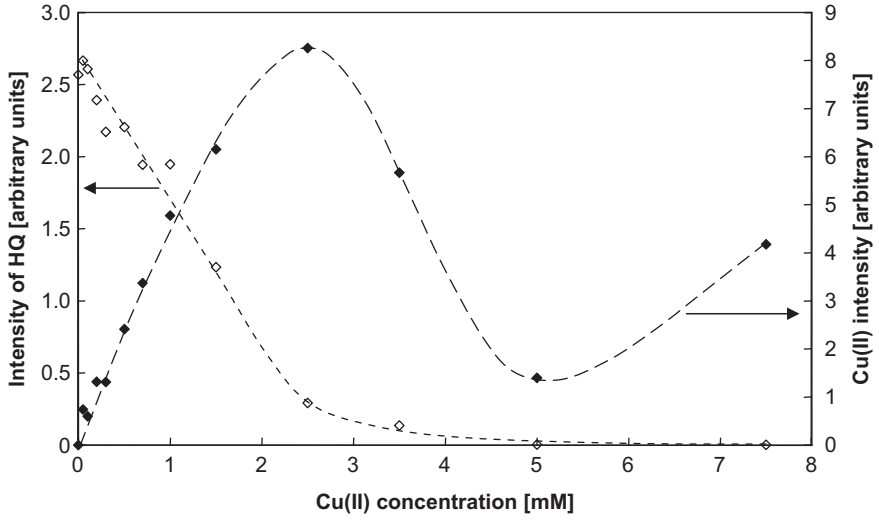


Figure 5. Dependence of the HQ· (◊) and Cu(II) (♦) EPR signal intensities on Cu(II) concentration in a solution of 5 mM GA at pH 13.

of Cu(II) to Cu(I) as interpreted by Oess et al. [5], although these authors also recognized that the loss of EPR signal intensity could also occur as a result of the formation of a di- or polymeric coordination complex of Cu(II) and GA [4]. The measurements in the present paper lend support to the dimerization/polymerization concept, and the results can all be understood in terms of the $pK_a$ values for gallic acid. Some cloudiness was observed in the solutions around pH 4. Since this is ~1.7 pH units below that at which hydrolysis of Cu(II) occurs in the absence of GA, the product must involve both Cu and GA. Also, although the EPR spectral intensity was very low in the pH range 5.0–8.0 for all samples containing GA, a weak but distinct EPR signal was observed for the Cu:GA ratio of 1:10. Above pH 8.0, the intensity of this signal increased with increasing pH for GA concentrations at or greater than twice that of the Cu, whilst remaining at or close to zero for lower GA concentrations.

Dissolution of the precipitate obtained at pH 5.5 in alkali at pH 11.5 (under $N_2$) resulted in the generation of a spectrum identical to that of Complex B, and this increased in intensity when more GA was added to the solution. Thus, increased concentrations of GA favours the formation of Complex B, and this result is consistent with the copper being distributed between this complex and an EPR silent Cu(II) species.

It is proposed that the coordination of Cu(II) in the initial complex, which starts to form at pH ~4.0, is similar to that in complexes with simple carboxylic acids, where di- or polymeric structures readily occur (Figures 7A–C). We have no evidence as to which of these co-ordination arrangements might apply to the present sample, but that in Figure 7A, in which four carboxylate groups bridge two Cu(II) ions is the most common. The formation of Cu–Cu bonds, which result in the formation of a singlet ground state [34] would then explain the virtual absence of an EPR

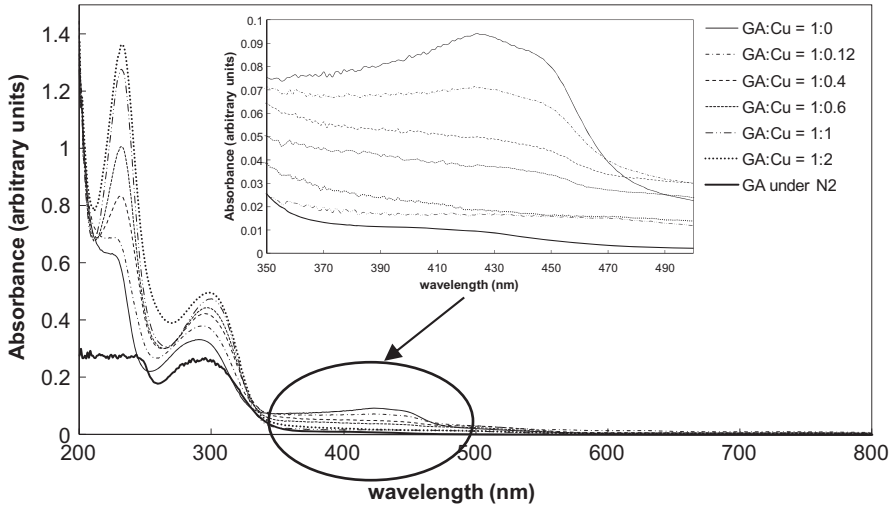


Figure 6. UV-visible spectra of 5 mM GA at pH 11 for GA:Cu concentration ratios of 1:0, 1:0.12, 1:0.4, 1:0.6, 1:1 and 1:2 and for pure GA (5 mM) at pH 12 prepared under $N_2$. All solutions were made in deionized $H_2O$.

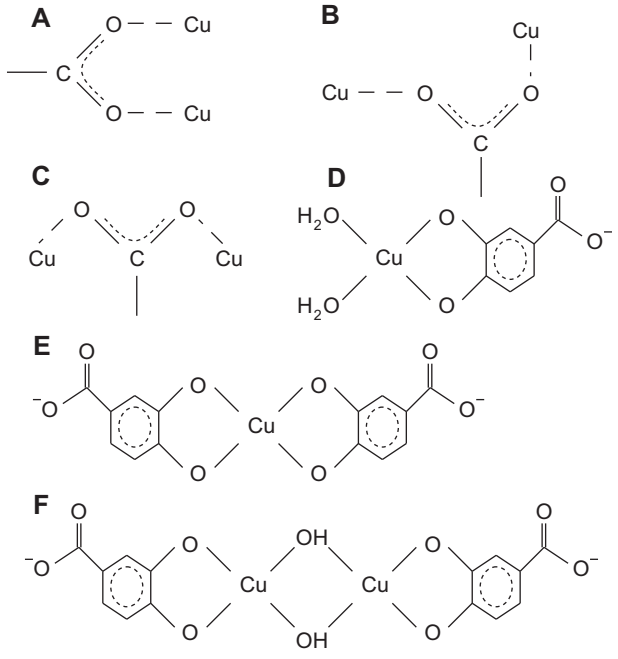


Figure 7. Proposed co-ordination arrangements for the various Cu/GA structures. (A–C) Modes through which the carboxylate group can function as a bridging ion (A) *syn–syn*, (B) *syn–anti* and (C) *anti–anti* arrangements, (D) Cu(GA), (E) $Cu(GA)_2$ and (F) $Cu_2(OH)_2(GA)_2$. Note: (A) results in dimeric structures with four equivalent bridging groups, whereas (B) and (C) both produce polymeric structures.

signal in the pH range 4.5–8.0. Although it is possible to explain the loss of Cu(II) signal as indicating that reaction (2) is faster than reaction (1), this mechanism is incompatible with the presence of a signal from a Cu/GA complex in the solutions with 1:10 Cu:GA ratio at this pH. The unimportance of reaction (2) at high pH is also discussed below.

At pH values greater than ~8.0, there was a progressive increase in Cu(II) signal intensity with increasing pH for the higher GA concentrations, but not for the 1:1 Cu:GA ratio. This observation is consistent with a competition between di- or polymerization and chelation reactions, with the latter gaining in importance at higher pH values as the GA di- and tri-anions increase in importance. The observation of increasing intensity for the Cu(II) signal with increasing GA concentration clearly indicates that the redox reaction between Cu(II) and GA is not important for pH values $> 8.0$.

Where Cu(II) EPR spectra were observed, they can all be interpreted in terms of mononuclear Cu(II) complexes. These showed at least two distinctly different sets of parameters in addition to those of the uncomplexed Cu(II) ion. By analogy with the results from Cu(II) amino acid complexes [35] and other published results for Cu(II) complexes with polyphenols [5], Complexes A and B in Figure 2 can be assigned to *mono* and *bis* complexes, respectively (Figures 7D and E); the *mono* complex with the larger *g*-value and smaller $A$(Cu)-value being formed at the lower pH value. The EPR parameters for complex C observed at high pH are also consistent with a *bis* chelate, and may involve partial coordination to the Cu(II) of GA dimers which have been observed to form in GA solutions at alkaline pH [36]. An alternative assignment of Complex C to a *tris*-bidentate GA-Cu complex was made by Oess et al. [5]. However, the addition of a third GA ligand to the Cu complex would be expected to cause a considerable change in the geometry of the complex, which should then also have a corresponding effect on the *g*- and *A*-values.

The progressive decrease in intensity of the HQ· EPR signal with increasing Cu(II) concentration could occur if either (i) the auto-oxidation reaction is inhibited by Cu or (ii) the semiquinone radical is destabilized and further oxidized by Cu(II), as suggested by reaction (2). However, the fact that the shape of the intensity decay curve for the HQ· EPR signal was similar in both the absence and presence of Cu(II) (Figure 4A) suggests that reaction (2) is unimportant. This conclusion is further supported by the UV/visible spectra, which showed a decrease in the intensity of the quinone peak at 425 nm in the presence of Cu(II). The present measurements, therefore, provide no evidence to support either reactions (1) or (2) occurring between Cu(II) and GA over a wide range of ratios and pH values in aqueous and aqueous methanol solutions, and we conclude that inhibition of the auto-oxidation reaction is the reason for the decreasing intensity of the semiquinone EPR signal with increasing Cu(II) concentration. Since the EPR spectra indicate Cu(II) complex formation, chelation with the (deprotonated) phenolic groups would appear to stabilize the unoxidized GA; the slope of the line for HQ· EPR signal intensity vs Cu(II) concentration (Figure 5) provides strong evidence that this is a *bis* chelate and hence provides additional support for Complexes B and C having similar co-ordination. It is well-known that various metal ions can stabilize one or other of the species in the hydroquinone, semiquinone, quinone redox system [37] and complexation with Zn(II) is an established method for stabilizing semiquinone radicals [38]. It appears that Cu(II), like Al(III) [37], stabilizes the hydroquinone moiety and, as a result of the complexation reaction, less GA is available for the auto-oxidation reaction.

A decrease in free radical production from GA in the presence of Cu(II) has previously been reported by Satoh and Sakagami [9] and this was accompanied by an enhancement of the cytotoxicity of GA. However, the reported free radical spectra do not correspond to that of the semiquinone from oxidized gallic acid [13]. The fact that the reaction system was controlled by Tris-HCl buffer may be a factor because this buffer exerts a strong influence on the chelation

chemistry of Cu(II) [12] and its role is not simply one of controlling pH.

The changes in the Cu(II) EPR signal with Cu(II) concentration at pH 13 can be explained by changes in the copper speciation. When GA is present in ≥ 2-fold excess, the Cu(II) is in the form of a *bis*-complex $CuL_2$ ([26] and this paper) and, as shown in Figure 1, most, if not all of the copper is in the Cu(II) form. The decrease in intensity of the signal in Figure 5 with increasing Cu(II) concentration from 0.5:1–1:1 Cu:GA ratios indicates the progressive formation of an EPR silent species, whose contribution is at a maximum for a 1:1 Cu:GA ratio. At this ratio the intensity of the semiquinone radical signal had decreased to ~10% of its value in the absence of Cu (Figure 5), thus indicating that there was little free GA present in the system. Furthermore, the Cu(II) signal reduction is not the result of precipitation of Cu(II) hydroxide, because the spectrum of $Cu(OH_4)^{2-}$ (Figure 3C) appeared with further increases in Cu(II) concentrations in the solutions. This then indicates that the most probable identity of the EPR silent species is a di- (or poly-)meric species containing equal amounts of Cu and GA and, although this cannot be positively identified from EPR measurements, a possible structure is shown in Figure 7F.

## Conclusions

The reaction between Cu(II) and GA is considerably more complex than is generally assumed. The present results clearly show that reduction of Cu(II) by GA does not occur under strongly acidic and strongly alkaline conditions and strong evidence is presented for the loss of the Cu(II) EPR signal in the pH range 4–8, being the consequence of the formation of di- or poly-meric structures involving reaction with the carboxylate group of the GA. In the pH range 4–8 mononuclear Cu(II) complexes make only minor contributions to the copper speciation, but they increase in importance with increasing pH and GA concentration. Free radical formation as a result of GA oxidation is, therefore, the consequence of auto-oxidation, and there was no evidence in the present measurements for the stimulation of GA oxidation by Cu(II). On the contrary, the presence of Cu(II) in solutions inhibited the auto-oxidation reaction at high pH, leading to decreases in both the semiquinone and quinone moieties, as a result of complexation between Cu(II) and the GA.

## Note

1. The hyperfine coupling constant represents a weighted average of the values for the $^{63}Cu$ and $^{65}Cu$ isotopes, because their individual spectra were not resolved.

## Declaration of interest

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