Iron and manganese surface complex formation with extracted lignin. Part 2: characterisation of magnetic interaction between transition metal and quinonic radical by EPR microwave power saturation experiments

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EPR spectra of a Lignocellulosic Substrate (LS) extracted from wheat straw, were obtained after surface complexation by the metal cations Fe(III), Cu(II) and Mn(II). For each system, we determined the geometrical environment of the metal centre at 15 K, 77 K and room temperature for Fe(III), Cu(II), and Mn(II) complexes respectively. Power saturation experiments were also performed in order to show the interactions between metal ions and organic radicals. Unfortunately, it was not possible to apply this technique to the Cu(II)–LS system due to the superimposition of the metal and radical signals. The fitting of the curves of iron and manganese systems led to the determination of the half-saturation power value ($P_{1/2}$), which is dependent on the spin–lattice and spin–spin relaxation time. The presence of a sorbed metal induces a change in the relaxation time of the radical, which is evidence of interactions between these two paramagnetic species. Comparisons of the two metal centres Fe(III) and Mn(II) behaviour towards LS were made. This study gives useful information about interaction processes, which can be interpreted in terms of lignin degradation in soil influenced by transition metal ions.

Introduction

The metal complexation capacity of organic matter in soils and redox processes are known to play an important role in the immobilisation, transport and bioavailability of metals in the environment.[1,2] Indeed, the knowledge of the association of metals with complexing agents in natural systems is of great interest to various scientific disciplines, such as ecotoxicology, water chemistry, and plant nutrition. The distribution of metals over different physico-chemical phases strongly influences their bioavailability and mobility.[3] In this regard, multi-charged macromolecular ligands, such as lignin-containing substrates play a key role in the speciation pattern of metals. Although metal–polyelectrolyte interactions have extensively been investigated, they are still poorly understood. Here, we propose a spectroscopic approach, which is based on electron paramagnetic resonance (EPR) experiments, in order to vary the strategies for studying metal–polyelectrolyte interactions. Our main purpose is to point out the role of metals in the biodegradation of lignin following organic radical content, which is dependent on the presence of metal.

EPR spectroscopy is a fine tool for the study of structures containing free radical species (such as lignin-containing substrates) or paramagnetic metal ions (present in soils for example). This technique is very interesting for the study of the interactions between these moieties. Lignocellulosic Substrate (LS), like other natural organic matter, gives a permanent EPR signal due to stable semiquinonic radical species,[4] that could take part in reactions with metal ions and pollutants. The quinonic nature of the radical has been previously described by pulsed EPR spectroscopy (2D SECSY).[5] It is difficult to apply this technique to such a complex polymeric structure of lignin, because it usually gives rise to a variety of free radicals and surface complexes. However, EPR spectroscopy gave useful information on metal ions implication in the biodegradation of lignin in soils, which still remains far from being fully explained.[6] Despite this, it has been proposed that Mn-peroxidase attacks the lignin polymer: Mn(II) would be oxidised to Mn(III), which, in turns, acts as a diffusable oxidising intermediate.[7] So, metals should have a key role in the biodegradation of lignin, simultaneously with enzymes.

This article was written in continuity with a classical EPR study published in a previous paper[8] that dealt with iron(III) and manganese(II) surface complexation properties with LS extracted from wheat straw. In this study, variations of semiquinonic radicals were observed as a function of the pH and metal concentration. But, some uncertainties remained to be explained about the interactions between metal ions and the organic radicals and also about the distribution of the metal sites on the surface of LS. This is the reason why, in this article, we furthered the study in order to obtain more information. We used EPR microwave power saturation experiments to study three abundant ions in soils, liable to be implicated in the biodegradation of lignin: copper(II), iron(III) and manganese(II). It was interesting to compare these ions, whose sorption

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properties differ markedly.[8,9] The three metal ions were studied at different temperatures in order to obtain optimised signals.

Experimental

Potassium nitrate KNO_3, metallic salts $Fe(NO_3)_3{\cdot}9H_2O$, $Cu(NO_3)_2{\cdot}4H_2O$, and $Mn(NO_3)_2{\cdot}4H_2O$ were purchased from Fluka.

All chemicals from commercial sources were of the highest available purity and have been used without further purification.

Sample

The LS sample was obtained from wheat straw as previously described.[9] It was analysed by various techniques[10] and its composition was 25% lignin and 75% cellulose.

Metal surface complexes were prepared by sorption of metal cations onto lignin. A hydrated LS sample was introduced at a concentration of 2 g L^{-1} in a suspension of 25 mL, and the ionic strength was fixed to 0.1 mol L^{-1} (KNO_3 medium). The pH was adjusted with 0.1 mol L^{-1} KOH or HNO_3 to 3.30, 5.75, and 8.50 for iron, copper and manganese complexes respectively, in order to avoid hydrolysis reactions. Metal solutions were introduced as their nitrate salts at concentrations ranging from 2×10^{-6} to 1×10^{-3} mol L^{-1} and 2×10^{-6} to 2×10^{-4} mol L^{-1} for manganese and iron ions respectively. The same experiments were achieved for copper in the range 2×10^{-6} to 1×10^{-3} mol L^{-1} but the data is not shown for reasons further explained.

EPR technique

The surface complexes spectra were recorded at 15, 77 and 293 K for iron, copper and manganese respectively, using a Bruker ELEXSYS 500 spectrometer. LS spectra were recorded at each temperature. The following parameters were chosen in order to avoid signal saturation : modulation amplitude of 4 G (at 293 and 77 K) and 3 G (at 15 K), modulation frequency of 100 kHz and microwave frequency of about 9.4 GHz (X-band). For each system, spectra were recorded on powdered samples for microwave powers ranging from 0.0002 to 201 mW. Probe temperature was regulated with a liquid nitrogen or helium cryostat (ESR 900 Oxford) equipped with a temperature control unit and maintained at 77 and 15 K for copper and iron complexes spectra, respectively. The spectra of the LS radical and metal-LS systems were achieved with the same instrumental and experimental conditions in order to allow comparisons of the radical signal intensities.

Determination of $P_{1/2}$ values

Following the mathematical eqn. (1), initially proposed by Rupp *et al.*,[11] the curve obtained by plotting the intensity of the semiquinone radical signal as a function of the square root of microwave power was fitted using a non-linear least-squares fitting program. This equation was initially adapted for inhomogeneous broadening cases and was modified by Innes and Brudvig[12] for intermediate cases between homogeneous and inhomogeneous broadening.

$$I = \frac{C \times \sqrt{P}}{\left(\left(1 + \frac{P}{P_{1/2}}\right)^{b/2}\right)} \tag{1}$$

For our purpose, we modified this equation, in which I is the EPR signal intensity of the semiquinonic signal and this term is split up into two sub-equations (2). Indeed, we have considered that the signal intensity at $g = 2.0025$ was the result of non-interacting and interacting semiquinonic radicals with metal ions

$$I = \frac{C_{\mathrm{LS}} \times \sqrt{P}}{\left(\left(1 + \frac{P}{P_{1/2\mathrm{LS}}}\right)^{b_{\mathrm{LS}}/2}\right)} + \frac{C_{\mathrm{M-LS}} \times \sqrt{P}}{\left(\left(1 + \frac{P}{P_{1/2\mathrm{M-LS}}}\right)^{b_{\mathrm{M-LS}}/2}\right)} \tag{2}$$

sorbed on the surface of the sample. The intensity of the radical signal was extracted directly from the spectra because of its clear distinction from the manganese(II) and iron(III) lines. P is the applied microwave power and $P_{1/2}$ is the microwave power at half saturation. The inhomogeneity parameter is called b and varies from 1 to 3. It is determined by the ratio of the Lorentzian spin packet width to the Gaussian envelope width. The term C is the proportionality factor and regroups all the terms that are expected to remain constant. Fitting has been performed using the fitting program Excel.

The parameters that belong to the first member of eqn. 2 concern independent radicals in the sample, and were determined by fitting the power saturation curve of the LS sample before metal sorption. Then, for metal–LS systems, these parameters were fixed in the first member and we fitted the ones in the second member of eqn. 2.

Applying the Bloembergen[13] and Abragam[14] theory, which describes the enhancement of the relaxation rates due to dipolar interactions, we measured $P_{1/2}$ values as a function of the metal concentration. $P_{1/2}$ value depends on both the spin–spin and spin–lattice relaxation times. The interaction with a metal facilitate radical relaxation. Thus, we were able to evaluate the metal concentration effect on the spin–lattice relaxation time of the semiquinonic radicals through $P_{1/2}$ value changes.

Results and discussion

Electron paramagnetic resonance spectroscopy was first used to investigate the geometrical environment of some metal ions when they are sorbed on the LS, as well as their implication in its biodegradation. In this perspective, the LS substrate in suspension was submitted to iron (1×10^{-5} mol L^{-1}), copper (1×10^{-5} mol L^{-1}) and manganese (2×10^{-4} mol L^{-1}) nitrate solutions introduced at pH 3.30, 5.75 and 8.50 respectively. Then, the subsequent EPR spectra were recorded and compared with the spectrum of the LS substrate before metal sorption and achieved at the same temperatures as with sorbed metal, *i.e.* 15, 77, 293 K for Fe, Cu and Mn respectively.

Figs. 1, 2, and 3(a) show an EPR single line at a g value of 2.0025 that corresponds to the presence of free semiquinonic radicals from lignin, which are coexistent with quinhydrone and quinone moieties.[15,16] These free radicals, which give rise to this signal, are formed by cleavage of some ether linkages

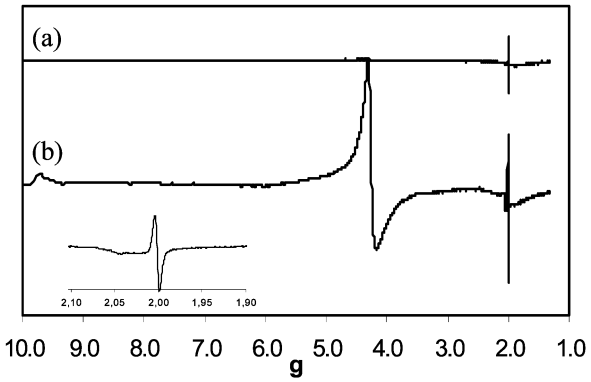

Fig. 1 Continuous wave EPR spectrum of LS before (a) and after (b) sorption of Fe(III) 1×10^{-5} mol L^{-1}, with an enlargement spectrum of the $g = 2$ region. $T = 15$ K, microwave power: 1.005 mW, $\nu = 9.4429$ GHz.

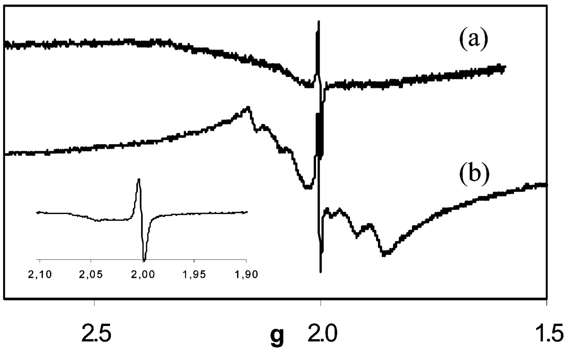

Fig. 2 Continuous wave EPR spectrum of LS before (a) and after (b) sorption of Mn(II) 2×10^{-4} mol L^{-1}, with an enlargement spectrum of the $g = 2$ region. $T = 293$ K, microwave power: 5.04 mW, $\nu = 9.4429$ GHz.

or oxidation of hydroxyl moieties. The Fe–LS spectrum (Fig. 1b) contains features of iron(III) held in an inner-sphere complex in a rhombic symmetry at $g = 4.3$ (strong) and $g = 9.7$ (weak). Fig. 2b shows the characteristic 6-line feature of octahedral manganese(II) ion held in an outer-sphere complex around $g = 2.017$ arising from the hyperfine coupling of Mn^{2+} with its $I = 5/2$ nuclear spin, with a hyperfine coupling constant $A = 9.02$ mT. Finally, the simulated spectrum of the Cu–LS system (Fig. 3b) gives the following spin-Hamiltonian parameters: $g_x = 2.058$, $g_y = 2.062$, and $g_z = 2.290$; $A_x = 2.22$, $A_y = 2.22$, and $A_z = 16.83$ mT. These results are consistent with a $d_{x^2-y^2}$ ground state for Cu^{2+} ions held in inner-sphere complexes with a slightly distorted square configuration with four coordinating oxygen donor atoms.

As expected, a variation in the quinonic radical signal intensity was observed, which can be attributed to redox processes or magnetic interactions between a quinonic radical and the metallic centre. The biodegradation of lignin in LS can be followed by the amount of semiquinonic radicals present in the matrix. So, in order to investigate the processes responsible for such reactions, we have carried out power saturation experiments focused on the unpaired electron spin system signal. These experiments consist of recording spectra for increasing microwave power values ranging from 0.00020 to 201 mW. The theory relative to these experiments is generally applied to homogeneous complexes, but it has already been applied to a cell wall protein system (with Dy metal centre)[11] which is also a high-molecular weight and heterogeneous system. So, we extended it to our substrate, a polymeric natural organic system.

Figs. 4 and 5 show the intensity variation as a function of the square root of the power applied for Fe–LS and Mn–LS systems respectively. The profile curves were obtained for the semiquinonic signal in LS alone, compared with the ones in metal–LS systems. It appears from the two curves that correspond to the two systems (Fe–LS and Mn–LS) that saturation curves of metal–LS systems differ markedly from the one in LS, which reflects their different relaxation properties. The relaxation of the radical is facilitated when it is in interaction with fast relaxing metal ions, even when it is introduced in a very low concentration, which results in the signal saturation at a greater power value. This enhancement of the spin–lattice relaxation of the free quinonic radicals is due to dipolar interactions with neighbouring exogenous metal ions. These results show, for the first time, the existence of interactions between metal ions and radical species from lignin, and consequently prove their proximity. We can compare the metal ion influence on the radical relaxation: the more the metal ion is a fast-relaxing agent, the more the radical relaxation is facilitated. The strength of the effect on the radical follows the order: Fe > Mn.

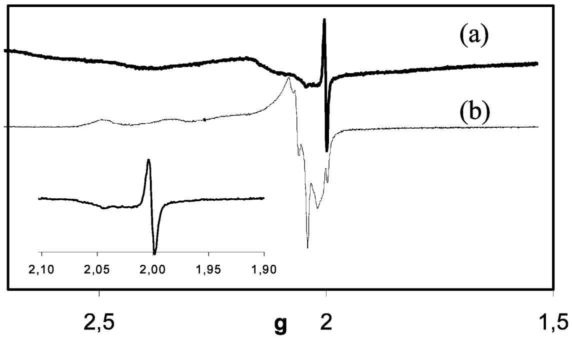

Fig. 3 Continuous wave EPR spectrum of LS before (a) and after (b) sorption of Cu(II) 1×10^{-5} mol L^{-1}, with an enlargement spectrum of the $g = 2$ region. $T = 77$ K, microwave power: 5.05 mW, $\nu = 9.4517$ GHz.

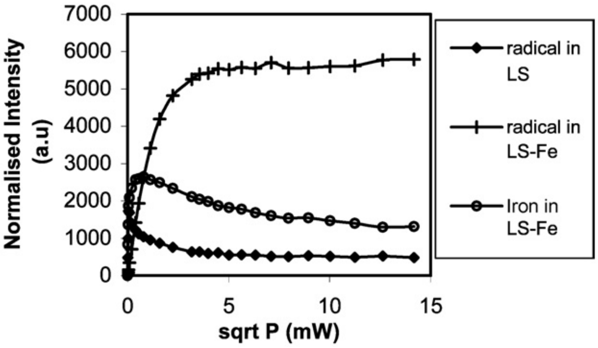

Fig. 4 Saturation curves for the Fe–LS system ([Fe] = 5×10^{-6} mol L^{-1}).

The relaxation properties depend on a specific parameter: the half-saturation power value $P_{1/2}$. From the previous saturation curves (Figs. 4 and 5), it was possible to calculate this value by fitting these curves on the basis of eqn. 2. Thus, we can numerically evaluate the enhancement of the relaxation time of a radical in interaction with metal ions. The results are given in Tables 1 and 2. The greater $P_{1/2}$ value obtained in the case of the Fe–LS system, compared with the one of the Mn–LS system, indicates faster relaxation processes, which is in accordance with the very fast relaxing properties of iron(III) cation, already well known. The values of the Fe–LS system are similar to those found for iron–sulfur proteins by Rupp *et al.*[11] Moreover, as expected, the increase of $P_{1/2}$ values from LS alone to metal–LS systems (Tables 1 and 2) is in accordance with a magnetic interaction between the metals and semiquinonic radicals in the LS substrate.

In the case of copper(II), the semiquinonic signal clearly appears only for very low metal concentrations and

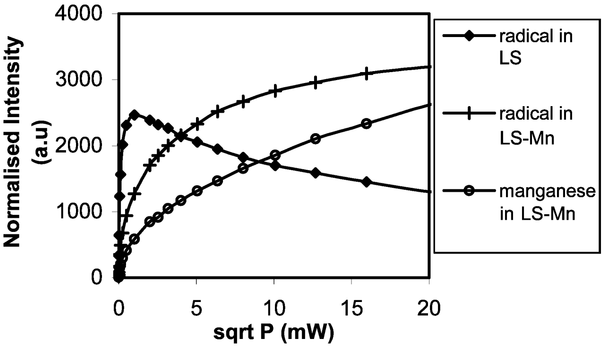

Fig. 5 Saturation curves for the Mn–LS system ([Mn] = 2×10^{-4} mol L^{-1}).

Table 1 $P_{1/2}$, b, C parameters determined using eqns. 2 to fit the experimental data for LS sample before and after sorption with different concentrations of Fe(III) at 15 K

[Fe]/mol L^{-1}	C_{LS}	$C_{M\text{–}LS}$	$P_{1/2\ M\text{–}LS}$	$b_{M\text{–}LS}$
0	50 443	0	0.0022	1.0
2×10^{-4}	2317	250	15.30	3.0
1.5×10^{-4}	3696	746	15.88	3.0
7.5×10^{-5}	4492	763	15.85	3.0
4×10^{-5}	5149	902	16.63	2.6
2×10^{-5}	2100	600	12.00	2.3
5×10^{-6}	1525	964	5.67	1.4

low microwave power values, due to the superimposition of the perpendicular component of copper. It disappears from 0.02 mW of microwave power, which is very low. This is the reason why we were not able to plot the semiquinonic intensity as a function of the microwave power, as we did in the two other cases, and thus not able to determine the value of $P_{1/2}$.

The microwave progressive power saturation method has also been used to evaluate the electron spin-relaxation rate and to distinguish between exchange and dipolar contributions to spin-relaxation enhancement. This process occurs when a slow relaxation spin is near a fast relaxing species.[17,18] With this aim in view, we plotted ΔH (peak-to-peak line width) as a function of $P_{1/2}$ (Fig. 6). $P_{1/2}$ has an inverse relationship with T_1T_2, in which T_1 is the spin–lattice relaxation time, and T_2 the spin–spin relaxation time.[17] In the case of iron– and manganese–LS systems, the values of ΔH showed a relatively high correlation with the $P_{1/2}$ parameter (Fig. 6). $P_{1/2}$ increasing with ΔH indicates that variation of this parameter was simultaneously due to T_1 and T_2 changes, which means that we have a dipolar interaction between spin–lattice and spin–spin relaxation. However, the fact that the b parameter is over 1 (Tables 1 and 2) indicates that eqn. (2) is still valid.[12]

In order to extend the study, we observed the effect of the metal concentration on the spin–lattice relaxation properties of free quinonic radicals. When the metal concentration increased, the $P_{1/2}$ value of the semiquinonic signal increased rapidly. It reached a plateau at an iron concentration of 5×10^{-5} mol L^{-1} (Fig. 7), while it increased with manganese concentration (Fig. 8). The break in the slope is very clear in the case of the Fe–LS system and seems to correspond to the saturation of acid sites at the surface of LS. Thus, by means of the EPR technique, it was possible to determine the exact concentration of metal necessary to saturate the LS surface under our experimental conditions, which was not possible by means of the batch technique in adsorption experiments.[8] In the case of the Mn–LS system (Fig. 8), the $P_{1/2}$ value seems to increase with the metal concentration, in accordance with batch experiments that indicated a co-precipitation process.

The nature of these interactions can be detailed as discussed by Innes and Brudvig.[12] Three scenarios can be expected:

Table 2 $P_{1/2}$, b, C parameters determined using eqns. 2 to fit the experimental data for LS sample before and after sorption with different concentrations of Mn(II) at 293 K

[Mn]/mol L^{-1}	C_{LS}	$C_{M\text{–}LS}$	$P_{1/2\ M\text{–}LS}$	$b_{M\text{–}LS}$
0	50 443	0	0.0022	1.0
1×10^{-3}	8572	4000	5.86	2.2
6×10^{-4}	6313	5572	3.13	1.2
4×10^{-4}	7031	5067	4.38	1.2
1.7×10^{-4}	4983	4736	2.98	1.3
1×10^{-4}	1707	2799	2.56	1.6
5×10^{-5}	3080	5879	1.65	1.4
2×10^{-5}	0	2049	1.75	1.6

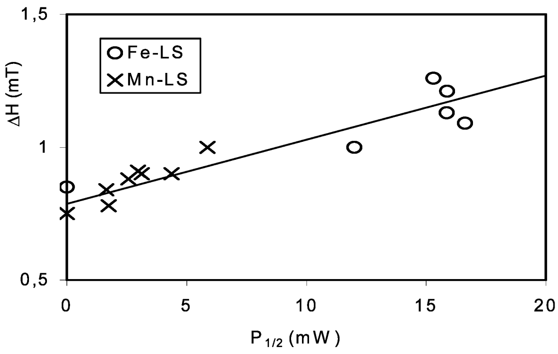

Fig. 6 Line width (ΔH) of organic free radical signal in metal–LS systems as a function of microwave power at half-saturation ($P_{1/2}$).

(1) The metal centre is bound to a specific site on the substrate, and only the metal bound to this site is contributing to the enhanced relaxation of the radical.

(2) The metal is not bound specifically to the surface of the substrate, and all the metal ions bound to the substrate contribute differently to the enhanced relaxation.

(3) The metal randomly remains in solution, and all metal ions contribute to the enhanced relaxation. This case will correspond to the formation of outer-sphere complexes. (*i.e.* the metal is bound with its hydration sphere).

The expected theoretical curves obtained for these scenarios are shown in Fig. 9. In the case (1) of specific sites, $P_{1/2}$ values have to be constant because the same metal environment surrounds the radical. In case (2), the $P_{1/2}$ value is expected to increase as metal ions are bound to the surface sites and then a plateau is reached when all the sites are occupied. Finally, case (3) corresponds to non-specific binding with the formation of outer-sphere complexes. The increase of the value of $P_{1/2}$ with the metal concentration reveals a continuous sorption of metal ions on the surface.

We can explore the distribution of the complexation sites, on the basis of these scenarios, from the curves shown in Figs. 7 and 8. If we focus on the general features of the curves, we can deduce that the metal sites are statistically scattered on the sample surface. Indeed, $P_{1/2}$ values vary depending on the metal concentration. Metal ions are complexed to the acid sites of lignin which are heterogeneously scattered on the solid surface. These results seem to indicate that we are in case (2) for iron complexes and in case (3) for manganese complexes. Indeed, $P_{1/2}$ values reach a plateau in the Fe–LS system and seem to increase with the metal concentration in the Mn–LS system. These results are in agreement with the low stability of the outer-sphere complexes formed with manganese ions.[8]

From the parameters C_{LS} and $C_{M\text{–}LS}$ determined by fitting eqn. (2), it was possible to calculate the percentage of radicals coupled with metal (Figs. 10 and 11) in iron and manganese

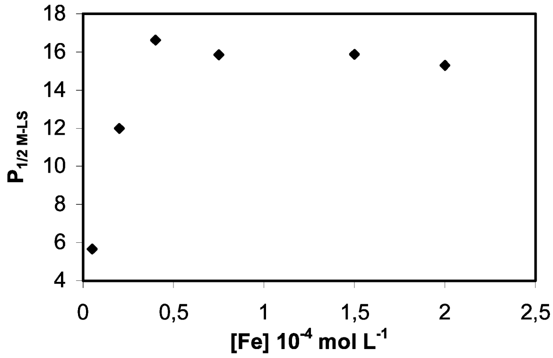

Fig. 7 $P_{1/2}$ values as a function of iron concentration.

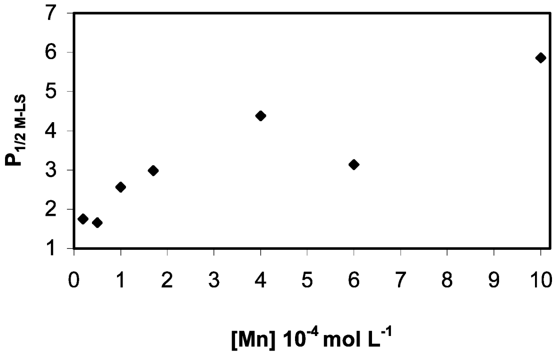

Fig. 8 $P_{1/2}$ values as a function of manganese concentration.

systems. The percentage of radicals in interaction with the metal increased until it reached a plateau at about 20% for an iron concentration of 1×10^{-4} mol L^{-1} (5×10^{-5} mol g^{-1}). This plateau indicates that we reached the saturation of surface sites before the hydrolysis of iron. In the case of the Mn–LS system, the percentage of interacting radicals increases with the metal concentration, and is equal to 60% for a concentration of 10^{-3} mol L^{-1}. Manganese is sorbed progressively as its initial concentration increases until surface complexation and hydrolysis of the metal occurred simultaneously during the adsorption experiments. Moreover, it is important to note that it was necessary to prepare the surface complexes at higher pH, equal to 8.5, due to the low stability of Mn–LS complexes. Contrary to iron, we did not reach the saturation of the surface acid sites. The lack of a plateau can be explained by an increasing metal content on the LS when the metal concentration introduced increases, which corresponds to a co-precipitation phenomenon.

Conclusion

In conclusion, our study shows that iron(III) and manganese(II) are in magnetic interaction with quinonic radicals contained in the LS substrate, even when metallic cations are present in very low concentrations. Iron is bound statistically at the surface on non-specific sites of lignin, while manganese is sorbed as outer-sphere complexes (with its hydration sphere) probably on non-specific sites. Despite the low stability of these complexes, the interactions with lignin semiquinonic moieties are clear. $Mn(NO_3)_2$ can oxidise phenols, including hydroquinone indirectly, either through Mn^{3+} formed from Mn^{2+} ions oxidation or through oxide or mixed oxide-hydroxide moieties. Indeed, Mn^{2+} ions are not oxidising species when they are in this form.

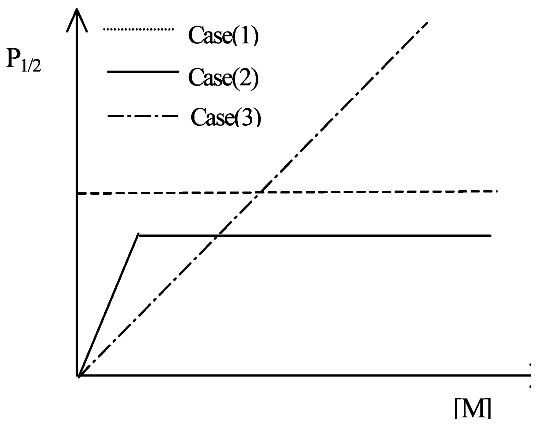

Fig. 9 Theoretical curves expected for the three cases of $P_{1/2}$ evolution as a function of metal concentration.

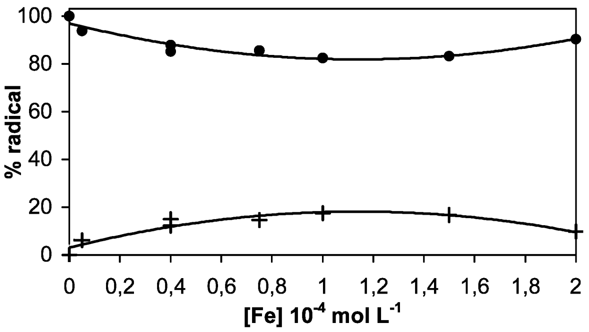

Fig. 10 Percentage of interacting (+) and non-interacting (•) radicals with metal as a function of iron concentration.

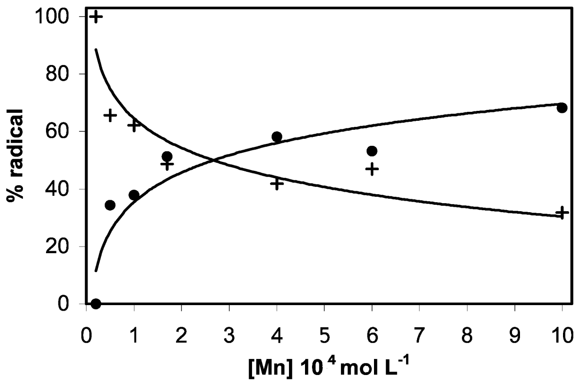

Fig. 11 Percentage of interacting (+) and non-interacting (•) radicals with metal as a function of manganese concentration.

It is possible that hydroquinone was converted to semiquinone probably *via* an autooxidation catalysed by trace amounts of free metal ions. The oxidation of the substrate might proceed through electron transfer between metal and surface quinonic species. These results provide a starting point for the understanding of the role of metals in the degradation of natural organic matter in soils. The evidence of interactions between quinone and metal centres is a key point in natural polymer chemistry.

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