Proton Electron Nuclear Double Resonance Study of Oxovanadium(IV) Complexes of D-Galacturonic and Polygalacturonic Acids

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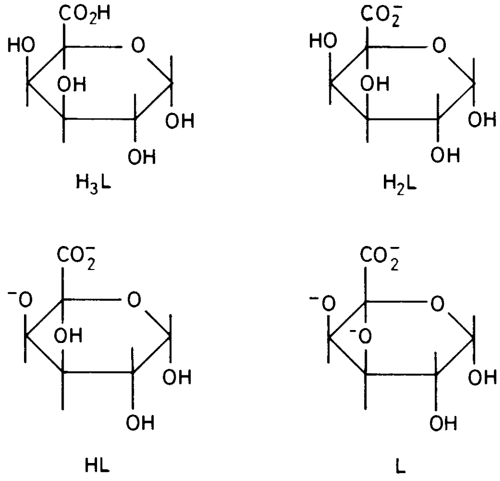
The ^1H electron nuclear double resonance (ENDOR) spectra of the VO^{IV}–D-galacturonic acid system in aqueous solution have been studied as a function of pH. Couplings between the paramagnetic ion and the protons of ligand or water molecules have been distinguished by comparative examination of the spectra recorded in water and D_2O. Comparison of data with results available from potentiometric and other spectroscopic measurements allowed interpretation of the pH dependence of ENDOR spectra and the assignment of the main observed resonances, *e.g.* those due to methine protons on the carbon atoms bearing deprotonated carboxyl and/or hydroxyl groups co-ordinated to the metal ion.

In the course of an investigation on the metal complexes of acid carbohydrates, we have undertaken the study of oxovanadium(IV) complexes formed by D-galacturonic acid (H_3L) in aqueous solution.

The combination of spectroscopic [e.s.r., absorption, and circular dichroism (c.d.)] and potentiometric analysis allowed us to identify the individual species taking part in complex equilibria and to obtain information on their structure.[1] According to the potentiometric data, at a ligand-to-metal ratio of 2:1, only the aqua-ion $[VO(H_2O)_5]^{2+}$ exists below pH 4. A first complex species $[VO(H_2L)_2]$, hereafter denoted as (**1**), which is never a major species, is formed above pH 4 and it involves two ligand molecules co-ordinating through the dissociated carboxyl groups. As the pH is increased, deprotonation of the sugar C^4 hydroxyl group occurs to yield the $[VO(HL)_2]^{2-}$ complex, (**2**), where the ligands chelate the metal ion *via* carboxyl and adjacent deprotonated hydroxyls. This species is nearly 100% abundant at pH 6.5—7.5. In more basic media, potentiometric data indicate the progressive formation of two species, $[VO(HL)L]^{3-}$ and $[VOL_2]^{4-}$, where one or both ligand molecules, respectively, insert a second deprotonated hydroxyl, in place of a carboxyl, into the metal co-ordination sphere. The $[VO(HL)L]^{3-}$ and $[VOL_2]^{4-}$ complexes yield indistinguishable e.s.r. spectra and thus are denoted as species (**3**).

As anticipated above, a valuable contribution to the interpretation of the examined complex system was given by e.s.r. spectra, which allowed us to obtain information on the nature of the major species present, but mainly as far as the metal chromophore is concerned. However, as is common in these cases, the superhyperfine interaction with ligand nuclei is unresolved in the e.s.r. spectra and some molecular information, *e.g.* that concerning the long-range interactions, is missing.

From this point of view, electron nuclear double resonance (ENDOR) spectroscopy is a more powerful technique, which detects the interaction of the paramagnetic centre with nuclei (N, H, *etc.*) of co-ordinated ligands, including solvent molecules, and discriminates between them by use of selective isotopic substitutions. In addition, in the case of oxovanadium(IV) complexes, because of the large hyperfine anisotropy, the e.s.r. peaks can be reliably assigned to well defined orientations of the complex with respect to the magnetic field and thus crystal-like spectra may be observed. In some cases, the coupling tensors may be easily extracted from the spectra and reliable structural information on the interacting nuclei of ligands is obtained.[2–15]



Scheme.

With this in mind, we have followed the complex formation in the VO^{IV}–D-galacturonate system through the ^1H ENDOR spectra, in order to establish whether this technique may be reliably used to characterize the binding sites and changes in them when the pH is varied and, possibly, to obtain molecular information which is otherwise not available.

The results of an ENDOR investigation on a related system, the vanadyl complex of polygalacturonic acid, are also reported. The spectroscopic results of our previous work[15] substantiated the formation of complexes between the metal ion and the polysaccharide through the direct insertion of dissociated carboxyl groups into the co-ordination sphere of oxovanadium(IV). These findings were in contrast with previous hypotheses which, instead, suggested an 'outer-sphere' electrostatic interaction between the hydrated ion and the charged polyelectrolyte.[16] In this context, and for sake of

Table. E.s.r. parameters for oxovanadium(IV) complexes[a]

Complex	$g_{\parallel}$	$A_{\parallel}$	$g_{\perp}$	$A_{\perp}$
(1)	1.94	170	1.98	61
(2)	1.95	154	1.98	54
(3)	1.96	150	1.98	49
VO^{IV}–polygalacturonate[b,c]	1.94	176	1.99	68
VO^{IV}–polygalacturonate[c,d]	1.93	184	1.98	63
VO^{IV}–Dowex[c,e]				

[a] A values in 10^{-4} cm^{-1}. [b] Air-dried or suspended into water. [c] Spectra recorded at room temperature. [d] Dehydrated. [e] $g_0 = 1.96$, $A_0 = 109 \times 10^{-4}$ cm^{-1}.

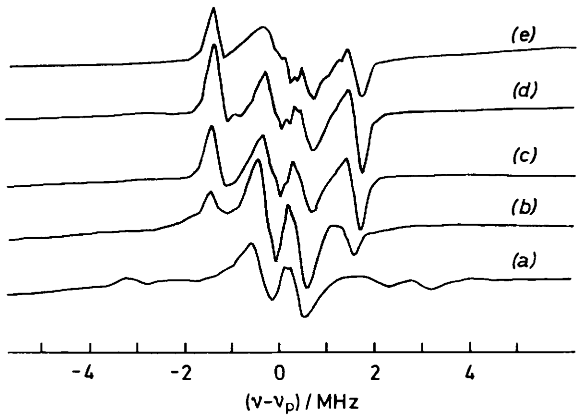


Figure 1. Frozen-solution parallel ^{1}H ENDOR spectra of the VO^{IV}–D-galacturonic acid system in water. pH (*a*) 2.3, (*b*) 4.7, (*c*) 5.9, (*d*) 7.0, and (*e*) 9.0. ν_p is the proton Zeeman frequency

comparison, the ^{1}H ENDOR spectra of the $[VO(H_2O)_5]^{2+}$ ion adsorbed onto a sulphonic-type cationic exchanger (Dowex) has also been examined.

Results

E.S.R. Spectra.—E.s.r. data for vanadyl complexes with D-galacturonic acid, in frozen solution, are listed in the Table. The e.s.r. spectra of the VO^{IV}-polygalacturonate system are of the rigid-limit type, even at room temperature, indicating that metal ions are immobilized in the polysaccharide matrix. In particular, for the latter system the e.s.r. parameters (Table) of the air-dried samples are almost the same as those of suspensions in water. Small spectral differences are, however, observed for the dehydrated samples as an effect of the drying process. The spectrum of VO^{2+} adsorbed on Dowex is isotropic at room temperature (Table) demonstrating that the metal ions, although electrostatically bound to the functional groups of the polymeric matrix, are in a rapid-tumbling mode.

ENDOR Spectra of the VO^{IV}*–D-Galacturonate System.*—Proton ENDOR measurements with a magnetic field set on the parallel e.s.r. components are expected to yield the components of the metal–proton interaction along the V=O direction (molecular *z* axis). Spectra recorded in water, Figure 1, show that the resonances observed below pH 4 may be assigned to the $[VO(H_2O)_5]^{2+}$ aqua-ion in frozen solution, as widely reported in the literature.[10–14] At pH values around 4.7 the e.s.r. spectra indicate the formation of the complex species **(1)** and **(2)** while the ENDOR spectra show a new pair of resonances with coupling constant of 3.0 MHz and, in addition, a complex pattern in the central part, where splittings around 0.4—1.2 MHz appear. The ENDOR spectra obtained from the e.s.r. resonances of species **(2)**, *ca.* pH 7, indicate that the outer peaks, which now exhibit a splitting of 3.1 MHz, gain intensity compared with those at lower pH values. The ENDOR spectrum of species **(3)** is similar to that of species **(2)** except for the presence of broader bands in the central part.

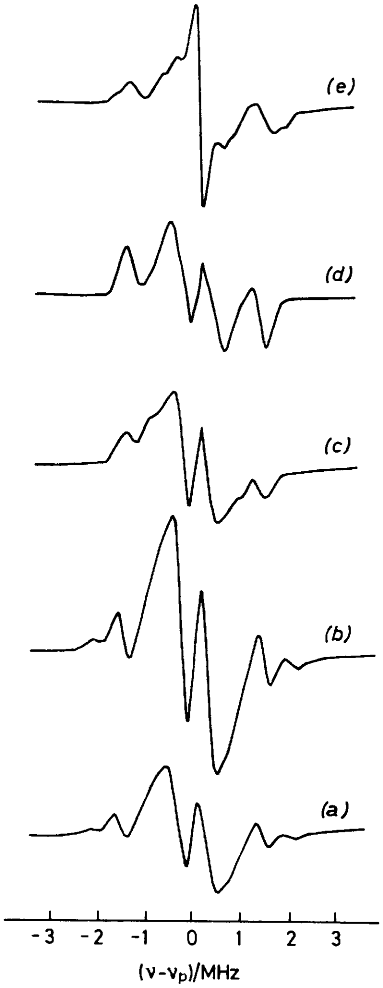


Figure 2. Frozen-solution perpendicular ^{1}H ENDOR spectra of the VO^{IV}–D-galacturonic acid system in water. pH (*a*) 2.3, (*b*) 4.3, (*c*) 5.0, (*d*) 7.0, and (*e*) 9.0

The perpendicular setting of the magnetic field, Figure 2, yields ENDOR spectra which consist of resonances which, at pH below 4, are again characteristic of the aqua-ion. At *ca.* pH 5 three pairs of signals with coupling constants of 3.0, 2.1, and 0.6—1.0 MHz are clearly seen. The 2.1-MHz resonances disappear when species **(2)** is dominant in solution, *ca.* pH 7, while the other ones become stronger. The increase of pH above 9 [complex **(3)** becomes dominant] modifies distinctly the ENDOR spectrum which now exhibits a peak around the free-proton frequency, together with pairs of lines with coupling constants of 0.9, 1.7, 3.0, and 3.4 MHz.

ENDOR Spectra of the VO^{IV}*–D-Galacturonate System in Deuteriated Water.*—The use of deuteriated solvent removes coupling with the labile protons from the ^{1}H ENDOR spectra. Thus, the spectra become simpler because they show only the interaction with the non-exchangeable protons being close to the vanadyl ion, *e.g.* those of the bound sugar ligand. As expected, at the pD range in which the aqua-ion is the dominant species no significant ENDOR spectrum is observed. At pD

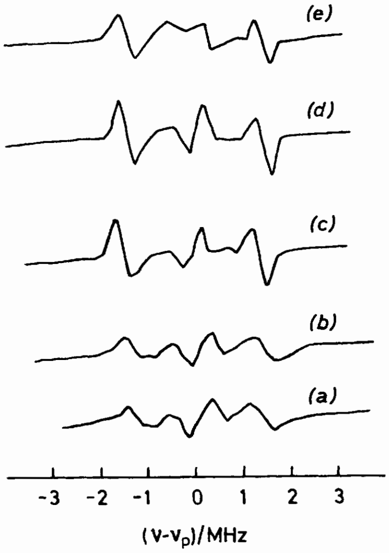


Figure 3. Frozen-solution parallel ^{1}H ENDOR spectra of the VO^{IV}–[^{2}H$_5$]D-galacturonic acid system in D_2O. pD (*a*) 4.7, (*b*) 5.1, (*c*) 6.7, (*d*) 8.0, and (*e*) 9.5

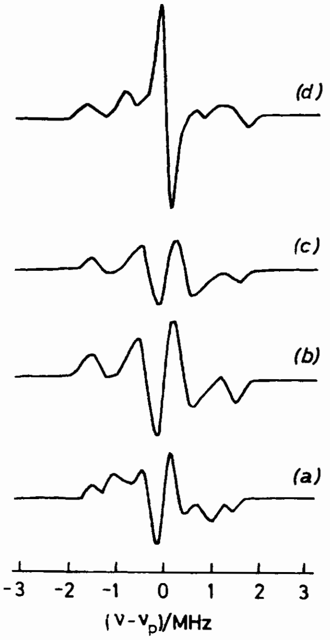


Figure 4. Frozen-solution perpendicular ^{1}H ENDOR spectra of the VO^{IV}–[^{2}H$_5$]D-galacturonic acid system in D_2O. pD (*a*) 5.1, (*b*) 6.7, (*c*) 8.0, and (*d*) 9.5

above 4.5, the clear resonances with coupling constants 3.0—3.1 MHz, the same as reported above for the aqueous solution, appear for the magnetic field set either on the parallel or perpendicular e.s.r. components, see Figures 3 and 4. In addition, the inner pair with coupling 1.2 MHz is distinguished in the parallel spectra. As pD increases and the complexes (**2**) and (**3**) are formed, the coupling 3.1 MHz is observed for both complexes in the parallel ENDOR spectra. Instead, the coupling constants for the inner peaks decrease noticeably for (**2**) and almost vanish for (**3**). With the perpendicular field setting, the couplings 3.0 and 1.0 MHz are assigned to (**2**), while complex (**3**) exhibits only the pairs at 3.4 and 1.7 MHz, together with the free-proton band. The peaks observed at 0.9 and 3.0 MHz for aqueous solutions above pH 9 may thus be assigned to protons exchangeable with D_2O.

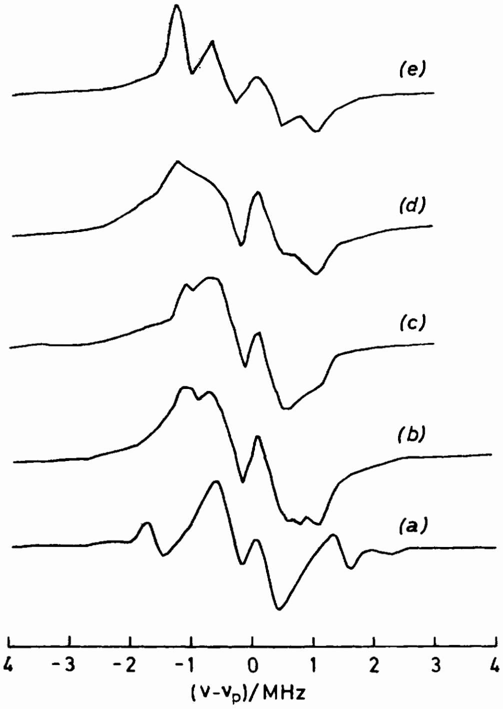


Figure 5. Perpendicular ^{1}H ENDOR spectra recorded at 116 K on (*a*) VO^{2+}–exchanged Dowex and on the VO^{IV}–D-polygalacturonate complex after (*b*) air-drying, (*c*) suspending in water, (*d*) dehydrating at 80 °C under vacuum, and (*e*) suspending in D_2O for 12 h

ENDOR Spectra of the VO^{IV}*–Polygalacturonate System.*—Proton ENDOR spectra of this system were of sufficient quality only when the magnetic field was set on a perpendicular component of the e.s.r. powder patterns (Figure 5). The spectra are considerably different to those recorded for the [VO(H$_2$O)$_5$]$^{2+}$ ion adsorbed on a Dowex resin (Figure 5). In particular, the ENDOR spectrum of an air-dried VO^{IV}–polygalacturonate sample exhibits two pairs of signals with coupling constants of 2.1 and 1.1 MHz. The inner resonances strongly diminish when the sample is dehydrated or treated with deuteriated water, while the intensity increases when the sample is suspended in aqueous solution. This indicates that the pair derives from water molecules which may be removed by heating. Instead, the 2.1-MHz pair, being almost unaffected by either D_2O exchange or heating, can be attributed to unexchangeable CH protons of sugar molecules.

Discussion

Galacturonate Complexes.—According to theoretical considerations,[17] in the crystal-like spectra, each set of equivalent protons yields one pair of lines for the magnetic field directed parallel to the molecular *z* axis and two pairs, reflecting the anisotropy of superhyperfine interactions, when the applied magnetic field is set perpendicular to the *z* axis. Reliable molecular information can be obtained only if the sets of magnetic axes of the coupled nucleus (proton) and vanadyl ion are coincident. Misalignment between these sets of co-ordinates makes the tensors non-coincident and, in the absence of structural data, the correlation is not immediate.

However, comparative examination of the ENDOR spectra, *e.g.* those recorded in deuteriated and non-deuteriated solvents, may lead to information about the complexes formed. For example, it is important to establish the number of complexes formed in the studied system, as well as to assign which kind of ligand is bound to the metal ion. The comparison of ENDOR spectra of species (**2**) and (**3**) in water and deuteriated solvent [no significant deuteriation effects are seen for (**2**) and signals typical of protons 2—3 Å distant from vanadium, *e.g.* with the largest coupling constants of 4—6 MHz (see, for example, refs. 9 and 10) are absent for (**3**)] rules out the presence of water molecules bound to metal in both these complexes, *e.g.* in the apical position. The pair of lines with the perpendicular coupling constant of 2.1 MHz, detected at low pH, may be assigned to species (**1**) defined earlier [1] as the $[VO(H_2L)_2]$ complex in which the metal ion is bound to ligand molecules *via* carboxylate donors only. This set of resonances, which has the 1.2-MHz peaks as counterpart in the parallel spectra, vanishes when species (**1**) transforms into (**2**). Also, the changes of the ENDOR spectra with pH suggest that the line sets with couplings of 3.0—3.4 MHz derive from the complexes in which the co-ordination of deprotonated hydroxyl occurs, *i.e.* the species (**2**), $[VO(HL)_2]^{2-}$, or (**3**), $[VO(HL)L]^{3-}$ or $[VOL_2]^{4-}$ (see ref. 1). The main characteristics for species (**2**) are the perpendicular resonances with coupling constants of 3.0 and 1.0 MHz and the parallel peaks at 3.1 MHz. This species, as mentioned above and shown earlier,[1] differs from the other two species identified by ENDOR, (**1**) and (**3**), by having the carboxylate group involved in the chelate ring formation. This correlation of ENDOR spectra and earlier [1] potentiometric and spectroscopic data allows us to make tentative assignment of the ENDOR resonances to the species present in solution, as well as to the modes of co-ordination. The 2.1-MHz pair of lines attributed to the $[VO(H_2L)_2]$ complex derives from the methine protons on the carbon atom bearing the unidentate carboxylate. The formation of the chelated complexes with carboxylate involvement, $[VO(HL)_2]^{2-}$, changes the coupling of these protons to 1.0 MHz and the new pair of resonances with coupling around 3.0 MHz appears. The latter pair is easily attributed to the methine protons on the carbon atoms bearing hydroxyl groups bound to the metal ion. These protons are, according to molecular models, closer to the metal ion than those adjacent to the bound carboxylate group. When the $[CO_2^-, C(4)\text{–}O^-]$ chelated complexes transform into those with $[C(4)\text{–}O^-, C(3)\text{–}O^-]$ co-ordination, *i.e.* when (**2**) changes into (**3**), the couplings of protons adjacent to bound hydroxyl groups change slightly, from 3.0 to 3.4 MHz, and a new pair of lines with a coupling constant of 1.7 MHz appears. The latter lines could be assigned as being derived from protons on carbon atoms next to those bearing bound hydroxyls, or, alternatively, as the second component of the protons responsible for the 3.4-MHz coupling. Instead, the resonance around the free-proton frequency is accounted for by the interaction of vanadium with ligand protons which are very distant (*e.g.* > 5.5 Å). The couplings of 3.0 and 0.9 MHz detected for complex (**3**) in aqueous solution only are most likely attributable to water molecules which interact with vanadium, although they are too distant to be considered as bound to the metal ion.

Polygalacturonic Acid Complex.—The ENDOR spectra clearly show the absence of the aqua-ion in the polymeric matrix. This result supports our earlier interpretation [15] that the functional groups of polygalacturonate are directly bound to the vanadyl ion. The 2.1-MHz resonances are almost the same as those of complex (**1**) described above and, analogously, they can be assigned to the C(5) methine protons of polygalacturonate co-ordinating only through carboxylate donors. On the whole, in spite of the obvious differences which may arise from the different states of both systems, it seems that ^1H ENDOR spectra may also be valid in interpreting the co-ordination of the polysaccharide to vanadyl ion, due to the carboxylate groups only. As far as the 1.1-MHz coupling is concerned, the spectral behaviour suggests that it has contributions from distant water moleules, because, as discussed above, this splitting value is too low to indicate bonding to the metal ion.

Experimental

Materials.—D-Galacturonic acid monohydrate was purchased from Fluka and used without further purification. The salt $VO(SO_4)\cdot 3H_2O$ (Aldrich) was the metal source. Fresh solutions [$(2 \times 10^{-2}$ mol dm^{-3}, $VO^{2+})$, ligand-to-metal molar ratio of 2:1] were prepared in twice distilled water just prior to the commencement of any experiment. In order to minimize air oxidation of oxovanadium(IV), the solutions were prepared and titrated under nitrogen. For the experiments on the deuteriated system $[^2H_5]$D-galacturonic acid was obtained by repeated freeze–drying cycles on D_2O solutions of the ligand. Weighed amounts of $VO(SO_4)\cdot 3H_2O$ were dissolved in D_2O and the solution evaporated under vacuum. The procedure was repeated at least four times and, finally, a D_2O solution containing the stoicheiometric quantity of deuteriated ligand was added. The pH (pD) was adjusted by addition of H_2SO_4 (D_2SO_4, 99.5% D) and NaOH (NaOD, 99.8% D); pD = pH meter reading +0.4.[18] Dimethyl sulphoxide (dmso) ($[^2H_6]$-dmso, 99.5% D) was added to samples to obtain good glass formation.

Vanadyl polygalacturonate was obtained by suspending the ligand (0.300 g) in a 1×10^{-3} mol dm^{-3} $VO(SO_4)$ solution (50 cm^3) in water at pH *ca.* 4. After stirring for 1 h, the suspension was filtered and the resulting gel washed with water and then air dried. The vanadyl complex with Dowex (50 × 8-100, Aldrich) was prepared similarly.

Measurements.—E.s.r. and ^1H ENDOR measurements were carried out at *ca.* 116 K on a Bruker 220 D instrument operating at the *X*-band frequency (*ca.* 9.40 GHz) equipped with a Bruker ENDOR accessory. Instrumental settings: microwave power 50 mW; radiofrequency power at 14 MHz, 100 W; frequency modulation depth 100 kHz. ENDOR spectra were recorded at the field setting on the parallel $M_I = -\frac{5}{2}$ or perpendicular $M_I = -\frac{3}{2}$ components of the powder spectra. Spectra of the VO^{IV}–D-galacturonate system were recorded on frozen solutions. Those on VO^{IV}–polygalacturonate were obtained on solid samples (after air-drying or dehydrating at 80 °C under vacuum) or on suspensions in water (D_2O). Proton ENDOR couplings were measured from peak to peak.

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References

1 G. Micera, A. Dessi', H. Kozlowski, B. Radomska, J. Urbanska, P. Decock, B. Dubois, and I. Olivier, *Carbohydr. Res.*, in the press.
2 S. Kita, M. Hashimoto, and M. Iwaizumi, *Inorg. Chem.*, 1979, **18**, 3432.
3 C. Mulks and H. van Willigen, *J. Phys. Chem.*, 1981, **85**, 1220.
4 B. Kirste and H. van Willigen, *Chem. Phys. Lett.*, 1982, **87**, 589.
5 B. Kirste and H. van Willigen, *J. Phys. Chem.*, 1982, **86**, 2743.
6 B. Kirste and H. van Willigen, *J. Phys. Chem.*, 1983, **87**, 781.
7 C. P. Scholes, K. M. Falkowski, S. Chen, and J. Bank, *J. Am. Chem. Soc.*, 1986, **108**, 1660.
8 C. F. Mulks, B. Kirste, and H. van Willigen, *J. Am. Chem. Soc.*, 1982, **104**, 5906.
9 D. Attanasio, *J. Phys. Chem.*, 1986, **90**, 4952.
10 N. M. Atherton and J. F. Shackleton, *Mol. Phys.*, 1980, **39**, 1471.
11 H. van Willigen, *J. Magn. Reson.*, 1980, **39**, 37.

12 H. van Willigen, C. F. Mulks, and N. M. Atherton, *Inorg. Chem.*, 1982, **21**, 1708.
13 H. van Willigen and T. K. Chandrashekar, *J. Am. Chem. Soc.*, 1983, **105**, 4232.
14 L. Kevan, *J. Phys. Chem.*, 1984, **88**, 327.
15 S. Deiana, L. Erre, G. Micera, P. Piu, and C. Gessa, *Inorg. Chim. Acta*, 1980, **46**, 249.
16 B. Lakatos, T. Tibai, and J. Meisel, *Geoderma*, 1977, **199**, 149, 319.
17 B. M. Hoffman, J. Martinsen, and R. A. Venters, *J. Magn. Reson.*, 1984, **59**, 110.
18 R. G. Bates, 'Determination of pH, Theory and Practice,' Wiley, New York, 1964.

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