

Reaction of Vanadate with Aquatic Humic Substances: An ESR and ^{51}V NMR Study

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Electron spin resonance (ESR) spectroscopy and ^{51}V nuclear magnetic resonance (NMR) spectroscopy have been used to study the interaction of vanadate with aqueous solutions of humic substances (HS) at different pH values and at different concentrations. Under acidic pH conditions, ESR spectra show that humic substances reduce vanadium(V) to vanadium(IV) without further reduction to vanadium(III). The reduced vanadium(IV) ion is bound to oxygen donor atoms, probably at carboxylic acid sites in the humic substances. ^{51}V NMR spectra show that the VO_2^+ cation is immediately reduced and that the decavanadate cation decomposes to the VO_2^+ cation prior to reduction. The overall rate of reduction depends on both concentration and pH. There is no reduction above pH 6, which suggests that the standard reduction potential of humic substances is about +0.65 V. Near pH 7, vanadate is stabilized by binding to humic substances. As the concentration of humic substances increases, the total vanadium NMR signal intensity decreases. This is due to the quadrupolar nature of the ^{51}V nucleus that, when bound to humic substances, is invisible in NMR measurements. Quantitative models applied to intensity changes show that the vanadate monomer forms HS–V(V) complexes. The formation equilibrium constant is estimated to be 108 M^{-1} . At pH above 9, NMR signals appear at -623.6 and at -763.2 ppm when humic substances are added to vanadate solution. The intensities of the signals increase with increasing pH and with increasing concentration of humic substances. These signals appear to be associated with peroxyvanadate anions, which are not bound to humic substances.

Introduction

The aqueous chemistry of vanadium(V) oxyanions has, over recent years, been an area of considerable interest in chemistry and biochemistry (1–5). The reproductive vanadium toxicity, the maternal and embryo/fetal toxicity of vanadium, and the prenatal and postnatal effects of vanadium, as well as the prevention of vanadium-induced

developmental toxicity by chelating agents have been reviewed (6). Vanadium, a trace element in the environment and in biological systems, influences the behavior of enzymes. It also mimics and regulates growth factor activity, is a potential mutagenic and carcinogenic agent, and regulates gene expression (7).

While it has not been established with certainty that it is essential for living organisms, vanadium has become an increasingly important environmental element. In recent years, environmental interest in vanadium has increased because of environmental pollution by vanadium, an abundant element in crude oils, through the combustion of fossil fuel, oil refinery processes, domestic heating, and automotive traffic (8–11). Smoke containing vanadium salts eventually settles on the ground, imposing risks to human health such as an increased incidence of cancer (12).

Humic substances, as naturally occurring organic matter with different functional groups, can exert a major influence on the fate of a metal in a number of ways such as through reduction and complexation (13). Although vanadium is found at low concentrations in water, rocks, and soils, vanadium has been found to be naturally associated with humic substances (14–17). There have been several reports that the addition of humic substances to vanadate in acidic solution causes the reduction of vanadium(V) to the paramagnetic vanadium(IV) (18–20). However, there appears to be no detailed study of the interaction of vanadate with humic substances under different pH and concentration conditions. In this study, we have applied ESR spectroscopy and ^{51}V NMR spectroscopy to examine the interaction of humic substances with the various vanadium species found in aqueous solution at different pH and at different concentrations of vanadate and humic substances.

Experimental Section

Chemicals. Chemicals were reagent grade (Sigma, Fisher, and Aldrich). The humic substances used were collected from swamp water near Myall Lake, New South Wales, Australia. The extraction procedure and characterization of humic substances have been described in an earlier paper (21). The concentrations of humic substances used were based on the weight average molecular weight.

Preparation of ESR and NMR Sample. A stock solution of 0.01 M sodium vanadate was prepared and was diluted to the concentrations required in the experiments. The reaction of humic substances with vanadate was carried out in the pH range from 2 to 12 by adding sodium vanadate solution to the humic substances in solution at corresponding pH values of vanadate solution. Samples at pH below 7 were prepared by mixing both vanadate solution and humic substance solution at the same pH, and the samples were then immediately examined by ESR and NMR spectroscopies. Since there was no reduction above pH 6, solutions with pH greater than 7 were magnetically stirred for 24 h before NMR measurement. One sample of vanadate and humic substances at about pH 11 was purged of dissolved oxygen by bubbling nitrogen gas through the solution and sealing it in a NMR tube prior to NMR measurement. Measurements of pH were made on a Radiometer PHM61 equipped with a combination proton selective electrode and calibrated with standard buffer solutions at pH 4.0 and 7.0, respectively.

ESR Spectroscopy. X-band ESR investigation for vanadium(IV) ions was carried out on a Bruker ESR 300 spectrometer at ca. 9.40 GHz. ESR parameters were obtained by computer simulation using the program SIMER (22), which applies perturbation theory to ground order.

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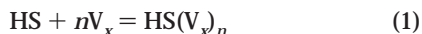
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⁵¹V NMR Spectroscopy. Vanadium-51 is NMR active and is 99.75% natural abundance. ⁵¹V NMR spectra were recorded at 52.6 MHz on a Bruker ACP-300 spectrometer operating at 78.94 MHz, equipped with a 10 mm broad band probe. Typical conditions were as follows: spectral width 30 kHz, pulse width 15 μs (90° pulse angle), acquisition time 139 ms. Between 2000 and 40000 scans were collected, and the free induction decay (FID) was exponentially line broadened (10–30 Hz) prior to Fourier transformation. All chemical shifts were relative to the external reference standard VOCl₃, which has been assigned to 0 ppm. Spectra were recorded at 25 ± 1 °C unless noted otherwise.

Data Analysis. The concentrations of vanadium(IV) ion formed in the reduction of vanadate by the humic substances were calculated by measuring ESR peak to peak intensity using ESR intensities recorded at different concentrations of vanadium(IV) nitrate solution. The concentrations of vanadate ion in the reduction were calculated by the difference between the total vanadate concentration added and vanadium(IV) concentration formed: $[V(V)] = [V(V)]_t - [V(IV)]$, where $[V(V)]_t$ was the total concentration of vanadate initially added. The concentrations of the vanadium(IV) calculated from ESR spectra are reproducible within 5%. The uncertainties of kinetic rate determinations are approximately 5%.

The concentrations of different vanadate species in the presence of and in the absence of humic substances were calculated by integration of ⁵¹V NMR spectra obtained under the different conditions. We used control samples containing a known total vanadate concentration to quantify vanadate species in the samples with different concentrations of humic substances. Because of the quadrupolar nature of the vanadium nucleus and the large molecular weight of the humic substances, the bound species of HS(V_x)_n was observed only at reduced intensity using ⁵¹V NMR spectroscopy (23–26). Thus vanadate anions (V_x) interact with humic substances, and the corresponding equilibrium is established:



By using settings defined by the control samples, we calculated the extent of disappearance of the vanadate NMR signal in the samples to which humic substances were added. The concentrations of the vanadate species calculated from ⁵¹V NMR spectra are reproducible within 5%. The overall concentration of total observable vanadate, and thus the concentration of invisible HS(V_x)_n, is accurate to within about 10%.

Results and Discussion

⁵¹V NMR Spectra of Vanadate Solution. The chemistry of aqueous vanadate is complex with many protonation equilibria and oligomerization equilibria occurring simultaneously. ⁵¹V NMR spectra of 0.5 mM solution recorded at different pH values showed resonances that have been assigned to the cation, VO₂⁺ (–543 ppm), the decavanadate anion, V₁₀O₂₈⁶⁻, (–425, –506, –524 ppm), protonated monomeric anions H₂VO₄⁻/HVO₄²⁻ (–555/–560 ppm), protonated dimeric anions H₂V₂O₇⁻/HV₂O₇²⁻ (–567/–571 ppm), a tetra vanadate species (–576 ppm), a pentameric species (–581 ppm), and the monomeric anion VO₄³⁻ (–536 ppm). The amounts of these species depend on the overall vanadium concentration, the pH, the ionic strength, and temperature (27, 28).

Since the reaction of the metal with humic substances involves different species at various pH values, it is therefore appropriate to review the main species in solution over a range of pH. Figure 1 plots the concentrations of the major species detected by ⁵¹V NMR versus pH for a total vanadate concentration of 0.5 mM. For simplicity, we have not separated out the dimer, tetramer, and pentamer species,

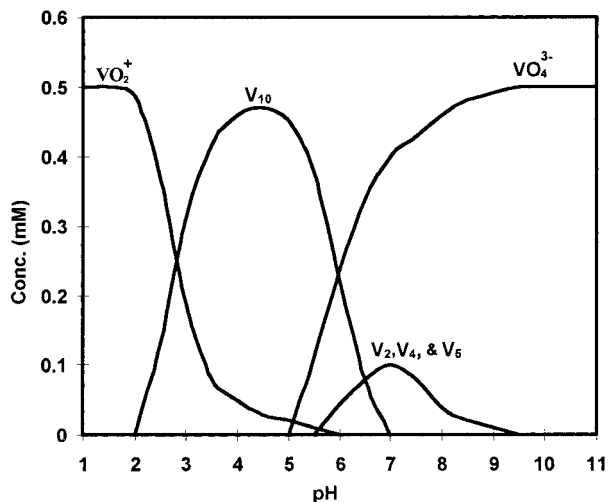


FIGURE 1. Distribution of vanadate species as a function of pH. Conditions: 0.5 mM vanadate in 0.5 M KCl as the electrolytic solution. The concentrations were obtained by calculating the mole fraction from the integrated ⁵¹V NMR spectra in samples where the total vanadate concentrations were known.

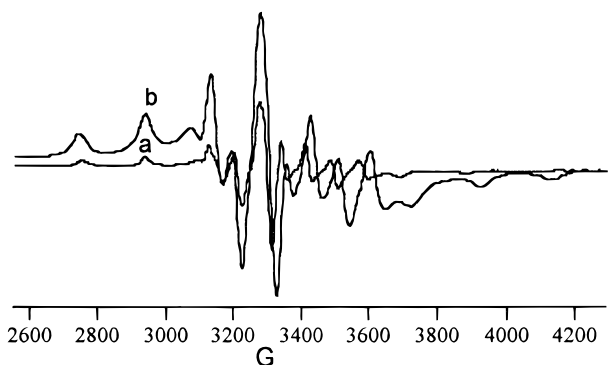


FIGURE 2. X-band ESR spectrum (a) of V(IV) reduced from V(V) by the humic substances recorded at 77 K in aqueous solution at pH 3.0 and (b) of V(IV) as the vanadyl ion was added directly to the humic substances at pH 3.0.

nor have we attempted to show variations in the degree of protonation as the pH is varied. At pH < 2.0, the cation (VO₂⁺) is predominant, and this species is gradually converted to the polymer (V₁₀O₂₈⁶⁻) as the pH of solution is raised. In the range of pH from 2.0 to 7.0, the V₁₀O₂₈⁶⁻ increases and then decreases, and the species has disappeared at pH about 7. As the solution pH is further increased, vanadate anions such as the monomer, dimer, tetramer, and pentamer are formed. At pH above 9, the unprotonated tetrahedral monomeric (VO₄³⁻) dominates.

Reduction of Vanadate by HS in Acidic pH. When humic substances were added to the vanadate solution at pH 3.0, reduction occurred, and the formation of vanadium(IV) species was confirmed by ESR spectroscopy. The ESR intensity of vanadium(IV) increased with time and can be correlated to the concentration of vanadium(IV) formed. The ESR spectrum of vanadium(IV) 8 h after the reaction of humic substances with vanadate is shown in Figure 2. There is little, if any, ESR-silent vanadium(III) present from the further reduction of vanadium(IV) since the ESR intensity of this spectrum remained unchanged for a long period. The corresponding ESR parameters listed in Table 1 agree with those of other workers (18–20). Table 2 lists the ESR parameters of vanadium(IV) with humic substances and with various organic acids.

⁵¹V NMR spectroscopy was used to follow vanadium(V) species in the reduction of the vanadate ion by humic

TABLE 1. ESR Parameters of Vanadium(IV) from Reduction of Vanadium(V) by Humic Substances and Formation of HS–V(IV) Complexes in Humic Substances

| complexes | T (K) | A _{iso} (G) | A (G) | A _⊥ (G) | g _{iso} | g | g _⊥ | ref |
|-----------|-------|----------------------|---------------------|--------------------|------------------|-----------------|----------------|-----|
| HS–VO | 77 | 112 | 188 | 74 | 1.975 | 1.966 | 1.993 | a |
| FA–VO | 77 | 116 | 199 | 75 | 1.961 | 1.929 | 1.979 | 18 |
| HA–VO | 77 | 103 | 174 | 67 | 1.974 | 1.943 | 1.990 | 19 |
| humate–VO | 77 | 106 | 185 | 67 | 1.968 | 1.942 | 1.981 | 20 |

^a ESR parameters of vanadyl ion reduced from vanadate ion by humic substance solution at pH 3. FA, fulvic acid; HA, humic acid.

TABLE 2. ESR Parameters of Vanadium(IV) Complexes with Organic Acids and Humic Substances in Aqueous Solutions

| complexes | T (K) | pH | L/VO ²⁺ | A _{iso} (G) | g _{iso} | ΔH _(-3/2) (Hz) |
|-----------------------------------|-------|-----|--------------------|----------------------|------------------|---------------------------|
| H ₂ O–VO ²⁺ | RT | 3.0 | | 116 ± 0.5 | 1.975 | 12.8 |
| HS–VO | RT | 3.0 | 5.6 | 112 ± 0.5 | 1.966 | 12.8 |
| HS–VO ^a | RT | 3.0 | 5.6 | 112 ± 0.5 | 1.975 | 11.4 |
| oxalic–VO | RT | 3.0 | 5.0 | 107 ± 0.5 | 1.974 | 8.60 |
| salicylic–VO | RT | 3.0 | 5.0 | 109 ± 0.5 | 1.974 | 14.2 |
| phthalic–VO | RT | 3.0 | 5.0 | 113 ± 0.5 | 1.979 | 12.9 |
| malonic–VO | RT | 3.0 | 5.0 | 110 ± 0.5 | 1.975 | 11.4 |
| maleic–VO | RT | 3.0 | 5.0 | 114 ± 0.5 | 1.977 | 12.9 |

^a Vanadyl ion reduced from vanadate ion by humic substances. RT, room temperature.

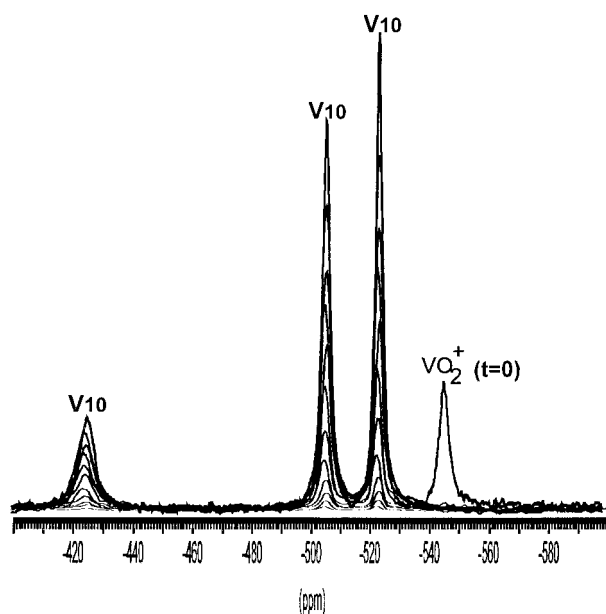


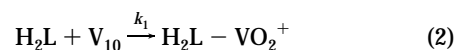
FIGURE 3. Reductive decay of ⁵¹V NMR spectra recorded for the vanadate solutions in the absence and in the presence of humic substances. Experimental conditions: 0.167 mM humic substances, 5 mM vanadate, pH 3.0. The spectra were recorded over a period of 100 min.

substances in acidic pH. Figure 3 shows ⁵¹V NMR spectra of a 5 mM vanadate solution at pH 3 before and after addition of the humic substances. The signal at –546 ppm assigned to VO₂⁺ cation is immediately lost on the addition of humic substances. The remaining signals at –425, –506, and –524 ppm, due to the decavanadate anion (27, 28), decay with time over a period of 100 min. The NMR spectra suggest that the “free cation”, VO₂⁺, is directly involved in the reduction.

The reduction of vanadate by humic substances under different conditions was investigated. The reduction of vanadate by humic substances at pH 3.0 was followed using

the concentrations of V₁₀ at different times obtained from integral areas of NMR spectra. The disappearance of V₁₀ gives a first-order plot with a rate constant (*k*₁) of 0.026 min^{–1} with a vanadate concentration of 5.0 mM and a humic substance concentration of 0.167 mM. With the vanadate concentration at 5.0 mM and the concentration of humic substances increased to 0.501 mM, the first-order rate constant is 0.077 min^{–1}, suggesting that the rate of reaction is first order with respect to humic substances. This is similar to the reactions of humic substances with chromium(VI) and manganese(VII) (29).

It appears that the first step in the reaction is the slow conversion of V₁₀ to VO₂⁺, forming a H₂L–VO₂⁺ complex in the presence of humic substances:



followed by the rapid reduction of the H₂L–VO₂⁺ complex to vanadium(IV):



where H₂L represents the ligand in the humic substances and L represents the oxidation product of the ligand, respectively. *k*₁ and *k*₂ represent the rate constants for the decomposition of V₁₀ and the rate constant for the reduction of VO₂⁺, respectively; where *k*₁ depends on the concentration of humic substances and the pH. Since NMR spectroscopy shows that the VO₂⁺ species is very reactive as compared to the V₁₀ species in the reduction, it can be assumed that *k*₂ is much greater than *k*₁ and that the steady-state hypothesis can be applied to the VO₂⁺ species. Therefore, the rate of reduction of V(V) to V(IV) can be given as follows (30):

$$\frac{-d[\text{V}_{10}]}{dt} = k_1[\text{V}_{10}] \quad (4)$$

$$\frac{-d[\text{VO}_2^+]}{dt} = k_1[\text{V}_{10}] - k_2[\text{VO}_2^+] = 0 \quad (5)$$

as *k*₂ ≫ *k*₁:

$$[\text{VO}_2^+] = [\text{V}_{10}]_0 - [\text{V}_{10}] - [\text{VO}_2^+] = [\text{V}_{10}]_0 \left\{ 1 - \left(1 + \frac{k_1}{k_2} \right) e^{-k_1 t} \right\} \quad (6)$$

where the [V₁₀]₀ is the initial total concentration of [V₁₀] before humic substances were added to vanadate solution and [V₁₀] is the concentration of decavanadate during reduction, which can be estimated by integration of the corresponding NMR peaks in the spectra.

Since concentration of VO₂⁺ over time is low, it appears that *k*₂ for the second step of reduction is much greater than *k*₁. With the assumption that *k*₂ is 10*k*₁, the distributions of the three species (V₁₀, VO₂⁺, and VO²⁺) involved in the reduction can be calculated using eqs 5 and 6. The distribution curves of the V₁₀, VO₂⁺, and VO²⁺ over time are shown in Figure 4 in which the concentrations of the three vanadium species are given in terms of the VO₂⁺ unit. The calculated concentrations of vanadium(IV) formed in the reduction are similar to the concentrations obtained by ESR spectra under the same conditions. A greater value of *k*₂ gives the same result, suggesting that *k*₁ is the rate-determining step.

The reduction of vanadate by humic substances depends on the pH. The ESR intensity of V(IV) recorded after 20 min with the same concentrations of vanadate and humic substances but at different pH values is shown in Figure 5.

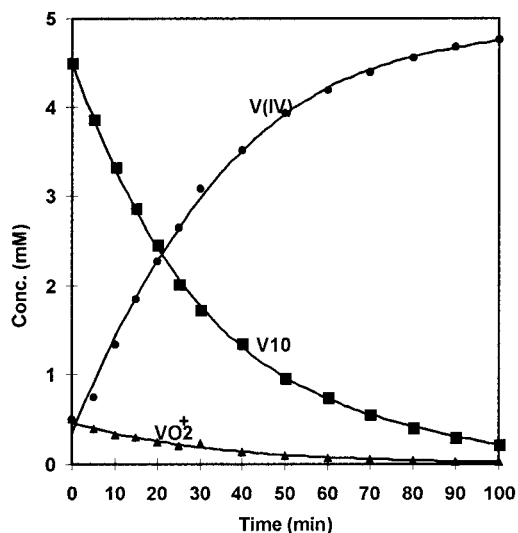


FIGURE 4. Distribution of vanadium species calculated from reduction of vanadate by humic substances assuming that k_2 equals $10k_1$ in aqueous solution at pH 3.0 with 5 mM vanadate and 0.167 mM humic substances.

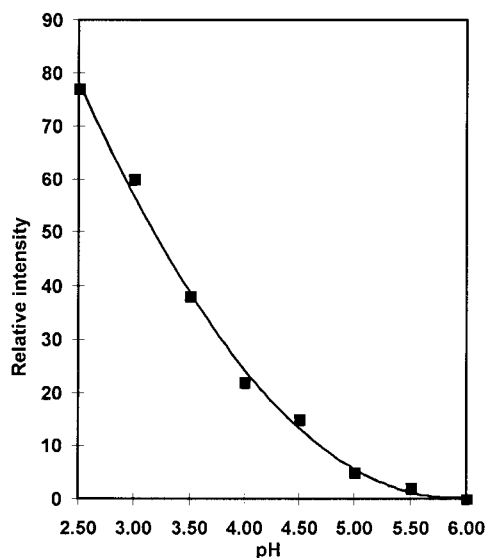


FIGURE 5. Intensity of the ESR spectra as a function of pH in the reduction of vanadate by humic substances. The spectra were recorded at room temperature 20 min after the addition of vanadyl to HS. Experimental conditions: 0.167 mM humic substances; vanadate concentration, 0.5 mM. The main ESR peak was used to calculate the concentration of vanadyl.

The intensity decreases with increasing pH until pH 6, and above this pH there is no detectable ESR signal. The ^{51}V NMR has shown that the VO_2^+ cation is the species that is reduced by humic substances. It is also the dominant species of vanadium(V) in acidic conditions. As the pH of the solution increases, the VO_2^+ species condenses to form $\text{V}_{10}\text{O}_{28}^{6-}$ (31), which is not directly involved in the reduction of vanadate. The rate of reaction also decreases with increasing pH. The first-order rate constant at pH 2.5 was 1.5 times the first-order rate constant at pH 3.0, and at pH 3.5 the first-order rate constant was half that at pH 3.0. The increase in rate with increasing acidity can be attributed to the decomposition of the decavanadate, as observed by Murmann and Giese (32). The observation that there is no reduction of vanadium(V) to vanadium(IV) above pH 6 can be attributed to the strength of vanadium(V) as an oxidizing agent as a function of pH. Assuming that the electrode potential of the overall

reaction is zero at pH 6 and assuming that the oxidation of humic substances is similar to the oxidation of hydroquinone to quinone, using the Nernst equation with the standard reduction potential ($\text{VO}_2^+ + 2\text{H}^+ + e = \text{VO}^{2+} + \text{H}_2\text{O}$; $E^\circ = 1.00$ V), it can be estimated that the standard reduction potential for the oxidized form of humic substances is +0.65 V, a value in the range of simple quinones such as quinone (+0.699 V), σ -benzoquinone (+0.792 V), β -naphthoquinone (+0.555 V), and α -naphthoquinone (+0.470 V) (33). In the same reference, it is also pointed out that substituents on the aromatic ring have an effect on the standard reduction potentials.

Wells and Kuritsyn (34) suggested that the reduction of vanadate by hydroquinone (QH_2) occurred via a semiquinone radical, which reacted further to quinone. Humic substances are chemically complex polymers, and many functional groups such as phenolic, enolic, and alcoholic OH; catechol; and other polyhydroxy derivatives have been identified in humic substances (35). We suggest that the vanadate ion may be reduced by a hydroquinone or a semiquinone in the humic substances to the vanadyl ion before it is bound to the humic substances. Such a mechanism has also been proposed by Szilagyi (36) and Alberts et al. (37) for the reduction of Fe(III) and Hg(II) ions by humic substances and by Lu et al. (29) for the reduction of permanganate by humic substances.

Infrared spectroscopy gives some indication of the bonding between vanadyl and humic substances. The FTIR spectrum extracted from the reaction mixtures of humic substances and vanadate after 2 day reduction reaction was similar to that extracted from the mixture of humic substances with vanadyl ion. Both spectra showed a decrease in the intensities of the bands near 1720 and at 1210 cm^{-1} and an increase in the intensities of the bands near 1630 and 1400 cm^{-1} , suggesting that the conversion of COOH groups into the COO^- form (38, 39) can be regarded as evidence that the vanadyl ions are bound to the carboxylic groups in humic substances.

The ESR parameters (Table 2) can also be used to examine the binding of the vanadyl ion, which was reduced by the humic substances. Initially, the A_{iso} value (116 G) of the reduced vanadyl ion was the same as that of vanadyl ion added to water at pH 3.0, indicating that this vanadyl ion is "free". A decrease in the value of the A_{iso} parameter was observed from 116 to 112 via time, indicating that the vanadyl ion is gradually bound to a ligand in the humic substances. The same ESR parameters were obtained when the vanadyl ion was directly added to humic substances. A detailed comparison was carried out on the ESR spectra of vanadyl solution added to humic substances and to several model organic acids containing both carboxylic and aliphatic hydroxyls. ESR parameters were similar to those reported in previous ESR studies of vanadyl ion bound to oxygen donor atoms, probably at carboxyl acid sites in fulvic or humic acids (17, 19, 20). There did not appear to be a clear correlation between the simple organic acids and the humic substances. Whereas when aluminum(III) ions and copper(II) ions were added to humic substances, the ESR and ^{27}Al NMR parameters indicated that the complex with humic substances is similar to complexes with oxalic acid (21, 40).

Binding of Vanadate by HS at Neutral pH. Figure 6 shows NMR spectra of a 0.5 mM vanadate solution at pH 7.2 in the presence of increasing humic substance concentrations ranging up to 0.583 mM. Addition of humic substances causes the disappearance of the pentamer of ^{51}V NMR resonance, and there is a significant decrease in the intensities of the dimer and tetramer NMR signals as the concentration of humic substances is increased. The decrease in the NMR resonance intensity of the monomer is smaller than that of the polymeric species, but the monomer decreases rapidly at humic substance concentrations greater than 0.333 mM.

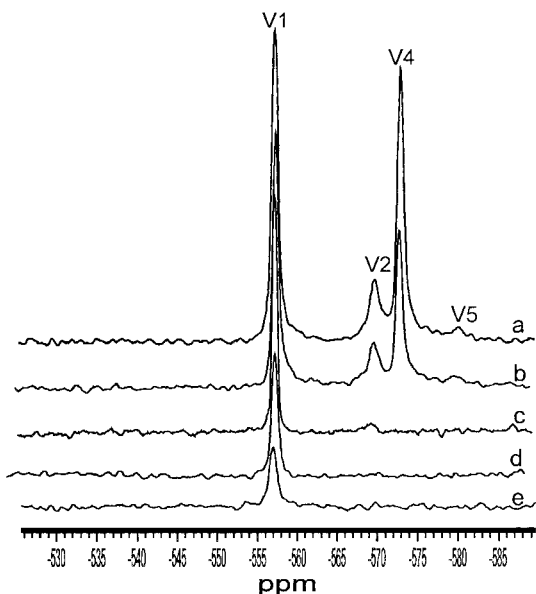


FIGURE 6. ^{51}V NMR spectra of 0.5 mM vanadate in various concentrations of humic substances at pH 7.2. HS concentration: (a) 0.000, (b) 0.167, (c) 0.333, (d) 0.500, (e) 0.667 mM.

TABLE 3. Chemical Shifts and Line Widths of Monomeric (V_1), Dimeric (V_2), and Tetrameric (V_4) with 2 mM Total Vanadate in the Absence and in the Presence of Humic Substances, at $25 \pm 1^\circ\text{C}$ and pH 7.2

| | V_1 | V_2 | V_4 |
|----------------------------|-------|-------|-------|
| No HS | | | |
| δ/ppm | -557 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 96 | 110 | 99 |
| 0.0167 mM HS | | | |
| δ/ppm | -557 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 100 | 120 | 135 |
| 0.0333 mM HS | | | |
| δ/ppm | -558 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 105 | 142 | 148 |
| 0.083 mM HS | | | |
| δ/ppm | -557 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 115 | 155 | 165 |
| 0.167 mM HS | | | |
| δ/ppm | -558 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 122 | 200 | 250 |
| 0.333 mM HS | | | |
| δ/ppm | -557 | -571 | -575 |
| $\Delta v_{1/2}/\text{Hz}$ | 150 | 245 | 300 |

Since the species of vanadate at neutral pH are exchanging with each other on a millisecond time scale, it is difficult to distinguish the species responsible for the bonding to the humic substances at this stage. Chemical shifts and line width of the monomeric, dimeric, and tetrameric species of vanadate in the presence of humic substances are summarized in Table 3.

While the loss of the NMR signals cannot be attributed to reduction of vanadium(V) to paramagnetic vanadium(IV), the binding of the vanadate anions to the high molecular weight humic macromolecules broadens the ^{51}V NMR resonances of vanadate species because of the quadrupolar nature of the ^{51}V nucleus, and the bound vanadate becomes invisible. A similar phenomena has been observed for vanadate bound to a high molecular weight protein (23). We also conclude that the observed changes in intensities and line widths of the ^{51}V NMR resonances of vanadate solution are caused by the presence of the humic substances.

TABLE 4. Concentrations (mM) of Vanadate Species in the Presence of Increasing Concentrations of HS (mM), pH 7.2, and 0.5 mM Total Vanadate at $25 \pm 1^\circ\text{C}$ ^a

| HS | $[V_1]$ | $[V_2]$ | $[V_4]$ | $[\text{HSV}_x]_n$ |
|--------|---------|---------|---------|--------------------|
| 0.0250 | 0.287 | 0.0292 | 0.0327 | 0.0238 |
| 0.0500 | 0.276 | 0.0253 | 0.0320 | 0.0454 |
| 0.125 | 0.244 | 0.0212 | 0.0242 | 0.117 |
| 0.167 | 0.236 | 0.0188 | 0.0208 | 0.142 |
| 0.208 | 0.206 | 0.0172 | 0.0188 | 0.184 |
| 0.250 | 0.186 | 0.0136 | 0.0165 | 0.221 |
| 0.292 | 0.165 | 0.0093 | 0.0124 | 0.267 |
| 0.333 | 0.154 | 0.000 | 0.00610 | 0.322 |
| 0.417 | 0.104 | 0.000 | 0.000 | 0.396 |
| 0.500 | 0.035 | 0.000 | 0.000 | 0.465 |

^a $[\text{HSV}_x]_n$ was estimated as $[V]_{\text{tot}} - [V_1] - 2[V_2] - 4[V_4]$.

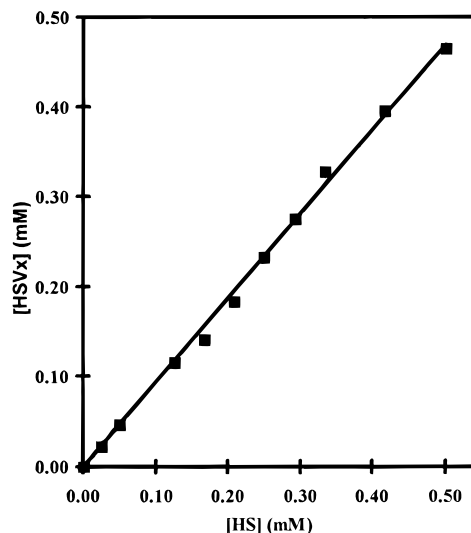


FIGURE 7. Total HS-V concentrations plotted against the total concentrations of humic substances at pH 7.2.

The number of vanadate atoms bound to humic substances was determined by measuring the concentrations of total invisible $\text{HS}(\text{V}_x)_n$ complexes at a constant vanadate concentration and varying humic substance concentrations. The concentrations of vanadate species in the presence of increasing concentrations of humic substances at pH 7.2 are listed in Table 4. Figure 7 shows a linear relationship between $\text{HS}(\text{V}_x)_n$ complexes and humic substance concentrations. The slope of the curve (0.929), which is close to 1, suggests that humic substances complex vanadate through the monomer.

To determine the nature and number of vanadate anions in the $\text{HS}(\text{V}_x)_n$ complexes, we investigated the proportion of vanadate species under conditions of varying vanadate concentrations and constant humic substance concentration using the approach of Wittenkeller et al. (26). The data are summarized in Table 5. Using these data, it is possible to examine which vanadate derivatives bind to the humic substances. A plot of $1/\text{HS}(\text{V}_x)_n$ as a function of $1/[V_1]$ is linear (Figure 8). From the vertical intercept in Figure 8, the limiting stoichiometry of one atom per molecule of humic substances was derived. These results confirm that the vanadate monomeric ion binds to humic substances. Both of the relationships of $1/\text{HS}(\text{V}_x)_n$ as a function of $1/[V_2]$ or $1/[V_4]$ are sigmoidal, suggesting that these dimer or tetramer species of vanadate at this pH do not bind to the humic substances.

There is substantial literature describing the binding of the diprotonated vanadate monomeric anion to various organic compounds, particularly those organic compounds

TABLE 5. Concentrations (mM) of Vanadate Species in the Presence of 0.333 mM HS, pH 7.2, at $25 \pm 1^\circ\text{C}$, Containing Varying Total Vanadium Concentrations

| V_{total} | $[V_1]$ | $[V_2]$ | $[V_4]$ | $[\text{HSV}_x]_n$ |
|--------------------|---------|---------|---------|--------------------|
| 0.25 | 0.0689 | 0.000 | 0.000 | 0.181 |
| 0.50 | 0.154 | 0.000 | 0.00610 | 0.322 |
| 0.75 | 0.272 | 0.000 | 0.0182 | 0.405 |
| 1.00 | 0.407 | 0.000 | 0.0226 | 0.501 |
| 1.25 | 0.499 | 0.000 | 0.0284 | 0.637 |
| 1.50 | 0.607 | 0.0116 | 0.0292 | 0.753 |
| 1.75 | 0.734 | 0.0514 | 0.0331 | 0.781 |
| 2.00 | 0.860 | 0.0644 | 0.0428 | 0.840 |
| 2.25 | 0.950 | 0.0740 | 0.0522 | 0.940 |
| 2.50 | 1.04 | 0.0882 | 0.0744 | 0.982 |
| 2.75 | 1.16 | 0.112 | 0.0860 | 1.02 |
| 3.00 | 1.34 | 0.122 | 0.094 | 1.04 |
| 3.25 | 1.60 | 0.130 | 0.098 | 1.05 |

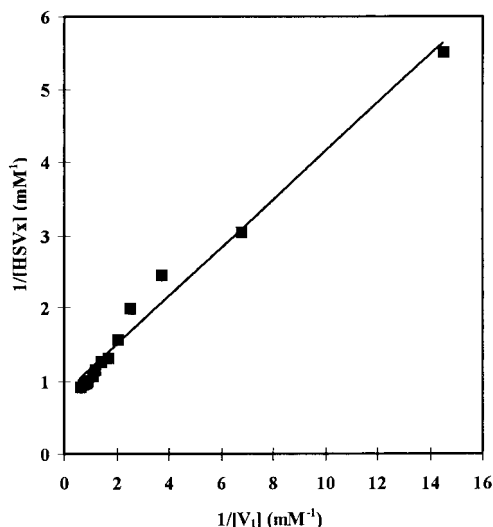
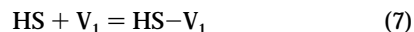


FIGURE 8. Double-reciprocal plot of the concentrations of total HS-V complex against the concentrations of vanadate monomer at pH 7.2.

having hydroxyl groups available for chelation (41–44). At neutral pH, the vanadate rapidly and spontaneously condenses with alcohols and carboxylic acids to form esters (41–44). It is then not surprising that many functional groups such as carboxyl (COOH), hydroxyl (OH), and carbonyl (C=O) in humic substances condense with vanadate to form esters.

The vanadate monomer bound to ligands in humic substances can be expressed in the following equation:



with the formation constant given by

$$K = \frac{[\text{HSV}_1]}{[\text{HS}][V_1]} \quad (8)$$

From eq 8, we can calculate the formation constant K directly from the ^{51}V NMR spectra. The average of the formation constant K is 108 M^{-1} . The formation constant is consistent with that obtained for mono-dicarboxylic acids (43) and alcohol-vanadate complexes (45) but much smaller than that of the EDTA- and EGTA-vanadate complexes (46), suggesting that the main ligands in humic substances involved in the binding of vanadate are oxygen atom ligands and not nitrogen atom ligands. This is expected because

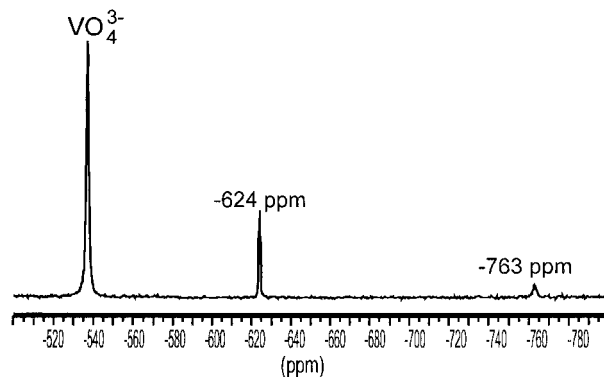


FIGURE 9. ^{51}V NMR spectra of 2 mM total vanadate in 0.167 mM humic substance solution at pH 11.

there are many more oxygen atom functional groups than there are nitrogen atom functional groups in the humic substances.

Vanadate Species in Alkaline HS Solution. As the pH increases from 7.2 to 12, the vanadate monomer (H_2VO_4^-) undergoes a decrease in chemical shift from -555 to -536 ppm associated with deprotonation of the anion to VO_3^- . Also NMR spectroscopy indicates that as the pH of the HS-vanadate solution is increased a sharp peak appeared with a chemical shift at -624 ppm ($W_{1/2} = \sim 85$ Hz) at pH 10.5, and then a second new peak with chemical shift at -763 ppm ($W_{1/2} = \sim 120$ Hz) is observed at pH 11.0. Figure 9 shows ^{51}V NMR spectra of a 2 mM vanadate containing 0.167 mM HS at pH 11. The intensities of the peaks increase with increasing pH and with increasing humic substance concentration. Also there is little (0.5 ppm), if any, chemical shift over the range of pH.

The two peaks appearing in alkaline conditions are considerably different from those of vanadate derivatives such as vanadate oligomers, vanadate esters, and cyclic vanadate esters at neutral pH (41–44) and those of vanadate derivatives with hydrocarboxylic acids and alcohols (27, 28). Moreover, these mentioned derivatives give rise to broad peaks whereas the observed peaks are sharp and appear to be associated with unbound vanadate. Both observed peaks correspond to some of the chemical shifts observed by Harrison and Howarth (47) and by Clague and Butler (48) in alkaline conditions when hydrogen peroxide was added to vanadate solutions. These chemical shifts have been attributed to peroxyanion vanadate (e.g., $[\text{VO}_3(\text{OO})]^{3-}$ and $[\text{VO}_2(\text{OH})(\text{OO})]^{2-}$) and peroxydivanadates (e.g., $[\text{HO}_2(\text{OO})\text{VOVO}(\text{OO})_2]^{3-}$), in which the vanadium is mainly octahedrally coordinated. Redox potentials mean that there is no hydrogen peroxide in aqueous solutions of humic substances. However, the ESR spectrum of the humic substances consists of a single sharp peak, devoid of fine structure with $g_{\text{iso}} 2.0032$, which is attributed to free radicals (49). We have found that the intensity of this peak increases with increasing pH. This can be related to an increase in free radical content as hydroxyl ligands in semiquinone and polyphenolic groups are deprotonated with increasing pH. The free radical content could be a factor in the formation of peroxyanion vanadate species at high pH, possibly through reaction with dissolved oxygen in solution. No sharp peaks with chemical shifts at -624 and -763 ppm were found in the sample that had been purged of dissolved oxygen with nitrogen gas.

Oxygen molecules are diradicals, and in the combustion of hydrocarbons (50), these oxygen diradicals join with organic radical R^{\cdot} to form peroxy radicals (RO_2^{\cdot}). A similar reaction at alkaline pH could be expected in that a free radical in the humic substances joins with an oxygen diradical to form a peroxy radical on the humic substances. This peroxy radical can then react with a vanadate ion to form a vanadate

peroxy anion. The reaction only occurs at high pH where there is greater free radical character in the humic substances.

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