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Interaction of *N*-hydroxyacetamide with vanadate in aqueous solution

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The equilibria between aqueous vanadate and *N*-hydroxyacetamide (acetohydroxamic acid, HL, $CH_3CONHOH$) have been studied at 25 °C in 0.15 mol dm^{-3} NaCl medium by combined potentiometric, spectrophotometric, ^{51}V and ^{17}O NMR methods. Complexes form in the range pH 3 to 11, and many of their formation constants have been determined. The neutral species HV(HL) and $HV(HL)_2$ predominate below pH 4.5 and with HL : V > 2 : 1, where V is an abbreviation for $[H_2VO_4]^-$. These notional formulae do not specify the possible co-ordination or loss of water molecules from the complex anions and the charges shown are the overall species charges rather than any algebraic sum. From pH 4.5 to 8.5 the main species are $[V(HL)_2]^-$ and, to a lesser extent, $[V(HL)]^-$. Oxygen-17 NMR data strongly suggest that $[V(HL)_2]^-$ has octahedral co-ordination, with a *cis*-VO_2^+ unit and two bidentate L^- ligands, one of which can lose another proton above pH 8.5 to form $[VL(HL)]^{2-}$. The species $[V(HL)]^-$ also deprotonates above pH 9. The minor oligomeric species $[V_2(HL)_3]^{n-}$ and $[V_2(HL)_4]^{n-}$ also form, at higher concentrations of V and HL.

The physiological relevance of vanadium has led to great interest in the complexes of organic ligands with vanadate. Much of this interest is concerned with the role played by vanadate as a phosphate analogue, allowing it to act both as an inhibitor of phosphate-metabolising enzymes as well as an activator of others. These as well as other aspects of the roles played by vanadium in living organisms have been discussed in recent publications.[1,2] In addition, hydroxamic acids and their complexation reactions with metal ions are of considerable interest and are the subject of a review.[3]

The main difficulty in studying the interaction of vanadate(v) with organic ligands in aqueous solution is the tendency of V^V to hydrolyse, forming both mono- and poly-nuclear species. Several authors have studied this hydrolysis, at different ionic strengths, using potentiometric,[4–8] spectrophotometric [9–12] and ^{51}V NMR methods.[4,5,13] These show that V^V exists as the VO_2^+ cation from pH 1 to 3, but forms several polymeric species, such as decavanadate, $[V_{10}O_{28}]^{6-}$, cyclic tetravanadate, $[V_4O_{12}]^{4-}$ and diprotonated monovanadate, $[H_2VO_4]^-$, in neutral medium. Mild alkali leads to further deprotonation and depolymerisation. Complexation in neutral medium is therefore a competition between this oligomerisation and the reaction of vanadate with the ligand in its prevailing form.

This work reports a detailed study, by three different techniques, of the interaction in aqueous solution of vanadate with *N*-hydroxyacetamide (acetohydroxamic acid, $CH_3CONHOH$). The stoichiometry and the formation constants of the complexes are established and structures are also proposed, on the basis of the NMR spectra.

In a recent paper, Pettersson and co-workers[14] reported the complexation of vanadate by maltol (3-hydroxy-2-methyl-4-pyrone) and noted both chemical and photolytic reduction of vanadate(v) in mildly acidic solutions. In the present study no paramagnetic species were detected, even after exposure of the solutions to light. Indeed, acetohydroxamic acid oxidises V^{IV} to V^V, above pH 4.[15]

Experimental

Potentiometric and ^{51}V NMR [in 0.15 mol dm^{-3} Na(Cl) medium] plus spectrophotometric [0.10 mol dm^{-3} $Na(ClO_4)$ medium] pH titrations were carried out on mixtures of vanadate ($[H_2VO_4]^-$, abbreviated below to 'V') and acetohydroxamic acid ($CH_3CONHOH$, 'HL'), along with a ^{51}V NMR titration over a wider pH range, in 0.6 mol dm^{-3} NaCl medium for comparison, plus further ^{51}V and ^{17}O studies at higher concentrations, to investigate oligomers and anionic structures.

Potentiometric measurements

Potentiometric measurements were carried out by the addition of NaOH to acidified mixtures of V and HL, in the following proportions: (1) [HL] = 0.015 mol dm^{-3} held constant plus [V] = 0.76, 1.53, 2.29 and 3.06 mmol dm^{-3}, and (2) [HL] = 0.023 mol dm^{-3} constant plus [V] = 1.53, 3.06, 4.59, 6.12 and 7.65 mmol dm^{-3}. A Metrohm 670 Titroprocessor was used to measure the electromotive force.

Solutions were added using a Metrohm Dosimat 665 autoburette, except that the acetohydroxamine solutions were added using a volumetric pipette. The ionic strength was adjusted to 0.15 mol dm^{-3} with NaCl solution and the temperature held at 25.0 ± 0.1 °C by circulating thermostatted water. A Metrohm 649 magnetic stirrer was used, and all measurements were done under N_2. Standardised, carbonate-free NaOH solutions were used throughout.

The above electrode system was calibrated for $[H^+]$ before and after each series of measurements, by titration of HCl with standardised NaOH, or *vice versa*, at an ionic strength of 0.15 mol dm^{-3}. A critical evaluation was always made of the parameters ($E°$, RT/F, A_j, B_j) which resulted from the potentiometric calibration procedure. The $-\log[H^+]$ values (represented by the symbol pH) were then obtained by a computerised extrapolation.

Spectrophotometric measurements

The solutions undergoing titration were also circulated, by a peristaltic pump, through a continuous-flow quartz cuvette, installed in a model 8451A Hewlett-Packard spectrophotometer. This allowed simultaneous measurement of the volume of NaOH added, of pH and of the absorbance of the solution. The time interval between subsequent titrant additions as well as the volume increments were both pre-set to obtain both a spectrum and a pH value after each addition.

NMR measurements

The ^{51}V NMR spectra were obtained at 105.2 MHz with a

Bruker ACP400 spectrometer. Typical spectra required 1000 transients, obtained in *ca.* 10 min, and were referenced to capillary $VOCl_3$. However, the most dilute solutions required longer accumulations. Solutions were prepared in 0.15 and 0.6 mol dm^{-3} Na(Cl) medium, prepared with 10% D_2O, with [V] being 4.5 and 10 mmol dm^{-3} respectively. The solutions, once formed, showed no further time dependence, provided that sufficient acetohydroxamic acid was present (at least two hydroxamate molecules per V) to prevent any formation of decavanadate. All baselines were corrected by spline fitting before integration of the spectra. Estimated errors in concentration for the main species, used for estimating formation constants, were ± 0.0002 mol dm^{-3}, as indicated by the data bars in Fig. 5. Computer deconvolution was used to separate the integrals for overlapping $[V(HL)]^{n-}$ and $[V(HL)_2]^{n-}$ peaks around pH 9.

Oxygen-17 NMR spectra were obtained at the modest threefold oxygen-17 enrichment of the solvent D_2O, at 56.2 MHz. They typically required 10^5 transients over *ca.* 3 h, and careful baseline corrections.

Chemicals

All solutions were prepared using deionised water. Carbonate-free NaOH solutions were prepared from saturated solutions and standardised with potassium hydrogenphthalate. Acetohydroxamic acid (Sigma) solutions were always prepared just before use and their concentrations checked by direct potentiometric titrations with NaOH solutions. Stock solutions of V were prepared by dissolving sodium metavanadate $NaVO_3$ (Carlo Erba) in a known excess of HCl.

Computer calculations

The protonation and formation constants from the potentiometric data were refined by a rigorous least-squares routine using the computer program SUPERQUAD.[16] The standard deviations computed by this program (for random errors only) were used as evidence of the presence of a species and of the adequacy of the equilibrium model proposed. Theoretical titration curves were calculated from the SUPERQUAD constants using software developed in house from an earlier program SCOGS, written by Sayce.[17] Species distribution diagrams were calculated using the program SCECS.[18] Approximate pK_a values were obtained from the NMR data by fitting the shift *vs.* pH data using the Henderson–Hasselbalch equation, and the main formation constants were calculated, independently of the potentiometric data except as noted below, by fitting of the observed integrals by the standard equilibrium constant equations using Microsoft EXCEL 7 with Solver,[19] treating the concentrations of free HL and V as variables.

Results and Discussion

Titrations

A typical potentiometric titration curve showed three inflections, at −1 (*i.e.* +1 mol H^+), 0 and +3 mol of added $[OH]^-$ per V. The inflections corresponded approximately to the dominance regions of neutral species (pH *ca.* 3.5) and of decomposition to $[HVO_4]^{2-}$ and L^- (pH > 10). The complexation reaction was checked independently by ^{51}V NMR spectroscopy at pH *ca.* 7. This confirmed that the V at this pH and concentration was indeed largely $[H_2VO_4]^-$, and that the products upon addition of dry HL were very largely the mononegative anions $[V(HL)_2]^-$ and $[V(HL)]^-$, for they had the shifts discussed below, and there was only a small change in the pH. This experiment defined the protonation states of all the complexes observed.

Fig. 1 shows typical spectrophotometric titration curves, for HL : V = 10 : 1. The initial spectrum, from an acidic solution, has a peak around 450 nm. As the pH is raised this maximum is replaced by a peak around 300 nm, thus creating two partial isosbestic points. At still higher pH the spectrum simply becomes that of vanadate. The curves at lower pH, plus the likelihood that all the complexes present are coloured, probably indicates the dominant presence of only two complexes. If one considers this to be the result of a single deprotonation process, a pK_a equal to 4.14 is calculated. However, additional complexes were apparent from NMR spectra of solutions with lower HL : V ratios, and further peaks were detectable at higher concentrations, >25 mmol dm^{-3}.

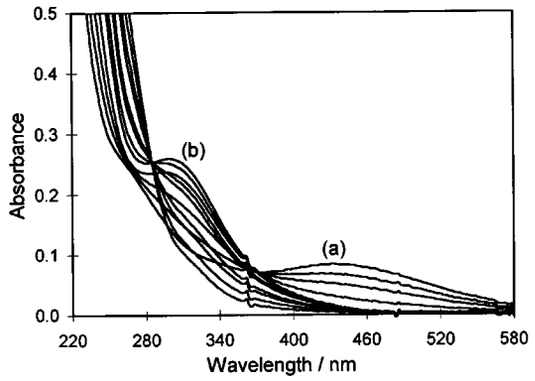


Fig. 1 Spectrophotometric titration data for a solution containing 10.1 mmol dm^{-3} HL and 1.06 mmol dm^{-3} V, from pH 2.4 to 10.3. Line (a) corresponds to pH 2.4 and line (b) to pH 8.2

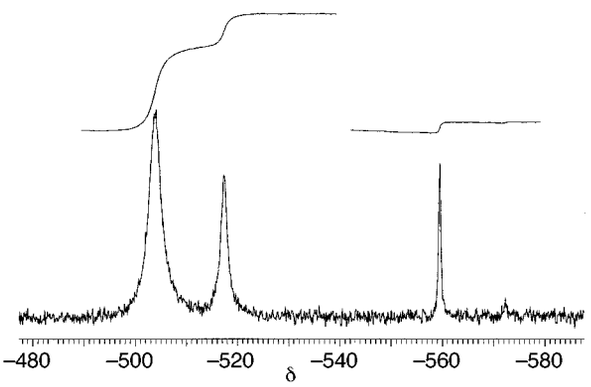


Fig. 2 The ^{51}V NMR spectrum of a pH 6.08 solution, 0.0016 mol dm^{-3} in V and 0.0080 mol dm^{-3} in HL. Peaks from left to right arise from $[V(HL)_2]^-$, $[V(HL)]^-$, V^- and V_2^{2-} (δ −572)

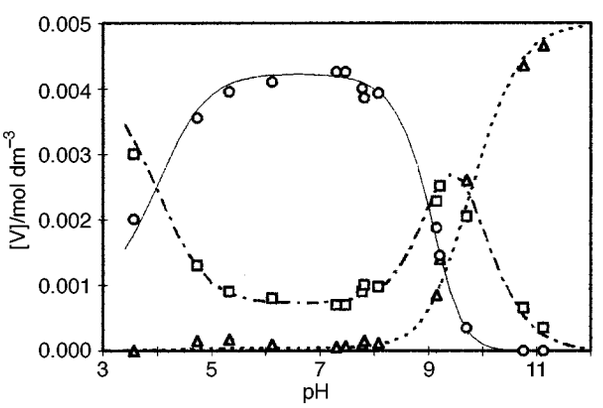


Fig. 3 Dependence on pH of the concentrations of the main species in 0.6 mol dm^{-3} Na(Cl), deduced from ^{51}V NMR integrals. [HL] = 0.025 and [V] = 0.005 mmol dm^{-3}; △, V, *i.e.* $[H_nVO_4]^{n-3}$; □, V(HL); ○, $V(HL)_2$

A ^{51}V NMR spectrum of a dilute solution at pH 6.08 is shown in Fig. 2. From left to right, the peaks arise from $[V(HL)_2]^-$, $[V(HL)]^-$, V^- and V_2^{2-}. The latter peak, at δ −572, is very weak in this example. The ^{51}V NMR integrals and shifts for the monomeric species are illustrated in Figs. 3 and 4 (0.6 mol dm^{-3} medium, [HL] = 0.025 and [V] = 0.005 mol dm^{-3})

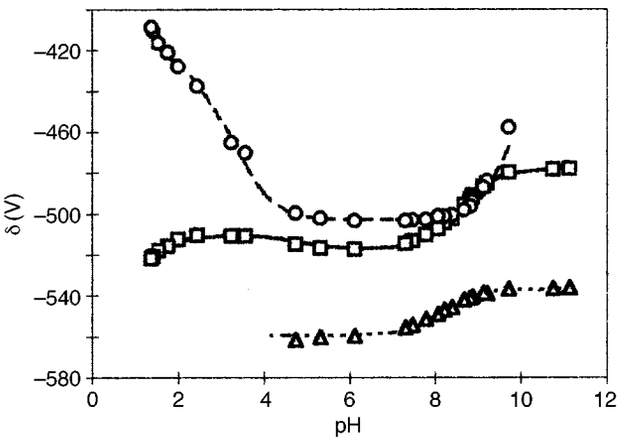


Fig. 4 Dependence on pH of the ^{51}V NMR shifts for the main species in 0.6 mol dm^{-3} Na(Cl). Symbols and concentrations as Fig. 3

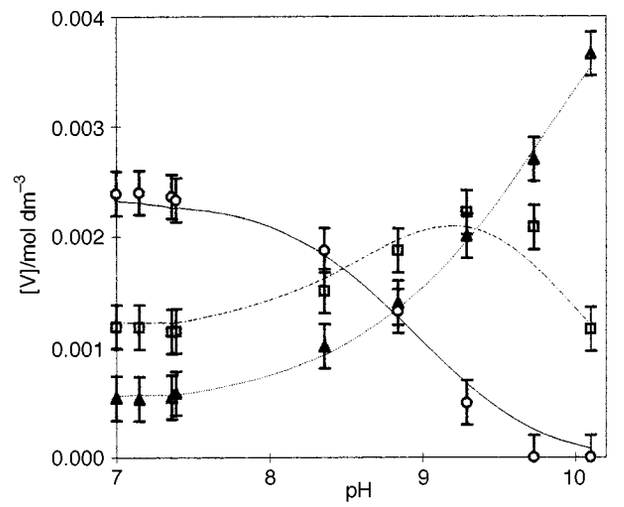


Fig. 5 Dependence on pH of the concentrations of the main species in 0.15 mol dm^{-3} Na(Cl), deduced from ^{51}V NMR integrals, with error estimates. [HL] = 0.010 and [V] = 0.0045 mmol dm^{-3}; symbols as Fig. 3

and 5 (integrals, 0.15 mol dm^{-3} medium, [HL] = 0.010 and [V] = 0.0045 mol dm^{-3}). They not only show a series of $[V(HL)]^{n-}$ as well as $[V(HL)_2]^{n-}$ anions, but also that these protonate to form cations beyond the neutral state, at pH <3.5, and also deprotonate to give dianions at high pH.

Formation constants

The formation of these species can be represented by the general equation (1). As in other investigations of complex

$$p[H^+] + q[H_2VO_4] + r[HL] \rightleftharpoons [(H^+)_p(H_2VO_4)^-{}_q(HL)_r]^{p-q} \quad (1)$$

systems, it proved impossible to determine all the formation constants from one single method. Some of the pK_a values were too close for determination from the NMR shifts alone. Conversely, some parts of the potentiometric titration curves were insufficiently sensitive to the relative proportions of the species present. However, very good data could be obtained by combining the two methods. The formation constants of the mononegative species were readily determinable by NMR spectroscopy. With these fixed, and also with the vanadate constants determined by Pettersson *et al.*,[5] as listed in Table 1, the constants for the remaining species were determined from the potentiometric data alone using the program SUPERQUAD. Fig. 6 shows the agreement between experimental and calculated data, which is so good that differences can only be seen in curve 5, between pH 3 and 4. The resulting distribution of species was calculated from the potentiometric data for 5

Table 1 Stability constants * (log β) of vanadium(v) hydroxo species at 25 °C and I = 0.15 mol dm^{-3}

Formula	log β
$[HVO_4]^{2-}$	−8.17
$[H_2VO_4]^-$	—
$[VO_2]^+$	7.00
$[V_2O_7]^{4-}$	−16.19
$[HV_2O_7]^{3-}$	−5.85
$[H_2V_2O_7]^{2-}$	2.65
$[V_4O_{13}]^{6-}$	−9.98
$[HV_4O_{13}]^{5-}$	0.63
$[V_4O_{12}]^{4-}$	9.24
$[V_5O_{15}]^{5-}$	11.17
$[V_{10}O_{28}]^{6-}$	50.28
$[HV_{10}O_{28}]^{5-}$	56.90
$[H_2V_{10}O_{28}]^{4-}$	61.07
$[H_3V_{10}O_{28}]^{3-}$	62.93

* From ref. 5.

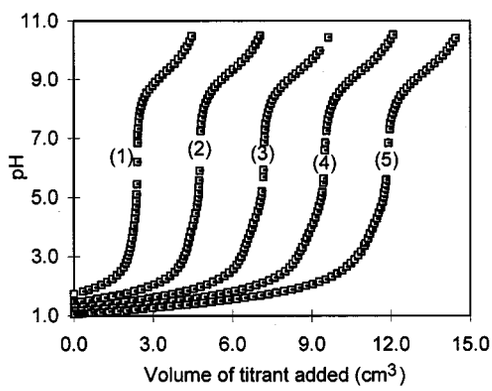


Fig. 6 Experimental (□) and calculated (line) potentiometric titration data points corresponding to titrations of 23 mmol dm^{-3} HL with (1) 1.80, (2) 3.60, (3) 5.40, (4) 7.20 and (5) 9.00 mmol dm^{-3} V

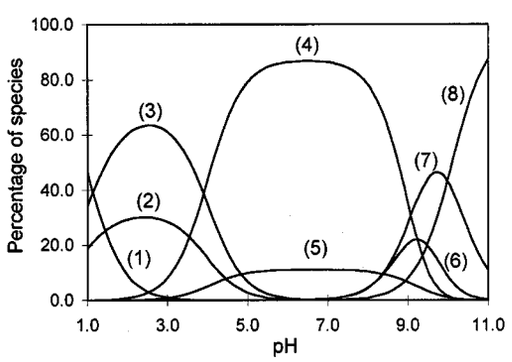


Fig. 7 A distribution diagram for the V–HL system, showing the percentages of species calculated for [V] = 5 mmol dm^{-3} and [HL] = 25 mmol dm^{-3}. (1) $[VO_2]^+$, (2) $HV(HL)_2$, (3) $HV(HL)$, (4) $[V(HL)_2]^-$, (5) $[V(HL)]^-$, (6) $[VL(HL)]^{2-}$, (7) $[VL]^{2-}$ and (8) $[HVO_4]^{2-}$

mmol dm^{-3} V and 25 mmol dm^{-3} HL, as shown in Fig. 7, and also separately from the NMR data (using 4.5 and 10 mmol dm^{-3} respectively) as the lines in Fig. 5. They show that the 1 : 1 complexes become more dominant at pH >9 and <3.

The lines in Fig. 3, *i.e.* for the integrals in 0.6 mmol dm^{-3} Na(Cl) medium, were calculated independently from the NMR data alone. The resulting, less accurate formation constants differ little from those given in Table 2. The pK_a value of the complexes are slightly higher and the log formation constant of $[V(HL)]^-$ increases by 0.3. This further indicates that no high ionic charges are involved.

Oligomers

Further NMR spectra were obtained as a function of concentration and at the approximately constant pH values of 3.0 and

Table 2 Stoichiometry, notation, formation (log β) and acidity (pK_a) constants calculated for the vanadate(V)–*N*-hydroxyacetamide system at 25 °C and $I = 0.15$ mol dm^{-3}

Species*	*p*, *q*, *r*	Simplified formula	log β	pK_a	δ(V)
1	0, 0, 1	HL	—	9.199	
2	−1, 0, 1	L^-	−9.199(3)		
3	1, 1, 1	HV(HL)	7.54(2)	4.84	*ca.* −560
4	0, 1, 1	$[V(HL)]^-$	2.70(2)	8.36	−517.3
5	−1, 1, 1	$[VL]^{2-}$	−5.66(3)		*ca.* −478
6	1, 1, 2	$HV(HL)_2$	8.95(5)	3.55	*ca.* −430
7	0, 1, 2	$[V(HL)_2]^-$	5.40(5)	9.23	−503.0
8	−1, 1, 2	$[VL(HL)]^{2-}$	−3.8(1)		> −460

* As numbered in Fig. 7.

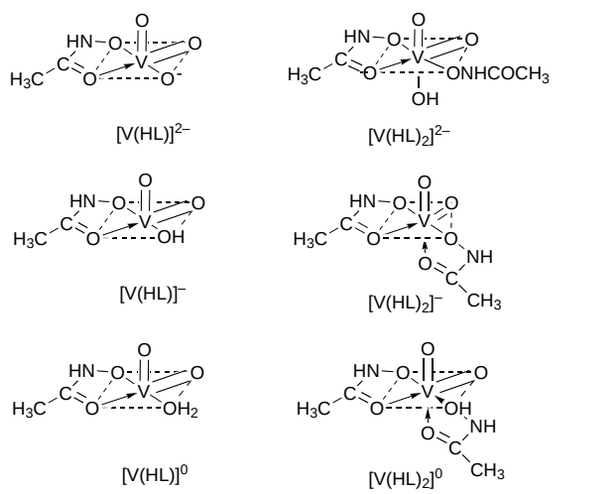
4.0, in order to investigate two minor resonances occurring only within this range. The first showed some dependence upon pH, with its shifts being δ −305 at pH 3.0 and δ −320 at pH 4.0. The second had the approximately constant shift of δ −443.9 (pH 4.0). The dependence of their integrals on concentration was strongly consistent with the formulations $[V_2(HL)_3]^{n-}$ and $[V_2(HL)_4]^{n-}$ respectively; other formulations did not yield satisfactory equilibrium constants over a wide range of dilutions at constant pH and ionic strength. The formation constants in 0.15 mol dm^{-3} NaCl could be conveniently expressed in terms of the $[V(HL)]^{n-}$ and $[V(HL)_2]^{n-}$ species present in the same solutions, *i.e.* without regard for the precise state of protonation. The value of $\log[V_2(HL)_3] - 2\log[VL] - \log[HL]$ was 2.80 ± 0.05 at pH 4, and that of $\log[V_2(HL)_4] - 2\log[VL_2]$ was 0.78 ± 0.05 at the same pH. Although these oligomeric species did not occur over a sufficient range for their charges or pK_a values to be determined reliably, it seems likely from their stability range that they are mononegative anions.

Structures

Table 2 also lists the ^{51}V NMR shifts for the main monomeric species present. However those at the extremes of pH could not be reliably determined. The ^{51}V chemical shifts of the main species vary with pH as shown in Fig. 4. The resonances and hence the shifts of the individual species in this figure were calculated by using the Henderson–Hasselbalch equation, although the resulting pK_a values do not differ significantly from those obtained from the potentiometric data. The shifts of compounds with *cis*-VO_2 groups fall into three general regions. Those below δ −540 are typical for tetrahedrally co-ordinated vanadium. The shift region from $\delta_V = -490$ to −540 is typical for vanadium co-ordinated by five or six oxygens, at normal distances. In contrast, the higher shifts either imply co-ordination involving ligands such as Cl, or else the presence of unusually long V–O bonds, such as might occur upon protonation of a co-ordinated NO^- moiety.

The linewidths also increase substantially at lower pH, from *ca.* 200 Hz at pH >7 to 550 Hz at pH 3, and are even wider at pH <2. If they are plotted against pH they display the same inflections as do the shifts shown in Fig. 4. The linewidths around 200 Hz are typical for five-co-ordinate vanadium, whereas the very wide resonances at low pH almost certainly correspond to six-co-ordination. In contrast, tetrahedral vanadium, in the absence of exchange processes, gives widths of less than 100 Hz, so this lower co-ordination number is unlikely for the present complexes.

We therefore tentatively propose the structures shown in Scheme 1, while remaining uncertain about the precise sites of protonation. These structures imply the likely loss of water, relative to the abbreviated formulae. We attempted to support these proposals by ^{17}O NMR spectra (in D_2O solvent, but otherwise not isotopically enriched). A solution 0.5 mol dm^{-3} in V, at pH 9.8, should correspond to a mixture of $[V(HL)]^-$ and $[VL]^{2-}$, for these high pH values minimise the presence of $[V(HL)_2]^-$. The solution gave a single resonance for oxo ligands



Scheme 1 Tentative structures for the monomeric species, based on shift and linewidth data

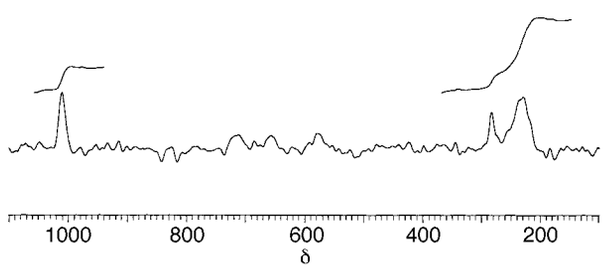


Fig. 8 The ^{17}O NMR spectrum in D_2O at pH 9.8, 0.5 mol dm^{-3} in V. The main complex ion present is $[V(HL)]^{2-}$, with resonances assigned in the text

at δ 1033, falling slightly to δ 1012 at pH 10.35, as shown in Fig. 8. There was also a free acetohydroxate resonance at δ 230, with a bound ligand resonance at δ 290. It had been hoped that the two oxo and one hydroxo ligands in the $[V(HL)]^-$ structure might give rise to separate resonances, but the data instead indicate rapid proton exchange. Nevertheless, the oxygen shifts rule out tetrahedral co-ordination.

A similar spectrum was obtained at pH 6.77, where $[V(HL)_2]^-$ predominates. A single oxo resonance appeared at δ 1067, consistent with the octahedral co-ordination proposed for the vanadium. Hydroxamate oxygen was also detected at δ 825 ppm, in this case. This relatively high shift is consistent with a weak bond to oxygen, such as a N–O bond. The area ratio of each of the two hydroxamate resonances to the oxo resonance was 1 : 3, as expected for the standard oxygen-17 enrichment of commercial D_2O. Thus the NMR shift and linewidth data offer partial confirmation of the proposed structures.

Conclusion

Our results show that *N*-hydroxyacetamide is a good ligand for vanadate over a wide range of pH. It is not oxidised by V^V,

even in the soluble neutral complexes that form under acidic conditions, and it tends to suppress the formation of oligomeric vanadates. The shifts and linewidths both indicate that it binds by chelating.

Acknowledgements

We thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico for financial support, including a CNPq scholarship for R. T. Y.

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Received 15*th August* 1997; *Paper* 7/05997C