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Combined Raman and ^{51}V NMR Spectroscopic Study of Vanadium (V) Oligomerization in Aqueous Alkaline Solutions

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The Raman spectra of aqueous vanadium(V) solutions at different pH values, ranging from 13.1 to 7.0, are presented. The dominant bands in the range 700–1100 cm^{-1} are assigned to the VO symmetrical stretching of the different oligomers, using additional information from ^{51}V NMR spectra and empirical models. The effects of pH, concentration and ionic strength on the oligomerization equilibria are discussed. The ionic strength is found to affect significantly the oligomer composition of vanadate solutions, which is of interest in biochemical and biological studies.

INTRODUCTION

Vanadium is a trace element, toxic at high concentration levels, and is well known for its biological impact.[1–3] In particular, it has been demonstrated that vanadium salts display insulin-like activity and may be used in the treatment of diabetes.[3] Most of the biological importance of vanadium is associated with the +5 oxidation state (vanadate) and is due to a similarity between vanadate and phosphate chemistries.[4] For instance, it has been suggested that vanadate species induce cardiovascular activity and hormonal action,[5] and there have been a number of reports concerning the influence of vanadate on the activity of various enzymes, inhibiting some[4,6–13] while stimulating others.[4,14,15]

Several studies involving the interaction of vanadate with small molecules in aqueous solution (e.g. ethanol and methanol, phosphate and pyrophosphate, amine derivatives, ADP and ATP) have been carried out in the last few years.[16–20] These studies are particularly useful since the enzyme regulatory properties of the oxyanion depend on its esterification with phosphate or hydroxyl groups of biological molecules.[16]

Vanadate aqueous solutions consist of complex mixtures of different oligomers (hereafter referred to as V_n, n being the number of vanadium atoms), with different states of protonation and, in some cases, with different forms [linear (L) or cyclic (C)] (Table 1, Fig. 1). According to an original report,[21] the main species in a solution with pH ranging from 12 to 7 are HVO_4^{2-} (HV_1), $H_2VO_4^-$(H_2V_1), $HV_2O_7^{3-}$ (HV_2) and $V_3O_9^{3-}$ (V_{3C}). However, in a later paper,[22] the same group proposed the occurrence of $V_2O_7^{4-}$(V_2), $V_4O_{13}^{4-}$(V_{4L}) and $V_4O_{12}^{4-}$(V_{4C}) species, in addition to the previous ones. Recent papers on the ^{51}V NMR spectra of vanadate

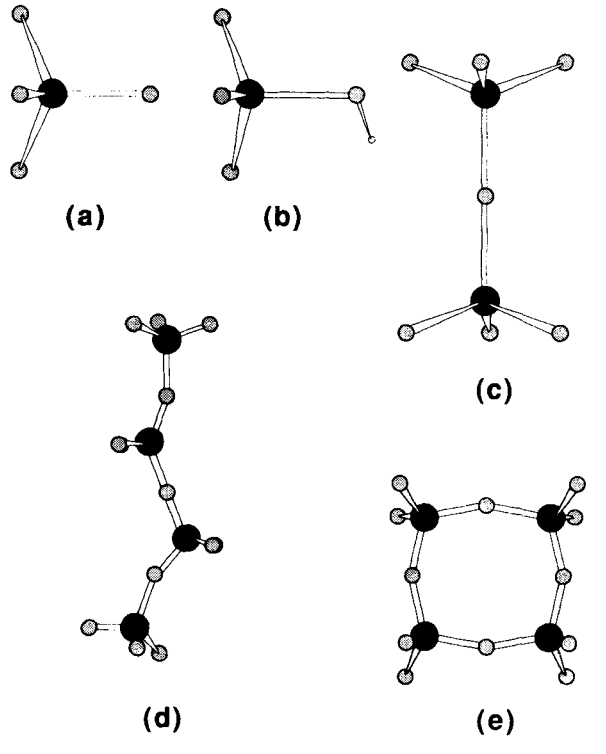

Figure 1. Representation of some vanadate oligomeric species: (a) V_1; (b) HV_1; (c) V_2; (d) V_{4L}; (e) V_{4C}.

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solutions[23,24] provide valuable information, although not without certain points of disagreement with Refs 21 and 22. For instance, according to Ref. 23, the most significant species in a vanadate aqueous solution are $VO_4^{3-}(V_1)$, HV_1, H_2V_1, V_2, HV_2, V_{3C}, V_{4C} and $V_{10}O_{28}^{6-}$ (V_{10}).

The concentration of each species depends on several factors, in particular pH, total concentration of oxyanion and ionic strength. Since several studies suggest that different biological systems are affected by different oligometric species,[8,10,11] an in-depth understanding of the biological activities of vanadate is likely to require a previous understanding of its chemistry. However, owing to the high complexity of the oligomerization equilibria, a knowledge of the various oligomers present at particular experimental conditions remains a challenge.

Raman spectroscopy has been widely used in conformational studies of biological molecules, especially, proteins.[25–29]. In particular, it has been used to evaluate conformational changes of proteins induced by other molecular systems, such as salts.[30] Hence it seemed appropriate to study the vanadate oligomerization process by this spectroscopic technique.

In this work the pH, concentration and ionic strength dependence of vanadate oligomerization were studied by Raman spectroscopy, in order to characterize the vanadate species present under experimental conditions which are specific to biochemical studies. Several Raman bands are tentatively assigned to individual oligomeric species, using additional information from NMR and empirical models.

EXPERIMENTAL

Vanadate ion solutions, with concentrations ranging from 50 to 100 mM, were prepared by dissolving sodium orthovanadate (Sigma) in water. Concentrations lower than 50 mM yielded unfavourable Raman signal-to-noise ratios and so were not considered.

The pH of each sample was adjusted using 1 M HCl or NaOH solutions. After pH adjustment with HCl, the solution was boiled (*ca.* 10 min.) in order to eliminate the yellow colour formed, indicative of oligomerization induced by the acid. The solution was cooled to room temperature prior to recording spectra.

Raman spectra were recorded on a Spex Model 1403 Ramalog double spectrometer, 0.85 m, *f*/7.8. The excitation source was a Spectra-Physics argon ion laser, the output of which at 514.5 nm was adjusted to provide 100–200 mW at the sample position. The sample solutions were kept in a cylindrical cell of i.d. *ca.* 6 mm.

NMR spectra were recorded at 52.6 MHz on a Varian XL-200 spectrometer equipped with a multinuclear broadband probe. A 90° pulse Fourier transform technique was used under the following conditions: 15 kHz spectral width, 2944 data points and 200 accumulated transients at 10 scans s^{-1} without relaxation delay. $VOCl_3$ (0 ppm) was used as external reference. To avoid the addition of D_2O, the different spectra were recorded after a pre-lock procedure was performed with a D_2O sample. The samples were kept in 10 mm NMR tubes.

RESULTS AND DISCUSSION

pH effect

Figure 2 presents the Raman spectra of a 50 mM vanadate solution, in the 700–1100 cm^{-1} region, for pH values ranging from 13.1 to 7.0. In this pH range, the spectra are dominated by three bands at 820, 870 and 945 cm^{-1}, ascribed to the VO symmetric stretching mode (ν_{VO}) of different oligomeric species.[31–33] In addition, a broad band of medium intensity is noticeable at *ca.* 936 cm^{-1} in a restricted pH range (9.6–9.2). As can be seen (Fig. 2), the intensities of these bands (820, 870, *ca.* 930 and 945 cm^{-1}) undergo a sequence of changes with pH change, enabling us to identify the major species present at specific pH conditions, as will be discussed below.

While some workers[34,35] have associated V_1 with a Raman band at 870 cm^{-1}, others[31,33] have correlated this species with a band near 820 cm^{-1}, ascribing the band at 870 cm^{-1}, to HV_1, V_2 and HV_2. By raising the pH above 11.6, the intensity of the band at 870 cm^{-1} tends to diminish whereas the 820 cm^{-1} band gradually increases in intensity (Fig. 2). These observations suggest that V_1 should be correlated with the band at 820 cm^{-1}, rather than with that at 870 cm^{-1}.

In a recent report,[36] a correlation between the bond order of the VO bond (ρ_{VO}) and ν_{VO},

$$\nu_{VO} = 21349 \exp\left[-3.434\,(\rho_{VO})^{-0.2}\right]$$

was derived, where ρ_{VO} is defined by n'/N, N being the number of equivalent VO bonds and n' the total

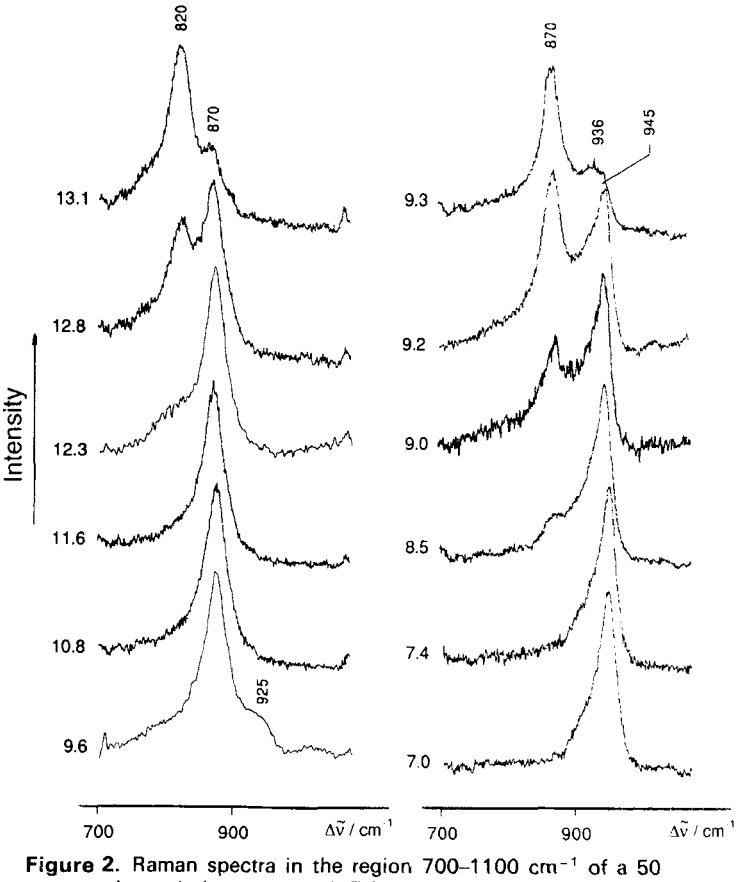

Figure 2. Raman spectra in the region 700–1100 cm^{-1} of a 50 mM vanadate solution at pH 13.1–7.0.

number of electron pairs assigned to them. Table 1 presents the approximate wavenumbers of the stretching mode calculated by the above relationship for several vanadate oligomeric species. This correlation agrees with the previous assignments in stating that the protonation leads to an increase in ρ_{VO}. In addition, the above correlation also agrees with the previously suggested[31] coincidence of $\nu_{VO}(HV_1)$ and $\nu_{VO}(V_2)$, since n'/N has the same value for these species (4/3 and 8/6, respectively).

Figure 3 shows the ^{51}V NMR spectra of a 50 mM vanadate solution at pH 12.3 (a) and 9.2 (b). At pH 12.3, the NMR spectrum presents two peaks centred at 537 and 561 ppm which were ascribed previously to HV_1 and V_2, respectively.[23] Since the Raman spectrum at the same pH value shows a single major band centred at 870 cm^{-1} (the shoulder at 820 cm^{-1} has already been assigned to V_1), it can be concluded that both HV_1 and V_2 contribute to the Raman band at 870 cm^{-1}, as predicted by the above-mentioned ν_{VO} *vs.* ρ_{VO} relationship (see Table 1). The coincidence of ν_{VO} (HV_1) and $\nu_{VO}(V_2)$ is certainly a reason for the persistence of the 870 cm^{-1} band over a wide pH range.

For pH values below 9.2, the most intense Raman band occurs at 945 cm^{-1} (Fig. 2). To the best of our knowledge, no specific assignment of this band to a particular vanadate species has been attempted before. The small number of Raman reports[31,32] ascribe this band to ν_{VO_2} of $(VO_3)_n^{n-}$, without referring it to any specific oligomer.

At pH 9.2, the most intense peaks in the ^{51}V NMR spectrum of the 50 mM vanadate solution [Fig. 3(b)] occur at 578 and 539 ppm and have been assigned to V_{4C} and HV_1, respectively.[23] Thus we find it reasonable to associate V_{4C} with the Raman band at 945 cm^{-1}. However, this band persists over a wide pH region, thus pointing to the possible contribution of other species. According to the results in Table 1, V_{3C}, V_{4C}, V_{5C} and V_{6C} should occur at the same wavenumber, higher than that of HV_1 and V_2. NMR results[23,24] point to the occurrence of a mixture of these oligomers below pH 9.0, excluding V_{3C} as a highly improbable species[23,37].

Table 1. Bond orders (ρ_{VO}) and empirical stretching wavenumbers (ν_{VO}) of vanadate species

Species	Abbreviation	ρ_{VO}	ν_{VO}/cm^{-1}
VO_4^{3-}	V_1	5/4	800
HVO_4^{2-}	HV_1	4/3	834
$H_2VO_4^-$	H_2V_1	3/2	900
$V_2O_7^{4-}$	V_2	8/6	834
$HV_2O_7^{3-}$	HV_2	7/5	861
$V_3O_{10}^{5-}$	V_{3L}	11/8	851
$V_3O_9^{3-}$	V_{3C}	9/6	900
$V_4O_{13}^{6-}$	V_{4L}	14/10	861
$V_4O_{12}^{4-}$	V_{4C}	12/8	900
$V_5O_{16}^{7-}$	V_{5L}	17/12	868
$V_5O_{15}^{5-}$	V_{5C}	15/10	900
$V_6O_{19}^{8-}$	V_{6L}	20/14	872
$V_6O_{18}^{6-}$ [a]	V_{6C}	18/12	900
$V_6O_{17}^{4-}$ [b]	$V_{6C'}$	16/10	937

[a] Cyclic structure.[23]
[b] Bicyclic structure.[24]

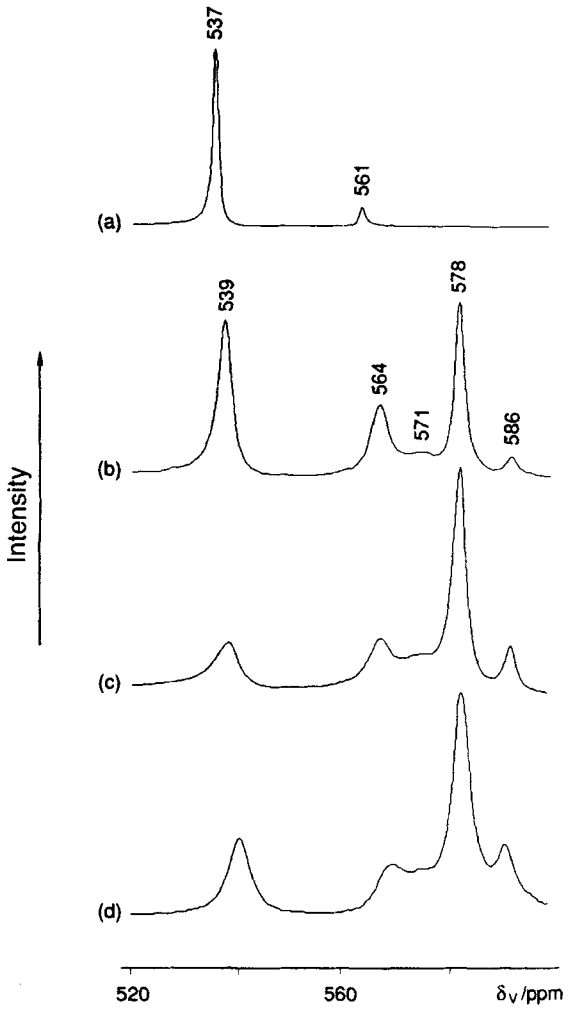

Figure 3. ^{51}V NMR spectra of (a) a 50 mM vanadate solution at pH 12.3, (b) a 50 mM vanadate solution at pH 9.2, (c) a 100 mM vanadate solution at pH 9.2 and (d) a 50 mM vanadate solution, with 0.6 M KCl, at pH 9.2.

In this way, it seems reasonable to correlate the Raman band at 945 cm^{-1} not only with V_{4C} but also with V_{5C} and, maybe, with V_{6C}.

The pH region 9.6–9.2 is the most sensitive over the total pH range analysed. In this pH range, a very broad Raman band is centred at about 936 cm^{-1}. In contrast to the bands discussed above, this band disappears rapidly on decreasing the pH, giving rise to that at 945 cm^{-1}. These observations point to the contribution of more than one species, less stable than HV_1, V_2 and V_{4C}. According to Ref. 37, both V_{3L} and V_{4L} are very unstable, rapidly yielding V_{4C}. Nevertheless, the momentary occurrence of both V_{3L} and V_{4L} should not be discounted, since both precede V_{4C} formation.

The empirical results (Table 1) predict very similar ν_{VO} values for V_{3L}, V_{4L}, V_{5L} and V_{6L}, which fall between those of HV_1, V_2, and V_{4C}. In addition, ν_{VO} for HV_2 coincides with that for V_{4L}. Hence, we tentatively assign the broad Raman band in the pH range 9.6–9.3 to these linear vanadate species (V_{3L}, V_{4L}, V_{5L} and V_{6L}).

The ^{51}V NMR spectrum at pH 9.2 [Fig. 3(b)] provides further support for these assignments. In fact, besides the peaks at 578 and 539 ppm (previously assigned to V_{4C} and HV_1, respectively), it also presents

several smaller features correlated with the less stable species HV_2 (564 ppm), V_{4L} (571 and 586 ppm) and, eventually, V_{5L} (571 ppm) and V_{5C} (586 ppm).[23]

Concentration effect

Figures 4(a) and (b) show the Raman spectra obtained for 50 and 100 mM vanadate solutions, respectively, at pH values ranging from 13.1 to 9.2, in the range 700–1100 cm^{-1}.

At pH 13.1, in the Raman spectrum of the 50 mM vanadate solution, the most intense band occurs at 820 cm^{-1} and was previously assigned to V_1. On the other hand, for the 100 mM solution at the same pH value, the most intense band occurs at 870 cm^{-1}, and was ascribed to HV_1, V_2 and HV_2. A similar trend is observed for pH 12.8. In fact, while two band maxima centred at 820 and 870 cm^{-1} are present in the Raman spectrum of the 50 mM solution, in the spectrum of the 100 mM vanadate solution a single intense band at 870 cm^{-1} occurs [Figs 4(a) and (b)].

At lower pH values, an increase in the vanadate concentration causes a decrease in the Raman intensity at 870 cm^{-1} and an increase in the 945 cm^{-1} band. In particular, at pH 9.2, the bands at 870 and 945 cm^{-1} have similar intensities in the spectrum of the 50 mM vanadate solution, whereas for the 100 mM solution the latter band is more intense.

Overall, these observations support the Raman band assignments proposed here for the various vanadate species, since they agree with the well known effects of concentration on oligomerization, namely that an increase in the total vanadate concentration increases the concentration of the higher oligomers. In addition, this general trend is also supported by the ^{51}V NMR spectra.

In Fig. 3, the ^{51}V NMR spectrum of a 100 mM vanadate solution at pH 9.2 (c) can be compared with the ^{51}V NMR spectrum of a 50 mM vanadate solution at the same pH (b). In fact, Figs 3(b) and (c) show that, at pH 9.3, an increase in total vanadate concentration greatly reduces the intensity of the 539 ppm (assigned to the monomeric species) and 564 ppm (assigned to the dimeric species) peaks, whereas it intensifies the peaks assigned to V_{4C} (578 ppm) and V_{5C} (586 ppm).

Ionic strength effect

Figure 4(c) presents the Raman spectra of a 50 mM vanadate solution with 0.6 mM KCl at pH values ranging from 13.1 to 9.2, in the 700–1100 cm^{-1} region. As observed previously for the variation of total vanadate concentration, the main effect of the ionic strength increase, due to KCl addition, seems to be a shifting of the equilibria towards an increase in the degree of oligomerization. In particular, at pH 12.8 the presence of V_1 is no longer detectable in the Raman spectrum of the KCl–vanadate solution whereas the pure vanadate solution spectrum still reveals a considerable concentration of that species. A similar effect can be observed at pH 9.2. Indeed, at this pH value there is an intensity inversion for the pair of Raman bands at 870 and 945 cm^{-1} with an increase in ionic strength, suggesting an increase in the V_{4C} and, probably, V_{5C} concentrations in relation to the concentrations of HV_1 and V_2.

The ^{51}V NMR results give further support to these general trends. Figure 3(c) presents the ^{51}V NMR spectrum of a 50 mM vanadate solution with 0.6 M KCl at pH 9.2. It is clear that the presence of 0.6 M KCl leads to a decrease in the concentrations of HV_1 and HV_2 species (as shown by the intensity decreace of the 540 and 564 ppm peaks, respectively). Moreover, the peaks at 578 and 583 ppm are intensified, pointing to an increase in the V_{4C} and of V_{5C} concentrations, respectively.

In the very sensitive pH region (9.6–9.2), however, an increase in the ionic strength seems to yield quite the opposite effect. In particular, at pH 9.3, in the absence of KCl, in addition to the Raman band at 870 cm^{-1}, a broad band centred at *ca.* 936 cm^{-1} is observe

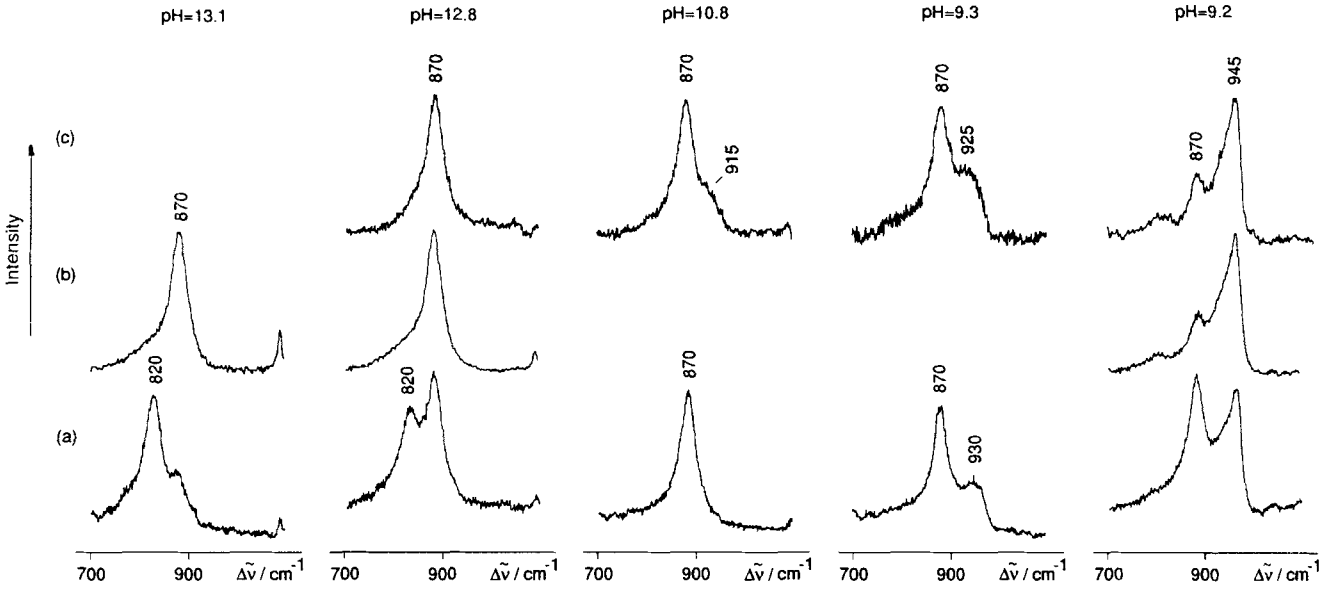

Figure 4. Raman spectra in the region 700–1100 cm^{-1} of (a) a 50 mM vanadate solution, (b) a 100 mM vanadate solution and (c) a 50 mM vanadate solution with 0.6 M KCl, at pH 13.1–7.9.

[Fig. 4(a)]. Addition of KCl shifts the centre of that broad band down to *ca.* 925 cm^{-1} [Fig. 4(c)]. Hence, in this pH region, an ionic strength increase seems to stabilize the linear intermediate species with higher negative charges per vanadium atom ($V_{3L} > V_{4L} > V_{5L}$).

CONCLUSIONS

Important conclusions can be drawn from this combined Raman ^{51}V NMR spectroscopic study. In particular, it has been shown that in a 50 mM vanadate solution at pH 13.1 the main species is still V_1, in contrast to other studies.[31,33] In addition, although the predominant species in a solution with that same concentration at pH 12.8 are HV_1 and, probably V_2, V_1 still occurs in a considerable amount. By decreasing the pH, several higher oligomers are formed in particular, the very stable form V_{4C}. However, in the pH region 9.6–9.2 some evidence has been found for the occurrence of less stable species, e.g. V_{3L}, V_{4L} and, maybe, V_{5L}.

It has been shown that the oligomerization equilibria are also significantly affected by the ionic strength. Like the concentration increase, the ionic strength also favours the formation of higher oligomers. However, in the pH range 9.6–9.2, an increase in ionic strength seems to stabilize the species with a higher negative charge per vanadium atom. These findings have important consequences for biochemical and biological studies whenever a particular vanadate oligomer may be desired and high ionic strengths are used.

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