Article

Model Compounds of Humic Acid and Oxovanadium Cations.
Potentiometric Titration and EPR Spectroscopy Studies

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As constantes de estabilidade e parâmetros isotrópicos de EPR (A_o e g_o) foram obtidos por titulações potenciométricas e espectroscopia de EPR, empregando-se soluções aquosas, 15% v/v etanol, de compostos modelo de ácido húmico - ácido salicílico (SALA) - e do artefato de laboratório, ácido nitrohúmico - ácidos 3-nitrossalicílico (3-NSA), 5-nitrossalicílico (5-NSA) e 3,5-dinitros-salicílico - e cátions oxovanádio.

Espectros de EPR foram obtidos de soluções dos compostos modelo e o íon VO^{2+} (V(IV)), e as constantes de estabilidade foram obtidas com solução de VO^{3+} (V(V)), sendo que os valores obtidos são 12.77 ± 0.04 até 7.06 ± 0.05 para as espécies ML, e 9.90 ± 0.04 até 4.06 ± 0.05 para as espécies $ML2$, de acordo com a diminuição na acidez dos grupos carboxílico e fenólico com a introdução dos substituintes nitro- no anel aromático dos compostos modelo estudados.

Os parâmetros de EPR demonstraram que os valores de g_o aumentam e os valores de A_o diminuem concomitante ao aumento dos logaritmos das constantes de formação globais encontrados para os complexos.

The stability constants and the isotropic EPR parameters A_o (hyperfine splitting constant) and g_o (g value) were obtained by potentiometric titrations and EPR spectroscopy, respectively, of 85% v/v aqueous solutions of model compounds of humic acids - salicylic acid (SALA) - and both nitrohumic acids, a laboratory artifact - nitrosalicylic acids, 3-nitrosalicylic acid (3-NSA), 5-nitrosalicylic acid (5-NSA) and 3,5-dinitrosalicylic acid (3,5-DNSA) and oxovanadium cations.

It was possible to record EPR spectra of those model compounds and the ion VO^{2+} (V(IV)), and the stability constants were obtained from a solution of VO^{3+} (V(V)), the values for the logarithms of the stability constants ranging from 12.77 ± 0.04 to 7.06 ± 0.05 for the species ML, and from 9.90 ± 0.04 to 4.06 ± 0.05 for the species ML_2 according to the decrease in the acidity of the carboxylic and the hydroxyl groups in the aromatic ring of the model compounds studied as the $-NO_2$ substituents were added. Species distribution diagrams were also obtained for the equilibria studied.

The EPR parameters showed that as the logarithm of the overall stability constants increase, g_o values also increase, while A_o values show a tendency to decrease.

Keywords: *humic acid, model compounds, equilibrium constants, oxovanadium cations*

Introduction

The importance of vanadium in biological systems[1] is imparted mainly by the rich oxygen chemistry, being VO^{2+} and VO_2^+ examples of oxocations being associated as important catalysts exerting defense and protection functions and being also a cofactor of nitrogenase in the fixation of dinitrogen. As a micronutrient in living organisms, vanadium is important to prevent growth retardation and skeletal deformations in animals, and possibly participates in regulation of enzymatic processes. Further some complexes of V(IV) and V(V) have shown insulin-mimetic actions[2-4].

Humic acids present heterogeneous compositions that depend on the chemistry of how they were generated. Thus to unambiguously identify their structures is a difficult task. However it is known[5] that within these geopolymers the main chelating groups are catechol, salicylate and phthalate

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derivatives which are distributed throught the humic acid structure.

The cation exchange capacity - CEC - which regulates the availability of metal ions in the soil, is dependent upon variations of pH values. These cation exchange reactions involve the labile protons on soil humus and are directly related to the functional groups present, such as carboxyl, carbonyl, amino, imidazole, phenolic and alcoholic hydroxyl and sulfhydryl groups[6] as represented generally in Eq. 1:

$$SH_{2(s)} + M^{2+}_{(aq)} \rightleftharpoons SM_{(s)} + 2H^+ \qquad (1)$$

Studies involving VO(IV) and coal nitrohumic acid[7] showed how this cation is important as a probe in EPR studies as well as unravelled the sites of complexation, which turned out to be through o-hydroxycarboxylic aromatic (salicylic) basic sites as equatorial ligands of VO^{2+} oxocation. A study[8] of the EPR parameters in natural and synthetic complexes of vanadyl ion - humic acids also revealed participation of the salycilate basic sites in the complex. Further reported studies employing potentiometric, spectrophotometric titrations[9-10] and EPR Spectroscopy[9-11] have investigated the complex formation of deferoxamine B and VO^{2+} and VO_2^+ oxocations[9], the complex formation of VO^{2+} and citric and tartaric acids[10] and the biosorption of VO^{2+} by the roots of some plants[11].

The present study involved potentiometric titration and EPR techniques in order to measure the interaction between salicylic basic sites present in humic acid and nitrosalicylic basic sites present in nitrohumic acids, the latter, a laboratory artifact[7], and oxovanadium cations by determining their stability constants. The obtained EPR parameters were compared with the stability constants in order to determine if there was a correlation.

The model compounds chosen are salicylic acid for humic acids and nitrosalicylic acids for nitrohumic acids (for the structures refer to Ref. 12).

Materials and Methods

All chemicals were used without further purification. KCl (J.T.Baker - USA) was the supporting electrolyte. Aqueous 85%v/v:15%v/v ethanolic (Merck - Brasil) solutions (0.1 mol L^{-1}) of the ligands salicylic acid (SALA - Reagen - Brasil), 3-nitrosalicylic acid (3-NSA), 5-nitrosalicylic acid (5-NSA) and 3,5-dinitrosalicylic acid (3,5-DNSA), TCI - Japan, and 0.1 mol L^{-1} KOH (Merck - Brasil) were prepared in double distilled and deionized carbonate - free water. The KOH solutions were standardized against potassium acid phthalate. The vanadyl solutions were prepared in two different ways as follows. In the first one, a proper mass of the VOSO$_4$.nH$_2$O (0.1 mol L^{-1} - Aldrich - USA) was dissolved in an aqueous 0.36 mol L^{-1} H$_2$SO$_4$ (Reagen - Brasil) solution (VO^{2+}). In the second one a mass of VOSO$_4$.nH$_2$O (0.1 mol L^{-1} - Aldrich - USA) was dis-

solved in an aqueous 0.4 mol L^{-1} HNO$_3$ (Merck - Brasil) solution (VO^{3+}). The H$^+$ content was measured by Gran's Plot[13] and the vanadyl ion by titration with KMnO$_4$[14].
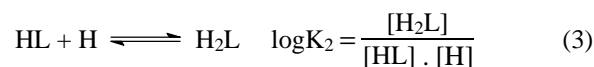
Potentiometric equilibrium measurements

Following the procedures described in the literature[12,13-15] all the possible errors arising in calculating unknown equilibrium constants by least-square techniques were minimized. Three titrations were performed with the metal ion and each ligand. All the potentiometric studies were carried out in a Micronal (Brasil) model B-375 research pHmeter fitted with blue glass and calomel reference electrodes calibrated with standard HCl 10^{-2} mol L^{-1} (μ = 0.100 mol L^{-1} (KCl)) and KOH aqueous 85%v/v: 15%v/v ethanolic solutions to read -log [H$^+$](p[H]) directly, under a stream of purified N$_2$ (White-Martins - Brasil). The temperature was maintained at 25.0 $\pm$ 0.1 °C and the ionic strenght adjusted to 0.100 mol L^{-1} by the proper addition of KCl.

All solutions were titrated with 0.1 mol L^{-1} standard CO$_2$-free KOH solutions in duplicate. The p[H] range was from 2.0 to 11.0. In the callibration step of the pHmeter the p[H] reproducibility is < 0.005 units of p[H] at acidi region and < 0.015 at basic p[H] region.

Computations

The protonation constants of the ligands employed were reported earlier[12] according to Eqs. 2 and 3. The hydrolysis constants of vanadyl ion were taken from the literature[16]. The species considered in the calculations were M:L (being M = VO^{2+} or VO^{3+} and L = sacilicylate derivatives) 1:1, 1:2; 2:1; 2:2 and their protonated ones.

$$L + H \rightleftharpoons HL \qquad \log K_1 = \frac{[HL]}{[L].[H]} \qquad (2)$$

$$HL + H \rightleftharpoons H_2L \qquad \log K_2 = \frac{[H_2L]}{[HL].[H]} \qquad (3)$$

The calculations employed the microcomputer program Best7[13] and the species distribution diagrams were calculated with the microcomputer program SPE[13]. The Best7 program calculation begins with the set of known and unknown (estimated) overall stability constants (Eq. 4) and computes [H$^+$] at the equilibrium for each quantity of added base. For each equilibrium point, the fitting process consists in the minimization of the differences between the observed and the calculated p[H] values by using a weighted least square method. The iterative process is repeated until no further minimization is obtained. All other mathematical aspects are described elsewhere[12-13,15].

$$M + nL \rightleftharpoons ML_n \qquad \log \beta_n = \frac{[ML_n]}{[M].[L]^n} \qquad (4)$$
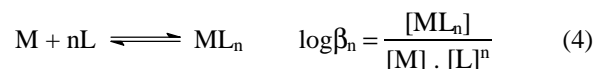
EPR spectra

A Bruker ESP 300E spectrometer was used to acquire the spectra of the oxovanadium complexes in aqueous solutions in the ratio metal to ligand 1:1 to the nitrosalicylic acids and in the ratio 1:1 and 1:2 (M:L) for salicylic acid and VO^{2+} at room temperature (300 K) in thin quartz tubes operating at a frequency of $\cong 9.5$GHz (X-band) with a 100KHz modulation frequency. The p[H] of the solutions was controlled with aqueous solution of KOH (0.001 mol L^{-1}) trying to sample the maximum possible concentration of each formed species according to the distribution diagrams for each studied solution (refer to Figs. 5 to 8).

Results and Discussion

The first attempt to measure overall stability constants using potentiometric titrations was made with $VOSO_4$ in H_2SO_4 solution. The results have shown that due to the competition of the SO_4^{2-} ion in the equilibrium, only the first complexed species (ML) could be detected, before the whole system precipitated. These solutions however were investigated by EPR spectrometer in order to measure the A_o and g_o parameters. The results are presented in Table 1.

In order to delay the precipitation of this system, a solution of $VOSO_4$ in HNO_3 was investigated. However, in the presence of this acid, V(IV) would invariably be oxidised to V(V), an EPR silent valence. For the potentiometric titrations it turned out to be very useful, rendering not only values for the stability constants of ML, which were in perfect agreement with those obtained with the H_2SO_4 solution, but also values for MHL for some ligands, and ML_2 for all four ligands studied (Table 2).

The potentiometric equilibrium curves are depicted in Figures 1 for 4 of 0.1 mmole of SALA, 3-NSA, 5-NSA and 3,5-DNSA with 0.1 and 0.05 mmole of VO^{3+}, respectively.

In Fig. 1, both ratios present buffers until approximately p[H] 6.0, after which the formation of insoluble products prevented further acquisition of data. In Fig. 2 the buffer for the ratio 1:1 approached p[H] 4.9 and for the ratio M:L of 1:2, approached p[H] 3.1 with an inflection until p[H] 5.5, after which formation of insoluble products occurred. In Fig. 3 the buffering action was observed for the ratio 1:1 until precipitation began to occur at approximately p[H] 5.0. The ratio 1:2, besides acting as a buffer until approximately p[H] 4.0, presents an infection until near p[H] 8.2 after which further titration was prevented due to precipitation of products. Fig, 4 reveals the presence of a buffer for the ratio 1:1 until near p[H] 4.3 and the 1:2 ratio acts as a buffer until approximately p[H] 3.8 thereafter an inflection being observed until p[H] 6.2 after which precipitation occurred.

The calculated stability constants provided the diagrams for the species distribution (Figs. 5 to 8 for 2.5×10^{-3} mol L^{-1} of SALA and 1.25×10^{-3} mol L^{-1} for VO^{3+}, 3-NSA - VO^{3+}, 5-NSA - VO^{3+}, and 3,5-DNSA - VO^{3+}, respectively). The formation of ML starts at p[H] $\cong 3.0$ reaching its maximum formation near p[H] 5.3, disappearing at p[H] $\cong 9.0$; and ML_2 starts at p[H] $\cong 6.0$ reaching its maximum at p[H] $\cong 10.0$. For 3-NSA - VO^{3+} (Fig. 6) ML starts at p[H] $\cong 2.8$, reaching its maximum at p[H] 4.8, disappearing at p[H] $\cong 9.0$; ML_2 starts at p[H] $\cong 5.0$, reaching its maximum at 9.9. In Fig. 7 the formation of ML starts and ends around p[H] values of 3.0 and 9.0 respectively, reaching a maximum at p[H] $\cong 5.5$; and ML_2 starts at 6.5 and reaching its maximum at 10.4. Fig. 8 shows the formation of ML starting and ending at p[H] $\cong 3.0$ and 9.0 respectively, reaching its maximum at 4.8. MHL is at its maximum at p[H] 2.0, disappearing around p[H] 4.0. ML_2 starts at p[H] $\cong 5.0$, reaches its maximum at 8.2, disappearing after p[H] 10.0.

A closer look at the region of p[H] near 7.0, which besides being the phisiological p[H] is also the required value for a healthy soil and natural waters, the complexes species observed in this work (ML and ML_2) are present in
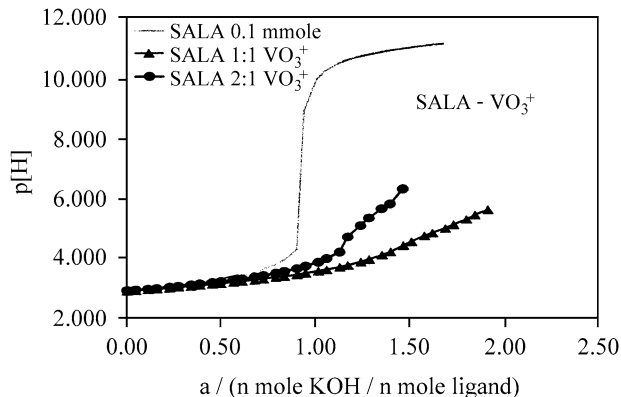
Table 1. EPR isotropic parameters for oxovanadium (IV) salicylate derivative complexes in aqueous solutions.

Ligand	M:L	g_o	A_o (Gauss)	$\log \beta$
3, 5-DNSA	1:1	1.9636	108	7.06
5-NSA	1:1	1.9654	108	9.00
3-NSA	1:1	1.9666	108	9.68
SALA	1:1	1.9685	107	12.77
SALA	1:2	1.9696	100	22.67

Table 2. Logarithms of the equilibrium constants of salicylate derivatives and VO^{3+} complexes (T = 25 °C; μ = 0.100 mol L^{-1} (KCl)).

$\log K$	SALA-VO^{3+}	3-NSA-VO^{3+}	5-NSA-VO^{3+}	3, 5-DNSA-VO^{3+}
[ML]/[M][L]	12.77[a]	9.68[b]	9.00[a]	7.06[c]
[MHL]/[ML][H]	n.d.	3.37[b]	n.d.	3.37[c]
[ML_2]/[ML][L]	9.90[a]	5.02[b]	5.64[a]	4.06[c]

[a]standard deviation = 0.04.
[b]standard deviation = 0.06.
[c]standard deviation = 0.05.
n.d = not detected.

Figure 1. Potentiometric p[H] profiles of 40 mL of SALA (0.01 mol L^{-1}; μ = 0.100 mol L^{-1} (KCl)) and the oxocation VO^{3+} (titrant KOH, 0.1 mol L^{-1}). (The ligand alone, and the ratios metal to ligand 1:1 and 1:2 have respectively 31, 35 and 27 points).
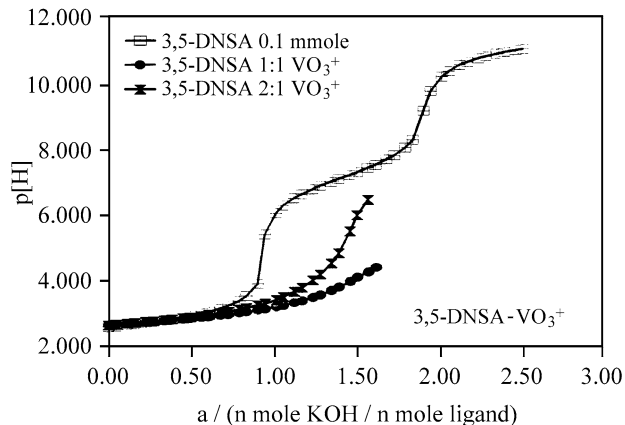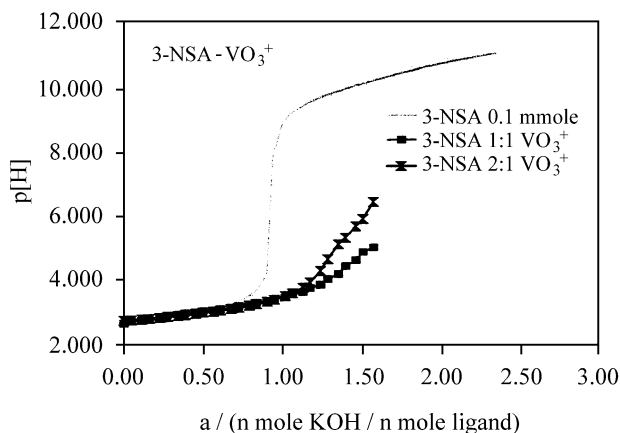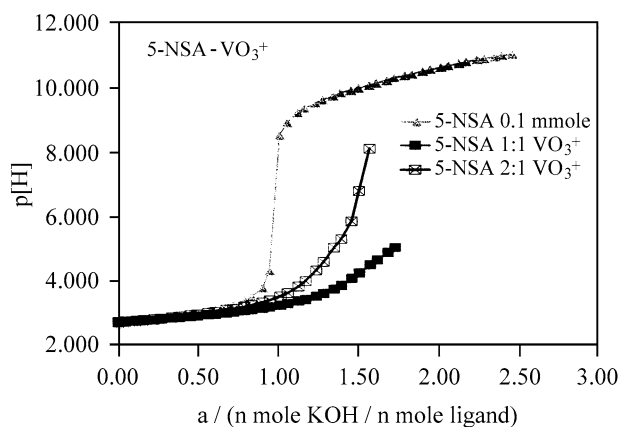

Figure 2. Potentiometric p[H] profiles of 40mL of 3-NSA (0.01 mol L^{-1}; μ = 0.100 mol L^{-1} (KCl)) and the oxocation VO^{3+}(titrant KOH, 0.1 mol L^{-1}). (The ligand alone, and the ratios metal to ligand 1:1 and 1:2 have respectively 43, 24 and 28 points).

Figure 3. Potentiometric p[H] profiles of 40mL of 5-NSA (0.01 mol L^{-1}; μ = 0.100 mol L^{-1} (KCl)) and the oxocation VO^{3+} (titrant KOH, 0.1 mol L^{-1}). (The ligand alone, and the ratios metal to ligand 1:1 and 1:2 have respectively 45, 32 and 29 points).
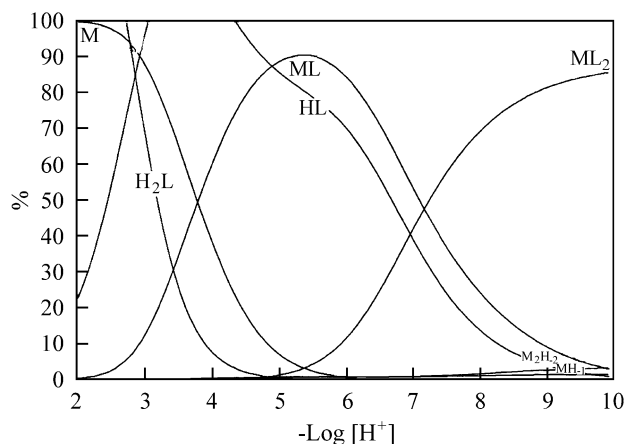

Figure 4. Potentiometric p[H] profiles of 3,5-DNSA (0.01 mol L^{-1}; μ = 0.100 mol L^{-1} (KCl)) and the oxocation VO^{3+} (titrant KOH, 0.1 mol L^{-1}). (The ligand alone, and the ratios metal to ligand 1:1 and 1:2 have respectively 46, 30 and 29 points).
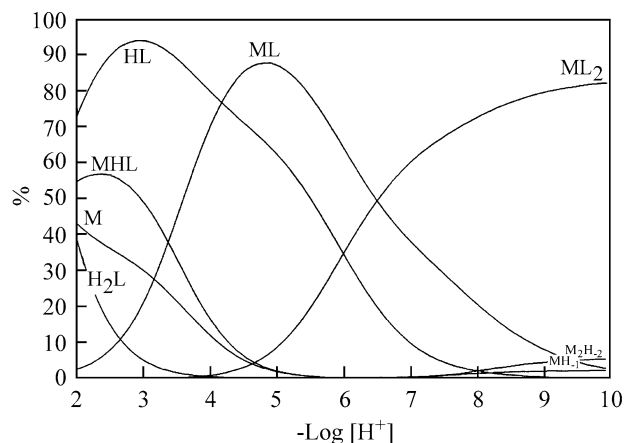

Figure 5. Species distribution diagram from p[H] 2.0 to 10.0 of a 2.5 x 10^{-3} mol L^{-1} solution of SALA - 1.25 x 10^{-3} mol L^{-1} VO^{3+}, with metal ion set at 100%. H$_{-x}$ represents (OH)$_x$.

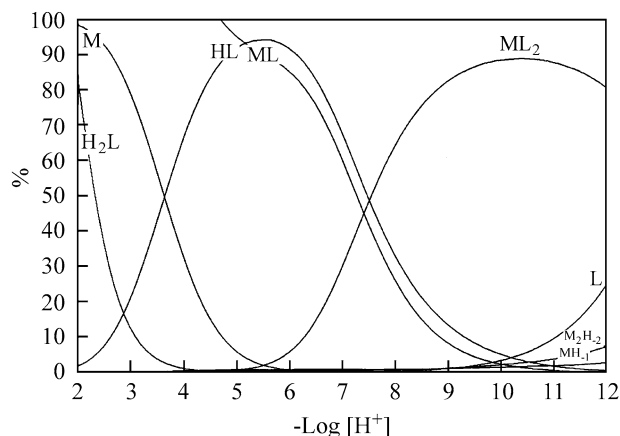**Figure 6.** Species distribution diagram from p[H] 2.0 to 10.0 of a 2.5 x 10^{-3} mol L^{-1} solution of 3-NSA - 1.25 x 10^{-3} mol L^{-1} VO^{3+}, with metal ion set at 100%. H$_{-x}$ represents (OH)$_x$.

Figure 7. Species distribution diagram from p[H] 2.0 to 12.0 of a 2.5 x 10^{-3} mol L^{-1} solution of 5-NSA - 1.25 x 10^{-3} mol L^{-1} VO^{3+}, with metal ion set at 100%. H_{-x} represents $(OH)_x$.

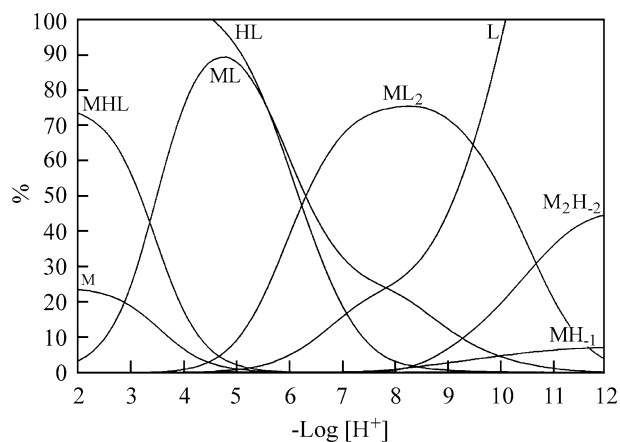**Figure 8.** Species distribution diagram from p[H] 2.0 to 12.0 of a 2.5 x 10^{-3} mol L^{-1} solution of 3,5-DNSA - 1.25 x 10^{-3} mol L^{-1} VO^{3+}, with metal ion set at 100%. H_{-x} represents $(OH)_x$.

significant quantities. The nitro- group in the ligands results in a shift of the ML_2 species has brought the species to slightly more acid values in comparison to the SALA ligand, the exception being 5-NSA.

Recent works[12,17] have shown that due to the more acidic behaviour of nitrosalicylic acids, a more pronounced difference in the distribution of species along with the variation of the p[H] values compared to salicylic acid would be expected, especially the formation of at least one complexed species at lower p[H] values for the nitrosalicylic acids than the salicylic acid. However, in the case of VO^{3+} this effect did not seem to be playing a major role, as this expected difference was not that remarkable.

The EPR parameters for the VO^{2+} complexes in aqueous solutions (Table 1) indicate the presence of oxygen donors in the equatorial plane of C_{4v} symmetry[7-8]. The complex of M:L ratio of 1:1 still bears two (or three in the case of MHL) molecules of water in the four equatorial

coordination sites. The complex M:L ratio of 1:2 for its turn, is through 4 oxygen atoms of the two basic sites of two salicylic type molecules, the $-COO^-$ and phenolic $-O^-$. Looking at the EPR isotropic parameters, g_o and A_o and comparing to the logarithm of the overall stability constants (β) taken as a measure of the ligand field force, it is seen that they follow a regular pattern according to a relation among those three parameters stated in the literature[18]. As log β values increase, the g_o values increase while A_o values show a tendency to decrease. This pattern may be accounted for the lower spin polarization of inner s electrons of vanadium atom as the ligand field becomes stronger.

Looking at the values of the stability constants in Table 2, the more basic ligand, SALA, presents the higher values for the stability constants of both ML and ML_2 species, followed by 3-NSA, 5-NSA and 3,5-DNSA. The latter is the less basic of the four, due to having two nitro- substituents.

The detection of protonated species in the ratio 1:1 (MHL) (Table 2) for only 3-NSA and 3,5-DNSA and VO^{3+}, the phenolic -OH group being protonated in the salicylic ring, leaving only the carboxyl group to complex, is probably because of steric effects imparted by the nitro substituent group in the C-3 position, present in both 3-NSA and 3,5-DNSA ligands. Also this protonated -OH may be hydrogen bridging the $-NO_2$ group in the vicinal position, forming a six membered ring with some stability. As the p[H] value increases, this stabilization effect disappears, leaving room for complexation through both $-COO^-$ and the phenolic $-O^-$ of one or two salicylic derivative molecules.

Conclusion

Although the molecules chosen as models are not sophisticated ones and do not to reproduce the inherently complex properties a geopolymer might have, as any model would[19], they have provided information concerning the aqueous equilibria for humic acid models for nitrosalicylic acids - oxovanadium complexes, as well as have shown the possibility of studying them by EPR spectroscopy in aqueous solutions, opening new perspectives in using them as slow-release fertilizers.

Acknowlegdments

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