

## Molybdenum (VI) Binded to Humic and Nitrohumic Acid Models in Aqueous Solutions. Salicylic, 3-Nitrosalicylic, 5-Nitrosalicylic and 3,5 Dinitrosalicylic Acids, Part 2

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Apresentam-se neste trabalho, estudos eletroquímicos e espectroscópicos na região do Ultravioleta-Visível de modelos derivados do ácido salicílico de ácidos húmicos e nitrohumicos, esse último um artefato laboratorial, na presença de molibdênio para determinar a afinidade desses modelos com esse íon metálico. Molibdênio tem um papel importante na química de solo e conjuntamente com as substâncias húmicas, promove fertilidade ao solo e água e é um elemento chave na enzima nitrogenase. Os resultados obtidos mostraram que pelo menos uma espécie complexada está presente na faixa de pH 6,3 a 8,0, mesmo para o modelo menos básico escolhido, dos ácidos nitrossalicílicos. Estudo anterior mostrou que modelos derivados do ácido ftálico e nitroftálico, modelos também para os ácidos húmicos e nitrohumicos, apresentaram espécies complexadas apenas até pH 6,5. As constantes de formação calculadas mostraram que a substituição do grupamento nitro na posição *orto* desfavorece a complexação, quando comparada à substituição *para*, devido provavelmente ao impedimento estérico no primeiro caso, uma vez que esse impedimento se manifesta indubitavelmente no composto duplamente substituído com o grupamento nitro. Os espectros obtidos por voltametria cíclica e por espectroscopia no Ultravioleta-Visível mostraram que a química do molibdênio em solução aquosa é muito complexa com a variação do pH, e que o ânion molibdato deixa de ser um ânion em valores de pH próximos a 4, quando então passa a se comportar como cátion,  $\text{MoO}_2^{2+}$  (M).

In this work electrochemical and Ultraviolet-Visible studies were performed in solutions of salicylic acid models of humic and nitrohumic acids, a laboratory artifact, and molybdenum in order to determine the affinity of these models towards the metal ion. Molybdenum, which plays a very important role in the soil chemistry, and together with humic substances, impart fertility to soil and water and is a key element in the activity of nitrogenase. The obtained results showed that at least one complexed species is present at the pH range of 6.3 to 8.0, even for the less basic chosen models, the nitrosalicylic acids. Previous study showed that phthalic and nitrophthalic, also humic and nitrohumic acids model compounds, presented complexed species with molybdenum only till pH 6.5. The calculated formation constants showed that the substitution of the nitro group in the *ortho* position was less favoured than in the *para* substitution, probably due to a steric hindrance in the former, which was clearly seen in the double substituted salicylic nitro derivative. The cyclic voltammetry as well as the Ultraviolet-Visible obtained spectra were able to show that the chemistry of molybdenum in aqueous solutions as the pH is increased is very complex, and the molybdate stops acting as an anion only after pH around 4, when it finally becomes a cation  $\text{MoO}_2^{2+}$  (M).

**Keywords:** humic acid models, molybdenum (VI), potentiometric titration, stability constants, slow release fertilizer, electrochemistry, complexation, UV-Vis

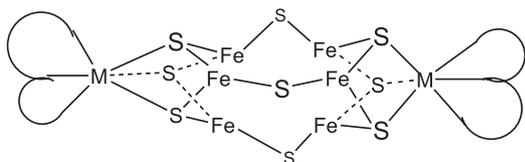
### Introduction

Chemistry of soil molybdenum (Mo) plays an important role in reactions where the nitrogenase enzyme is involved.

Thus, each Mo acts as a separate catalytic center and is a significant redox enzyme essential for microorganisms, plants and animals. At the centre of the nitrogenase enzyme is the FeMo cofactor, where the actual reduction of dinitrogen takes place. The Mo-Fe-S is biologically important in nitrogen fixation catalysed by nitrogenases.

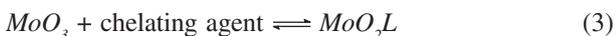
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One of its component is the Mo-Fe protein which contains a unique Fe-Mo-S cluster, which is believed to be the active site for the dinitrogen reduction. Figure 1 presents a structure of this nitrogenase FeMo cofactor where after core rearrangement there is the formation of two isomers ( $\text{Mo}_2\text{Fe}_6\text{S}_9$ ), having Mo or Fe as the metal ion.<sup>1</sup>

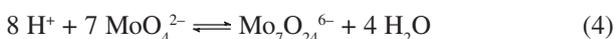


**Figure 1.** A proposed structure of a model of nitrogenase's cofactor.

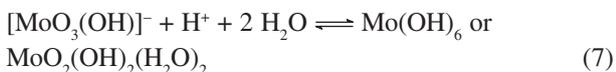
The molybdate anion undergoes certain reactions where there is the breakage of  $\text{M}=\text{O}$  bond. Some examples of such reactions are presented by the following equations:<sup>2</sup>



The dominant aqueous Mo species will depend on the equilibria as well as on the pH values. The anion  $\text{MoO}_4^{2-}$  undergoes the formation of many different polyanions in acidic solutions, particularly at pH 4.1 where there are the existence of both *para*  $\text{Mo}_7\text{O}_{24}^{6-}$  molybdate and *octa*  $\text{Mo}_8\text{O}_{26}^{4-}$  molybdate anions. The formation of polyanions may be represented as in equations 4 and 5:<sup>3,4</sup>



When a dilute solution of  $\text{MoO}_4^{2-}$  ( $< 10^{-4}$  mol  $\text{L}^{-1}$ ) is acidified it yields  $\text{HMoO}_4^-$  and  $\text{H}_2\text{MoO}_4$ . The tetrahedral ion  $\text{MoO}_4^{2-}$  transforms into an octahedral species at the second stage of protonation according to equations 6 and 7:<sup>3</sup>



At pH values higher than 4 the most important species is  $\text{MoO}_2^{2+}$ , when molybdenum is chemically acting as a cation according to previous equations 3 and 7.<sup>3,5</sup>

Soil organic matter (OM) promotes soil aggregation and its chemical function is recognized by its ability to interact

with metals, oxides, metal hydroxides and mineral clays to form organometallic complexes. Soil OM improves N, P, and S bioavailability, therefore it has the potential use as a slow release fertilizer. The OM is very heterogeneous and it can be functionally grouped into nonhumic (carbohydrate, proteins, fats, waxes, etc) and humic structures.<sup>6</sup>

It is widely stated in the literature that soil humic acid (HA) composition is dependent on soil composition and organic precursors, resulting in differences in HA molecular mass and chemical structures. Consequently metal binding will vary with HA sources.<sup>7-13</sup>

Soil fertility plays a role by improving metal ion mobility and transport in the environment.<sup>11-12,14-16</sup> Cation binding to humic substances include the extreme binding heterogeneity of these natural materials, the variable stoichiometry of binding, the competition between specifically-bound ions, especially protons and metal ions, and electrostatic effects which give rise to ionic strength effects and the non specific binding of couterions.<sup>17</sup> All the mathematical models presented in the literature so far<sup>18-25</sup> cannot deal with all those parameters, sometimes employing empirical equations and bacterial and HA competition. Also the complexation of metals by heterogeneous ligands is dependent on metal loading (the rate of bound metal to the binding site concentration). To overcome some of these difficulties this study deals with some molecular models of HAs according to the sorption sites following others in the literature<sup>5,26-28</sup> presenting data on those HAs main sorption sites that can reliably be used in those mathematical models as well as the speciation along a wide range of pH values.

Although higher valence cations such as molybdenum ions can undergo either hydrolysis or ligand binding, potentiometric titrations can provide the conditional stability constants as a way to try to understand how biological systems binds so selectively to ligands or metal ions. The study of model compounds has to be in such a way that they represent properly the known physical and chemical properties of the basic sites of the complex structure of the substance under investigation. Such is the case for humic and nitrohumic acids, geopolymers mainly composed of phenolic hydroxyl groups, hydroxycarboxylic and carboxylic aromatic groups as basic sites.<sup>7,9</sup>

Therefore, in this work the  $\text{MoO}_4^{2-}$  anion was chosen to be studied with the humic and nitrohumic acid models - salicylic and nitro-salicylic acids. The nitrohumic acids are a laboratory artifact. Although the high oxidation species of molybdenum (such as in molybdate) is not the only one involved in the biological reactions, it is water soluble and undergoes many interconvertible reactions (refer to equations 1 to 7). It is possible to determine how the pH values changes can act on the equilibrium of all species. In order to determine

all equilibrium possibilities, it was performed potentiometric, cyclic voltammetric (CV) and ultraviolet-visible (UV-Vis) titrations. A previous work reports a study with this same metal ion with models of phthalic, nitro-phthalic, catechol and nitro-catechol humic acids-like sorption or basic sites.<sup>5</sup>

## Experimental

### Materials

All chemicals were used without further purification. The supporting electrolyte KCl (J.T.Baker - USA) was used to maintain ionic strength (I) at 0.100 mol L<sup>-1</sup>. Aqueous 95% (v/v): 5% (v/v) ethanolic (Merck - Brazil) solutions (approximately 0.1 mol L<sup>-1</sup>) of the ligands salicylic acid (SALA - Reagen - Brazil), 3-nitrosalicylic acid, 5-nitrosalicylic acid and 3,5-dinitrosalicylic acid (3-NSA, 5-NSA and 3,5-DNSA, respectively, TCI - Japan), and 0.1 mol L<sup>-1</sup> KOH (Merck - Brazil) were prepared in double distilled and deionized, carbonate - free water. The KOH solutions were standardized against potassium acid phthalate.

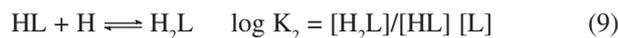
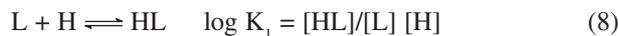
A proper mass of NH<sub>4</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (Carlo Erba - Italy) was dissolved in water and its content was measured by back titration with disodium edetate (Carlo Erba - Italy) following the literature.<sup>29</sup>

### Potentiometric equilibrium measurements

Following the procedures described in the literature<sup>30</sup> the errors arising in calculating unknown equilibrium constants by least-square techniques were minimized. Three titrations were performed with the metal ion and each ligand for all metal to ligand ratios analysed. The potentiometric studies were carried out in a Micronal (model B-375 - Brazil) pH meter fitted with a glass and a calomel reference electrode calibrated with standard HCl, 10<sup>-2</sup> mol L<sup>-1</sup> (I= 0.100 mol L<sup>-1</sup> (KCl)) and KOH solutions to read -log of the concentration of H<sup>+</sup> (pH) directly, under a KOH - aqueous saturated stream of N<sub>2</sub> (White-Martins - Brazil) and recording the pH values only after stabilization. The pH reproducibility was <0.005 pH at acidic pH region and <0.015 pH at the basic pH region. The temperature was maintained at 25.0±0.1 °C (Microquímica - MQBCT - 99-20, Brazil) and the ionic strength adjusted to 0.100 mol L<sup>-1</sup> with KCl.

### Calculations

The protonation constants of the ligands employed were reported previously<sup>26,27</sup> according to equations 8 and 9 with charges omitted for simplicity.



The hydrolysis constants of molybdenum (VI) were taken from the literature.<sup>31,32</sup> The species considered in the calculations were metal to ligand ratios (metal being MoO<sub>2</sub><sup>2+</sup>, hereafter represented as M, and ligand = salicylate derivatives, hereafter represented as L) of 1:1, 1:2; 2:1; 2:2; 1:3; 2:4 and their protonated counterparts as well as any possible μ-oxo compounds.

The calculations were made with Best7 program and species distributions were calculated with SPE program.<sup>5,30</sup>

Aware of the region where the uptaking of protons by molybdate is seen, only when MoO<sub>2</sub><sup>2+</sup> cations were present the calculations began. This initial portion of the pH profiles produced no complexed species rather than polyanions, under the experimental conditions employed. The mathematical model for the calculations took into account the possible rates of bound metal to the binding site concentration (the metal loading parameter)<sup>5</sup> in order to simulate an heterogeneous ligand such as humic and nitrohumic substances, the hydrolysis constants of molybdenum ions<sup>31,32</sup> and the standardized concentrations of KOH - the titrant employed - and the concentration of the mineral acid (HNO<sub>3</sub>) added when necessary.

### Cyclic voltammetry

The cyclic voltammetric measurements were obtained with a cyclic voltammeter (Bioanalytical Systems Inc. - USA, model CV-27). A three electrode system was utilized: vitreous carbon as the working electrode, saturated Ag/AgCl-KCl as the reference and a platinum wire, the auxiliary electrode. The voltammograms were obtained in aqueous solutions, at room temperature of 25 °C, in ionic strength of 0.10 mol L<sup>-1</sup> (NaNO<sub>3</sub>), and bubbled with ultra pure argon at pH range of 2.5 to 10.0. The initial solutions were acidified with HNO<sub>3</sub> (0.1 mol L<sup>-1</sup>) and titrated with KOH 0.1 mol L<sup>-1</sup> delivered by a microburet (Gilmont - USA) until the desired pH was reached. The cyclic voltammograms were obtained as a function of each pH value measured with a 5 mm diameter combined glass and Ag/AgCl reference electrode (Analyser - Brazil) and a Corning pH meter (UK), at an accuracy of 0.01 pH units. The final concentration in the reaction cell for all solutions was 10<sup>-4</sup> mol L<sup>-1</sup> and the ligand to metal ratios were 1:1, 2:1 and 3:1. The experimental conditions of the cyclic voltammogram were as follows. The optimum range of swept potential was from 0.0 V to -1.1 V. All scans were made from -1.1 to 1.0 V; the scan-rate = 10 mV s<sup>-1</sup>; and initial potential applied = 0.0 V.

### Ultraviolet-Visible spectra

Separated spectrophotometric titrations were carried out with the ligands alone and in the presence of the molybdate starting solution. By following the pH values where there is the presence of complexed species, aliquots were taken and analysed by UV-Vis spectroscopy and it was possible to monitor the formation and decomposition of species in the equilibria studied.

The UV-Vis spectra were recorded on a Hewlett-Packard model 8450-A diode array spectrophotometer (USA) in the range of 190-600 nm. Aliquots of about 3.0 mL of a separate titration in the ratios of 1:1, 1:2: 1:3 metal to ligand were performed in a quartz cell (1.000 cm path length) appropriately coupled to a 10 mL vessel where the pH values were adjusted by adding small volumes of KOH (0.1 mol L<sup>-1</sup>) and measured accordingly to cyclic voltammetry section.

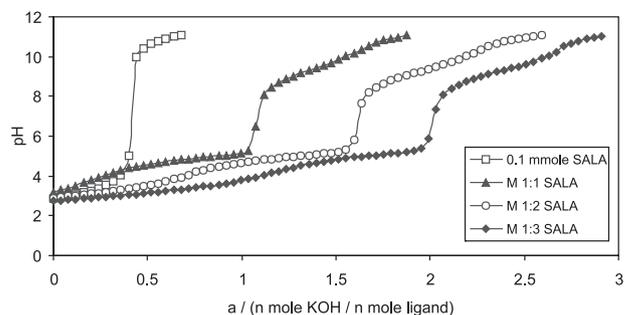
## Results and Discussion

Potentiometric titration profiles of the ligands (L) SALA, 5-NSA and 3,5-DNSA are presented in Figures 2 to 4 in the absence and in the presence of M in three different metal to ligand ratios. 3-NSA (Figure not shown) ligand presented very similar pH profiles with M as 5-NSA. The main striking feature of these profiles in the presence of metal is that at the beginning of the titrations, specially when the proportion favours the higher concentration of metal ion, the molybdate, an anion, first takes protons from the system, and then at pH values around 4.5 or greater, starts to act like a cation, by then complexing with the ligands.<sup>32</sup> Although the ML<sub>3</sub> species is highly thermodynamically unfavoured, potentiometric titration ratio M:L of 1:3 was conducted, but it was not possible to detect this species in percentages above 10% as required for the mathematical and chemical models employed in this study.<sup>30</sup> Formation constants for the complexed species were derived from the potentiometric data and are shown in Table 1.

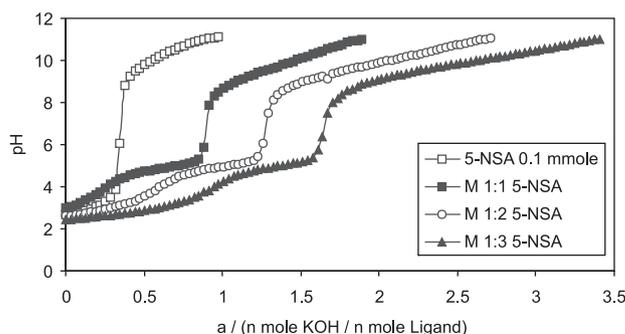
Looking at the values in Table 1 the non nitro ligand (SALA) has greater binding affinity among the four studied ligands because the electrons of the potentially binding

**Table 1.** Logarithms of the formation constants of molybdenum (VI) complexes and humic and nitrohumic acid like models (L). T=25.0 °C, I=0.100 mol L<sup>-1</sup>

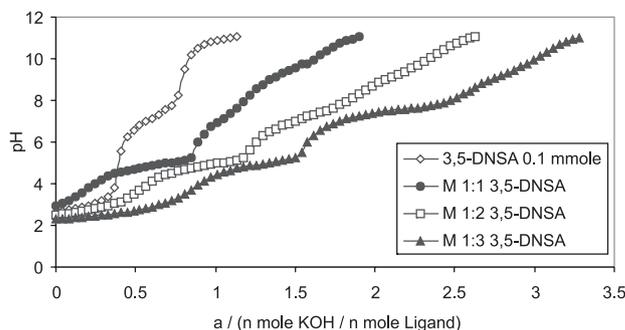
Species	log K ± 0.1			
	SALA	5-NSA	3-NSA	3,5-DNSA
[ML]/[M] [L] (1:1)	12.9	9.7	7.7	6.5
[MHL]/[ML] [H]	4.4	4.3	4.3	4.1
[ML <sub>2</sub> ]/[ML] [L] (1:2)	6.9	6.4	3.5	3.6



**Figure 2.** Potentiometric pH profile of molybdenum (VI) (M) and SALA (L). T=25.0 °C, I=0.100 mol L<sup>-1</sup>.



**Figure 3.** Potentiometric pH profile of molybdenum (VI) (M) and 5-NSA (L). T=25.0 °C, I=0.100 mol L<sup>-1</sup>.



**Figure 4.** Potentiometric pH profile of molybdenum (VI) (M) and 3,5-DNSA (L). T=25.0 °C, I=0.100 mol L<sup>-1</sup>.

sites (carboxylic and phenolic groups) are not involved in the resonance effect of the aromatic ring. Such is not the case of the nitro ligands (NSA's) since the electron withdrawing effect of the -NO<sub>2</sub> substituent pulls the electron from the binding groups towards the aromatic ring and consequently depleting the basicity effect of these groups. In addition when the -NO<sub>2</sub> group is in C-3 (3-NSA and 3,5-DNSA) there is the stereochemical influence in the formation of ML and ML<sub>2</sub>, making the complexation a little less favoured compared to when the electron pulling group is in *para* position (5-NSA). Both ML and ML<sub>2</sub> logarithms of the formation constants showed this trend.

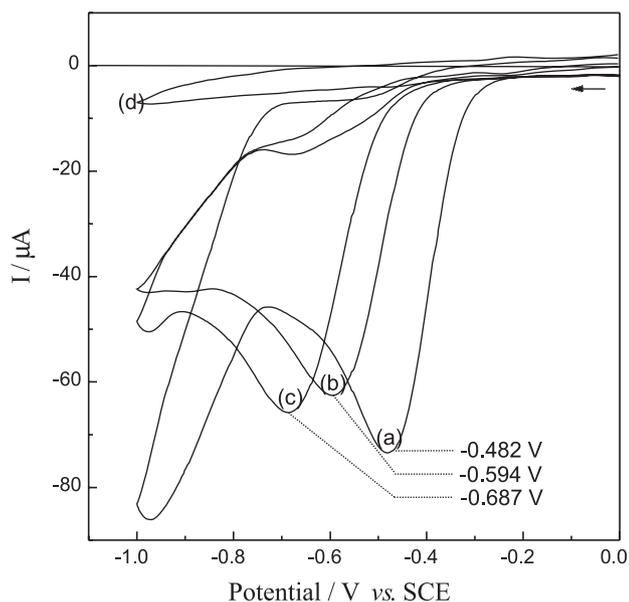
**Table 2.** Cathodic peak potentials of molybdenum (VI) complexes where humic acid models are the ligands (L) at 1:1 and 1:2 ratios of metal to ligand

Species	$E_{pc}$ (V vs. SCE)			
	$(NH_4)_2MO_2$	L = SALA	L = 5-NSA	L = 3-NSA L=3,5-DNSA
M	-0.584			
L		*	-0.687	-0.594
[ML] - (1:1)		-0.489	-0.494	-0.485
[ML <sub>2</sub> ] - (1:2)		-0.507	-0.532	-0.504

\* non electroactive ligand.

In the species distribution diagrams (Figures not shown) there was at least one complexed species at pH near 6.5 and until pH 8, for each ligand tested.

The cyclic voltammetric titrations added only limited information due to the irreversibility of the obtained voltammograms. In Figure 5 it is shown the voltammograms of all ligands,  $10^{-4}$  mol L<sup>-1</sup>. SALA is not electroactive and the salt  $(NH_4)_2MO_2$  is pseudo-reversible at acidic pH values. All ligands caused a pH dependent cathodic peak ( $E_{pc}$ ), but the definition became lower with the increased pH. At very high pH values the voltammograms lost their original shape and the  $E_{pc}$  wave was no longer shown. All voltammograms showed a second wave at  $\sim -0.95$  V that was assigned to amine group formation and does not undergo an oxidation process at the cathodic sweep (positive portion of the voltammogram not shown). The irreversibility may be due to the reduction of the nitro group, according to what occurs to nitrobenzene, which

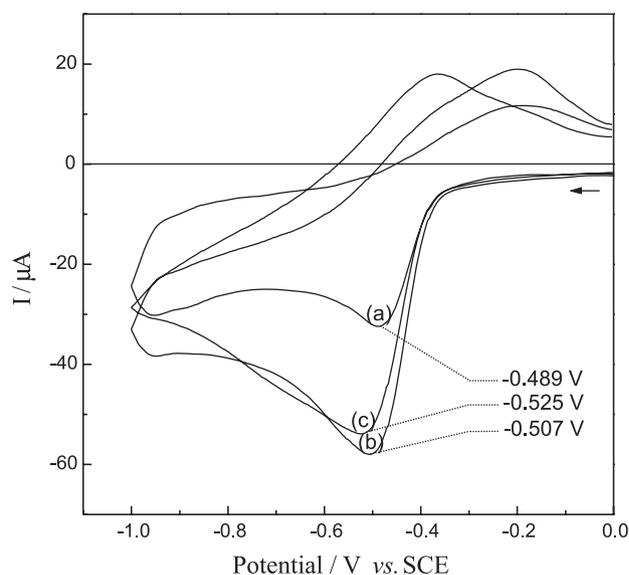


**Figure 5.** Cyclic voltammograms of (a) 3,5-DNSA:  $E_{pc} = -0.482$  V at pH = 2.53; (b) 3-NSA:  $E_{pc} = -0.594$  V at pH = 2.85; (c) 5-NSA:  $E_{pc} = -0.687$  V at pH = 2.80; and (d) SALA (not electroactive) at pH range of 2.90 to 10.97.

exhibits a single, pH-dependent and irreversible 4-electron polarographic wave in aqueous media below pH 5. This 4-electron wave is accompanied by a second 2-electron wave at more negative potentials giving way to p-aminophenol, formed by rearrangement of phenylhydroxylamine.<sup>34</sup>

The cathodic peak potential of all irreversible obtained voltammograms (Figures 5 to 9) showed a similar potential at acidic pH values (from 2.4 to 3.7) with  $E_{pc}$  varying from  $\sim -480$  mV to  $\sim -1000$  mV depending on the ligand, when the metal to ligand ratio increased from 1:1 to 1:3 metal to ligand ratios, indicating more difficulty in reducing ML<sub>2</sub> than ML.

In Figure 6 the SALA complexes voltammograms are shown with varying pH in 1:1 metal to ligand ratio. In all metal to ligand ratios studied, the voltammograms showed a pseudo-reversible curves at low pH values (from 2.88 to 3.32) and above pH = 5 it was observed undefined regions impossible to visualize neither the cathodic nor the anodic peaks.



**Figure 6.** Cyclic voltammograms of (a) [M(SALA)] (1:1):  $E_{pc} = -0.489$  V at pH = 3.32; (b) [M(SALA)<sub>2</sub>] (1:2):  $E_{pc} = -0.507$  V at pH = 2.96; (c) [M(SALA)<sub>3</sub>] (1:3):  $E_{pc} = -0.525$  V at pH = 2.88.

In equation 10:

$$E_{1/2} = E^0 - 0.059(m/n)pH \quad (10)$$

$E_{1/2}$  is the half wave potential,  $E^0$  is the standard potential at 298 K,  $m$  is the proton number, and  $n$  is the electron number transferred in the redox process. Rearranging equation 10, equation 11 is obtained:

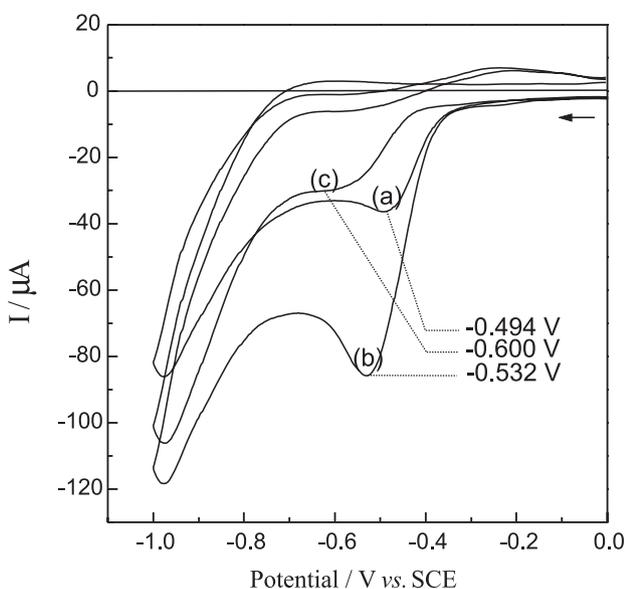
$$m/n = \Delta E_{pc} / -0.059 \Delta pH \quad (11)$$

The value of  $m/n=1.9976$  was found for the voltammogram of  $[M(\text{SALA})_2]$  considering  $E_{pc} = -0.507$  V for the voltammogram at  $\text{pH} = 2.96$  (Figure 6-b) indicating that there are 2 protons for each electron transferred in the redox voltammetric process of  $[M(\text{SALA})_2]$  (1:2 metal to ligand ratio), where one proton for each SALA ligand (total of 2 protons) and one electron for the complex may be transferred in this process. For the  $[M(\text{SALA})]$  complex (1:1 metal to ligand ratio) the  $m/n = 1.0015$  indicates that there was one proton and one electron transferred in the whole process (Figure 6a).

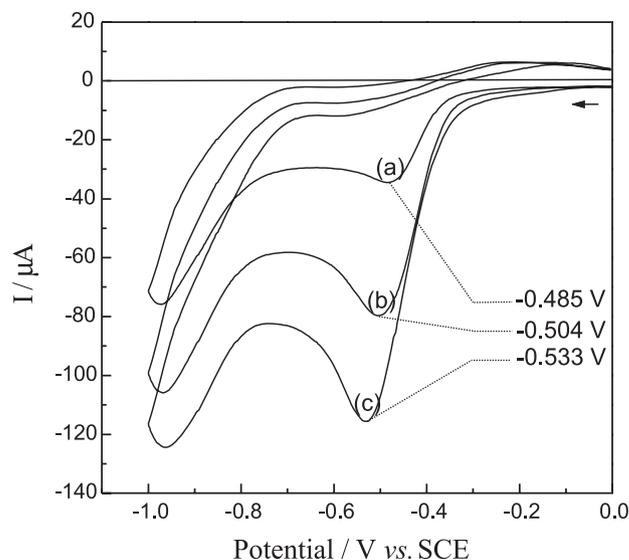
In Figure 7 it is shown the cyclic voltammograms of 5-NSA and molybdenum suggesting a 1:1 metal to ligand ratio. Considering  $E_{pc} = -0.494$  V for the voltammogram at  $\text{pH} = 3.17$  (Figure 7a) and  $E_{pc} = -0.575$  V at  $\text{pH} = 4.52$  of  $[M(5\text{-NSA})]$  complex, it was determined a  $m/n$  value of 1.0421, indicating that there is 1 proton for each electron transferred in the whole redox voltammetric process of  $[M(5\text{-NSA})]$  (1:1 metal to ligand ratio).

The cyclic voltammograms in Figure 8 present the 3-NSA in the 1:1 metal to ligand ratio. Considering  $E_{pc} = -0.485$  V for the voltammogram at  $\text{pH} = 3.11$  (Figure 8a) and  $E_{pc} = -0.564$  V at  $\text{pH} = 4.67$  of  $[M(3\text{-NSA})]$  complex, the  $m/n$  value was calculated, 0.9452. This indicates that there is 1 proton for each electron transferred in the whole redox voltammetric process of  $[M(3\text{-NSA})]$  (1:1 metal to ligand ratio).

Figure 9 presents the cyclic voltammograms of molybdenum and 3,5-DNSA in the ratio 1:1. A second and a third peaks were observed in this voltammogram,



**Figure 7.** Cyclic voltammograms of (a)  $[M(5\text{-NSA})]$  (1:1):  $E_{pc} = -0.494$  V at  $\text{pH} = 3.17$ ; (b)  $[M(5\text{-NSA})_2]$  (1:2):  $E_{pc} = -0.532$  V at  $\text{pH} = 2.71$ ; (c)  $[M:5\text{-NSA}]$  (1:3):  $E_{pc} = -0.600$  V at  $\text{pH} = 3.73$ .

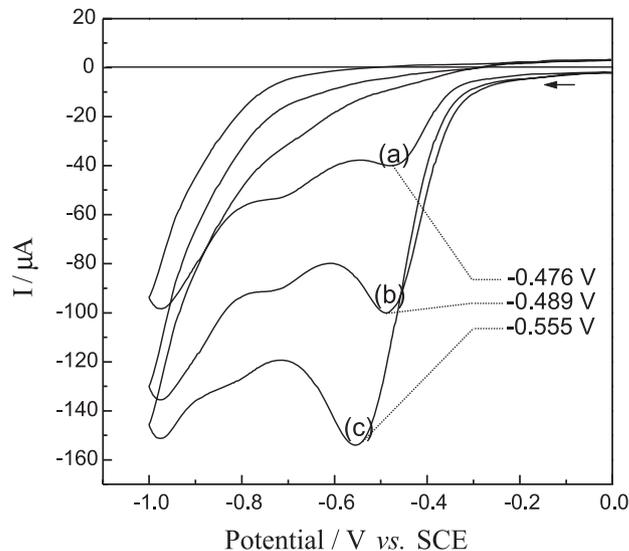


**Figure 8.** Cyclic voltammograms of (a)  $[M(3\text{-NSA})]$  (1:1):  $E_{pc} = -0.485$  V at  $\text{pH} = 3.11$ ; (b)  $[M(3\text{-NSA})_2]$  (1:2):  $E_{pc} = -0.504$  V at  $\text{pH} = 2.66$ ; (c)  $[M:3\text{-NSA}]$  (1:3):  $E_{pc} = -0.533$  V at  $\text{pH} = 2.41$ .

$E_{pc} = -0.75$  V and at  $-0.975$  V, the latter being assigned to the second nitro group being reduced in 3,5-DNSA ligand.

Although the metal to ligand ratios of 1:3 have shown different values for  $E_{pc}$  than any other metal to ligand ratio studied (Figure not shown), it was not possible to identify new species formed in this proportion neither by the cyclic voltammetry nor by potentiometric titrations. C items in Figures 5 to 9 are due to the uptake of protons by the molybdate anion as well as the complexation at some extent by the system of any metal to ligand ratio.

The species distribution diagrams of all ligands in the presence of the metal ion were obtained (Figures not



**Figure 9.** Cyclic voltammograms of (a)  $[M(3,5\text{-DNSA})]$  (1:1):  $E_{pc} = -0.476$  V at  $\text{pH} = 2.99$ ; (b)  $[Mo(3,5\text{-DNSA})_2]$  (1:2):  $E_{pc} = -0.489$  V at  $\text{pH} = 2.58$ ; (c)  $[M:3,5\text{-DNSA}]$  (1:3):  $E_{pc} = -0.555$  V at  $\text{pH} = 2.38$ .

shown) using the input of potentiometric data calculations in SPE program.<sup>30</sup>

The maximum peak absorbances, shoulders and isosbestic points of the UV-Vis spectra of all ligands and the metal ion are collected in Tables 3 to 6. Aliquots of pH varying from 2 to 12 were taken and analysed by UV-Vis spectroscopy of all ligands (Supplementary Figure S1, and each ligand in the presence of the metal ion, SALA, 3-NSA and 3,5-DNSA, supplementary Figures S2, S3 and S4, respectively) where it can be seen changes in the spectra due to complexed species in agreement with the species distribution diagrams. At pH values far below and far above those shown, no significant presence of complexed species were detected in the equilibria studied as the spectra in these values showed a sharp decrease in the charge transfer bands characteristic of the Molybdenum complexes with the nitro ligands studied (300-370 nm) and SALA (270-300 nm).<sup>35</sup>

The spectra of HL and L forms of SALA are at pH values of 4.67 and 12.19, where there are the presence of one deprotonated and the fully deprotonated forms, respectively (refer to Table 3). The spectra of  $\text{MoO}_4^{2-}$  ( $\text{MO}_2$ ) alone has shown at pH = 5.45 the cation  $\text{MoO}_2^{2+}$  (M) form which is the responsible for the complexed species found in all studied systems. The pH values of 4.07 and 5.52 represent the spectra of the complexed forms MHL and ML, respectively, in the 1:1 metal:SALA ratio. In 1:2 metal to ligand ratio it is seen the formation of  $\text{ML}_2$  (pH=6.86). In the 1:3 metal:SALA ratio it is seen both at pH 4.16 and 10.19 that other species rather than

ML, MHL or  $\text{ML}_2$  are present, but it was not possible to clearly identify them by potentiometric titrations under the experimental conditions employed, as the equilibrium in those pH values might be a mixture of all species involving polyanions in the former pH value and hydrolysis products in the basic pH value.

The UV-Vis spectra of HL and L were taken at pH 4.66 and 10.63 (refer to Table 4) of 3-NSA. The potentiometric profile and the complete UV-Vis spectra of 3-NSA are published elsewhere.<sup>26</sup> In pH values of 4.11, 4.77 in 1:1 metal:3-NSA ratio shows the complexed species MHL and ML. In 1:2 metal to ligand ratio the UV-Vis spectra taken at pH = 5.01 depict the ML species and in 1:3 ratio at acidic and basic pH values there is a mixture of species including hydrolytic ones in the equilibrium (refer to supplementary Figure S3).

The UV-Vis spectra of HL and L were taken at pH 4.74 and 10.81 of 5-NSA, respectively (refer to Table 5). The pH values of 4.10, 6.74 and 8.24 refer to the complexed forms MHL, ML,  $\text{ML}_2$  in metal:5-NSA ratios of 1:1, 1:1 and 1:2, respectively.

The UV-Vis spectra of HL and L forms of 3,5-DNSA was presented at pH = 4.42 and 7.32, respectively. The pH values of 4.71 and 7.01 refer to the complexed forms MHL, ML being coincidental the two obtained curves due to an almost equimolar presence of MHL and ML from pH 4 to 7. In metal to 3,5-DNSA 1:2 ratio,  $\text{ML}_2$  is present at pH around 7.6 (refer to Table 1).

The great number of isosbestic points in all taken UV-Vis spectra indicating interconversion of complexed

**Table 3.** UV-Vis spectra of SALA (0:1), SALA and M at different metal to ligand (L) ratios and metal (1:0), with varying pH values

	pH	4.07	5.52	6.86	4.67	12.39	4.34	5.45	10.34
$\text{MoO}_2\text{:L}$									
0:1	X	X	X		206m 300m 230sh 204i 216i	203m 298m 230sh 204i 216i	X	X	X
1:0	X	X	X	X	X	X	208m 206m	208m 225sh	198m 230sh 225sh
1:1		205m 280sh 240i	230m 295m 240i	X	X	X	X	X	X
1:2	X	X		208m 295m 194sh 225sh 220i	X	X	X	X	X
1:3		206m 295m 220sh	X	X	X	X	X	X	208m 295m 220sh

m= maximum peak absorbance; sh= shoulder; i=isosbestic point.

**Table 4.** UV-Vis spectra of 3-NSA (0:1) and 3-NSA and M at different metal to ligand (L) ratios, with varying pH values

MoO <sub>2</sub> :L	pH	4.11	4.77	5.01	10.93
0:1	X		200m 225m 350m 395i	X	198m 220sh 395i
1:1		207m 227m 348m 95sh 250sh 225i 237i	207m 227m1 195sh 225i 237i	X	X
1:2	X		X	210m 348m 227sh 225i 237i 246i 285i	X
1:3		203m 350m 220sh 218i 232i 244i 285i 310i 380i	X	X	206m 222sh 218i 232i 244i 285i 310i 380i

m= maximum peak absorbance; sh= shoulder; i=isosbestic point.

**Table 5.** UV-Vis spectra of 5-NSA (0:1) and 5-NSA and M at different metal to ligand (L) ratios, with varying pH values

MoO <sub>2</sub> :L	pH	4.10	6.74	8.24	6.05	10.21	4.74	10.81
0:1	X	X	X	X	X	X	198m 314m 220sh 366i	196m 406m 366i
1:1		208m 225m 305m 225i 355i	208m 228m 315m 225i 355i	X	X	X	X	X
1:2	X	X	X	208m 315m 229sh 215i 238i 276i 354i	X	X	X	X
1:3	X	X	209i	204m 315m 410m 220i 238i 354i	202m 315m X 222sh 354i	X	X	

m= maximum peak absorbance; sh= shoulder; i=isosbestic point.

species, coincided with the pH and species presented by the distribution diagrams. It was seen that 3,5-DNSA has a sterical difficulty in forming ML<sub>2</sub> species even presenting a stability constant similar to 3-NSA. This can be explained by the small percentage of formation in the equilibrium of this species.

The change in the CV and UV-Vis spectra caused by complex formation was used to support potentiometric data and was not used to calculate formation constants. Other complexed species were suggested by UV-Vis and CV analyses but it was not attempted to characterize them due to the large number of species in the equilibria as well as in the spectra. Some proposed complexes had a M:L ratio of 2:2, 2:1 and 4:2, as well as their protonated counterparts. The variation in the stoichiometry can be a direct consequence of the behaviour of molybdate and this metal ion towards  $\alpha$ -hydroxycarboxylate ligands as previously reported.<sup>35,36</sup>

**Table 6.** UV-Vis spectra of 3,5-NSA (0:1) and 3,5-NSA and M at different metal to ligand (L) ratios, with varying pH values

MoO <sub>2</sub> :L	pH	4.42	8.91	4.71	7.01	7.65
0:1		198m 330m 210sh 354i	198m 385m 354i	X	X	X
1:1	X		X	205m 225m 328m 240i 344i	205m 225m 356m 240i 344i	X
1:2	X	X	X	X	X	206m 230m 369m 220sh 215i 344i

m= maximum peak absorbance; sh= shoulder; i=isosbestic point.

## Conclusions

The use of this study showed the existence of at least one complexed model species of HA and NHA with molybdenum (VI) at a pH value commonly found in soil. Use of a salicylic acid model may contribute to the understanding of other complexed species involving this metal ion in aqueous solutions with varying pH values.

Making a comparison among salicylic HA and nitrosalicylic model compounds and ligands derived from phthalic and nitrophthalic based model compounds, the presence of the complexed species is seen in pH values until 8 only for the salicylic derived models and 4-

nitrocatechol, since the complexed species for phthalic derivatives are decomposed after pH 6.5.<sup>5</sup>

The nitro compounds are weaker Lewis bases than the non nitro model compound - SALA, due to the electron withdrawing effect of -NO<sub>2</sub> in the aromatic ring.<sup>5</sup> Nonetheless, the nitrohumic substances, laboratory artifacts, presented as an alternative fertilizer by providing more N content when the mineralization of the soil occur, can also bind differently to metal ions when compared to non nitro organic matter in a wider range of pH values.

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## Supplementary Information

UV-Vis spectra of all ligands, 10<sup>-4</sup> mol L<sup>-1</sup>. SALA, 3-NSA, 5-NSA, 3,5-DNSA and MoO<sub>2</sub><sup>2+</sup> (M) at various pH values (Figure S1), SALA and SALA and MoO<sub>2</sub><sup>2+</sup> (M), both at 10<sup>-4</sup> mol L<sup>-1</sup> at various pH values (Figure S2), of 3-NSA and 3-NSA and MoO<sub>2</sub><sup>2+</sup> (M) at various pH values (Figure S3) and 3,5-DNSA and 3,5-DNSA and MoO<sub>2</sub><sup>2+</sup> (M) at various pH values (Figure S4) are available free of charge as PDF file at <http://jbc.sbq.org.br>.

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## Molybdenum (VI) Bound to Humic and Nitrohumic Acid Models in Aqueous Solutions. Salicylic, 3-Nitrosalicylic, 5-Nitrosalicylic and 3,5 Dinitrosalicylic Acids, Part 2

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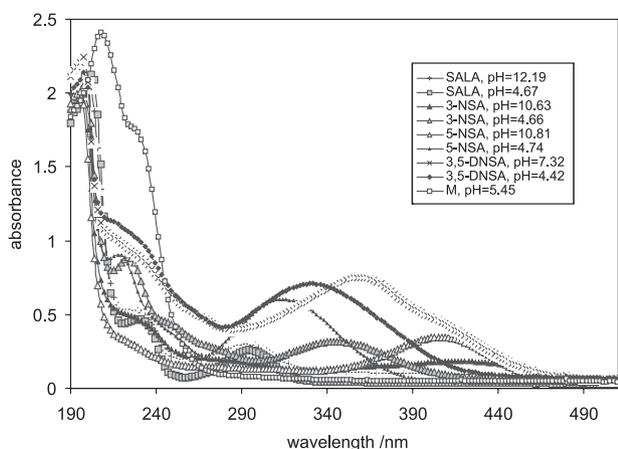


Figure 1S. UV-Vis spectra of ligands,  $10^{-4}$  mol L<sup>-1</sup>. SALA, 3-NSA, 5-NSA, 3,5-DNSA and M at various pH values.

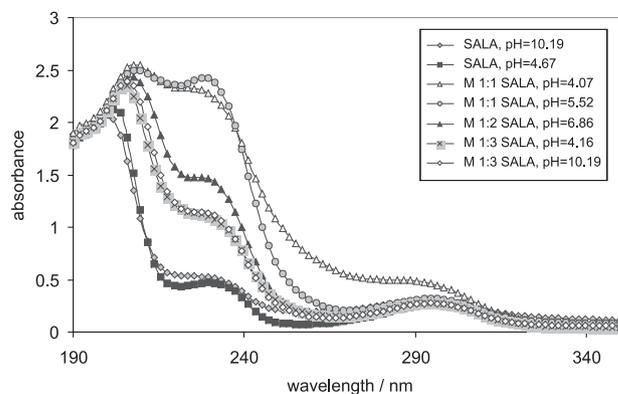


Figure 2S. UV-Vis spectra of SALA and SALA and M, both at  $10^{-4}$  mol L<sup>-1</sup> at various pH values.

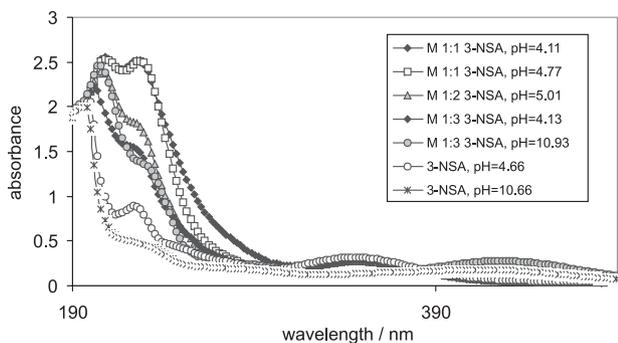


Figure 3S. UV-Vis spectra of 3-NSA and 3-NSA and M at various pH values.

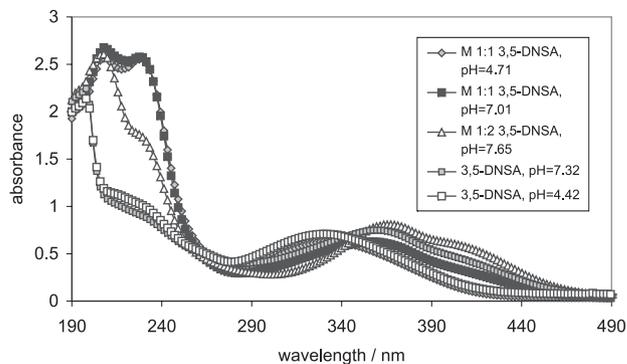


Figure 4S. UV-Vis spectra of 3,5-DNSA and 3,5-DNSA and M at various pH values.

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