

# Influence of the ligand concentration and pH on the complexation of Cu(II) by a soil fulvic acid

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**Abstract.** Anodic Stripping Voltammetry has been tested in a non-traditional, pH-variable procedure, to study the interaction between Cu(II) with a soil fulvic acid. The study was performed at 25 °C and  $I = 1.0$  M, and covered wide ranges of fulvic acid concentration ( $5 - 20$  mg L<sup>-1</sup>), Cu(II) concentration ( $2.10^{-5} - 4.10^{-6}$  M), and pH ( $3.5 - ca$  8). Mean complexation equivalent weights (between 800 and 5000 g mol<sup>-1</sup>, depending of experimental conditions) and apparent equilibrium constants (between ca. 3 and 0.02 for pH-independent constants), and their variations with pH and fulvic acid concentration were calculated through an iterative procedure. The results obtained are consistent with those published before for the implementation of the pH-variable procedure with Cu(II) ion selective electrode potentiometry, and show that Anodic Stripping Voltammetry at pH-variable conditions is suitable for the study of Cu(II) – fulvic acid interactions.

**Key words.** Fulvic acid – Cu(II) complexation – Anodic Stripping Voltammetry.

## Introduction

The interaction between heavy metals and organic matter in natural ecosystems influences markedly their availability to organisms, because the different chemical species of the metal are not all equally acceptable for animals and plants [1]. Humic substances (fulvic and humic acids) play an important role in this type of reactions owing to their complexation capacity and high concentration in the environment [2]. Moreover, the relative proportions of a metal ion bound to natural organic compounds depend on the acidity/basicity of the ligands, which also is influenced by the nature of the vegetal cover [3-4]. Therefore, the knowledge of the extent of the interactions between metal ions and organic matter is important to provide information on their states in soils and water.

Due to the inherent complexity of natural polyelectrolytes like fulvic and humic acids or humic-like substances, different techniques have been used for the analysis of their complexation reactions, depending on the information required [1]. Indeed, no technique alone provides a complete description of the behavior of fulvic or humic acids in presence of metal ions. Among other, electrochemical techniques have been extensively used in this type of analysis, and anodic stripping voltammetry (ASV) could be an useful choice to obtain information for the study of complex chemical interactions, due to its very low detection limit and the small changes induced in the equilibria. Therefore, it was decided to include ASV in a project that has been in progress in our Departments, to obtain data that can

complete information about humic and humic-like substances and their interaction with metal ions obtained by other techniques [5-14]. In the present paper, the results of a voltammetric study about the influence of the concentration of a soil fulvic acid (FAH<sub>n</sub>) on the stability of its complexes with Cu(II), and on the equivalent weight (EW) of the ligand are presented. The objective of the work was to test ASV for the implementation of a non-traditional pH-variable methodology used before with Cu(II) ion selective potentiometry [12], to analyze the interaction of metal ions with soil fulvic acids.

## Experimental

### Reagents and materials

All reagents were p.a. quality, and were used without further purification. For the preparation of the solutions, deionized water with resistivity  $> 16$  MΩ cm<sup>-1</sup> was employed.

The sample of FAH<sub>n</sub> used in the present work was isolated from a forest soil collected in Ermesinde (near Oporto, NW of Portugal), using a procedure similar to that of IHSS [15]. For that, the solid soil sample was dried at room temperature and treated with HCl 2 M until pH  $\approx 2$  was reached. After 15 days of agitation, the mixture was decanted and the soil was discarded. The liquid was then passed through an Amberlite XAD-8 column to concentrate the fulvic acids, washed with water until chloride ions were not detected, and then eluted with NaOH 0.1 M. The liquid obtained, which

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contains FAH<sub>n</sub> molecules in their ionized (basic) form, was passed through a Merck ion exchanger column, and freeze-dried to obtain a brown colored powder. The yield of the process was about 0.3 % w/w. The elemental analysis of the sample was: C 44.5, H 3.3, N 1.4, O (obtained by difference) 50.8 %. Ash content < 0.5 %. Neither S nor heavy metals were detected.

Cu(II) ion solutions were prepared daily from a 1000 mg L<sup>-1</sup> PANREAC standard solution. KNO<sub>3</sub> (Riel-de-Haen) was used as background electrolyte to adjust the ionic strength to 1 M. For pH adjustment 0.1 M NaOH (Merck) was employed.

Free Cu(II) concentrations were measured in Cu-FAH<sub>n</sub> solutions (see below) placed in a double wall cell (298.0 ± 0.2 K), and stirred with a Selecta magnetic stirrer, with a Metrohm E-506 Polarecord, connected to a Metrohm E-608 VA-Controller central unit, and to a Metrohm 663VA-Stand equipped with a SMDE. This instrument was calibrated with standard Cu(II) solutions. H<sup>+</sup> concentration was measured with a 52-03 Crison electrode and a Crison 2001 pH-meter calibrated by standard acid-base procedures [16]. For NaOH additions, a Methrom 1655 Dosimat burette was employed.

For the experimental determination of complexation parameters, 20.0 mL of a Cu-FAH<sub>n</sub> solution stabilized during 24 hours were placed in the cell. Initial concentrations were 5, 10, 15, 20, 25 and 30 mg L<sup>-1</sup> for FAH<sub>n</sub>, and 2.10<sup>-5</sup> – 4.10<sup>-6</sup> M for Cu(II), and these values were practically constants during the titration. Adequate volume of NaOH titrant were added in steps of 0.1 mL and, when the thermal and acid – base equilibria were reached, the pH was measured, the voltammogram was traced, and the procedure was then repeated adding new base aliquots until a final pH about 8 was obtained (20 experimental points for the complete curve). The titrations were performed twice, and replicate values of peak height were within 1% error. Voltammeter parameters were: electrodeposition time 60 s with agitation and 30 s without agitation, pulse amplitude 50 mV, step time 0.4 s, electrodeposition potential –0.6 V, current range 6.10<sup>-10</sup> A mm<sup>-1</sup>.

A Quattro-Pro spreadsheet was employed in the calculations describe in the following section.

## Theory

For the calculation of the apparent stability constants of the Cu(II)-FAH<sub>n</sub> complexes, a procedure similar to fully presented previously [12] was used.

When Cu(II) ions react with a polyelectrolytic ligand like FAH<sub>n</sub> at high FAH<sub>n</sub> / metal concentration ratios (*i.e.* in the starting of the titration), the 1:1 complex must be predominant as (charges omitted for the shake of simplicity)



where  $y$  represents the average number of hydrogen ions released when the complexes are formed. If these protons

react with a strong base (as NaOH) the equilibrium is disturbed, and further complex is formed, depending on the pH of the solution. Therefore, it is possible to calculate an apparent pH-independent stability constant ( $\beta$ ) using the equation

$$\beta = \frac{[\text{CuFAH}_{n-y}][\text{H}]^y}{[\text{Cu}]_f[\text{FAH}_n]} \quad (2)$$

where all the concentrations are expressed in mol L<sup>-1</sup>. As detailed in [12] this equation can be transformed to

$$\frac{|\text{FAH}_n|}{[\text{Cu}]_t - [\text{Cu}]_f} = \text{EW} + \text{EW} \frac{[\text{H}]^y}{[\text{Cu}]_f \beta} \quad (3)$$

where EW is the mean complexation equivalent weight of FAH<sub>n</sub>,  $|\text{FAH}_n|$  ( $= [\text{FAH}_n]$ ) is now measured in g L<sup>-1</sup> and the lower indexes  $t$  and  $f$  refer to Cu(II) total and free concentrations respectively.

The plot of the equation (3) above will be a straight line if the 1:1 complex predominates and the pH is constant, and, then EW can be obtained as the intercept and  $\beta$  from the slope. When the pH is variable like in the present work, both quantities can be calculated using a trial-and-error procedure, based in the transformation of (3) to

$$\log \left( \left( \left( \frac{[\text{FAH}_n]}{[\text{Cu}]_t - [\text{Cu}]_f} \right) - 1 \right) [\text{Cu}]_f \right) = -\log \beta - y \text{pH} \quad (4)$$

which allows the calculation of  $y$  starting from an initial value near the expected (1.0), and using equation (3) for the calculation of EW. The process is repeated with the new  $y$ , and so on iteratively, until differences are less than 0.01. From this value,  $\beta$  is also calculated by standard techniques [17].

The procedure can also be employed for complexes different than 1:1 by changing the stoichiometric proportion of the reaction, and applying the corresponding equations when  $n > 1.2$ , where  $n$  is the mean number of cooper ions bound to a FAH<sub>n</sub> molecule ( $n = ([\text{Cu}]_t - [\text{Cu}]_f) / [\text{FAH}_n]$ ).

Moreover, if the H<sup>+</sup> concentration and  $y$  are taking into account, apparent pH-dependent stability constants ( $\beta'$ ) can be calculated as

$$\beta' = \frac{\beta}{[\text{H}^+]^y} \quad (5)$$

## Results and discussion

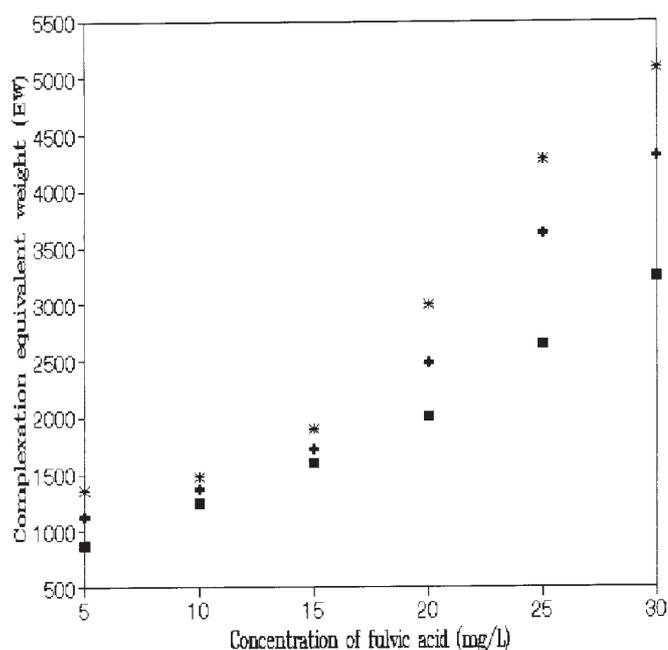
Although some  $n$  values greater than 1 were obtained from experimental data at the highest metal concentration conditions in characteristic experiments, only complexes with

1:1 stoichiometry were considered in the results, and values of EW,  $\gamma$  parameter and apparent pH-independent stability constants ( $\log \beta$ ) obtained for these systems were included in table I. This was due both to absolute predominance of 1:1 complexes in all experiments, and the impossibility to obtain accurate  $\beta$  values for complexes with higher  $n$  numbers (less than three experimental points). As the results in the table show a marked dependence on the concentrations of FAH<sub>n</sub> and Cu(II), hence the following discussion considers mainly to the influence of both variables on the magnitude of the equilibrium parameters.

In figure 1, a plot of the calculated EW against concentration of FAH<sub>n</sub> is presented. It shows a monotonous increase, which is in agreement with previous results obtained using the same procedure with a Cu(II) ion selective electrode [12]. On the other hand, the slopes of these plots are inverse related to the initial concentration of Cu(II) ion. Taken into account that the values obtained here are average values of different monomeric aggregates present when complex 1:1 predominate, the variation suggest that EW must be considered as a metal ion concentration depending equivalent weight, *i.e.* each value is only valid in the range Cu(II) / FAH<sub>n</sub> concentration ratio for which was calculated.

With respect to the  $\beta$  values defined in equation (2), the results indicate a continuous decrease when the concentration of FAH<sub>n</sub> rises. This variation is similar to other published for apparent stability constants of complexes formed by humic substances with metal ions [18-20]. Moreover, the range of values is the same range, despite the difference in the experimental conditions and techniques employed. No other comparisons are possible, as no results of the present type have been published before, to the best of our knowledge.

Although a complete theoretical explanation of the variation of stability constants with experimental solution conditions never has been reported before in the literature, the variations found here are consistent with an alteration of the FAH<sub>n</sub> molecules in the complexation process, owing to the variations of FAH<sub>n</sub> and Cu(II) ion concentration, which could change the aggregation state of macromolecules [10]. In fact, the values obtained here for



**Figure 1.** Variation of EW ( $\text{g mol}^{-1}$ ) with total fulvic acid concentration (see equations 2 and 4). Cu(II) concentrations: ■  $1.96 \cdot 10^{-5} \text{M}$ , +  $1.18 \cdot 10^{-5} \text{M}$ , \*  $0.392 \cdot 10^{-5} \text{M}$ .

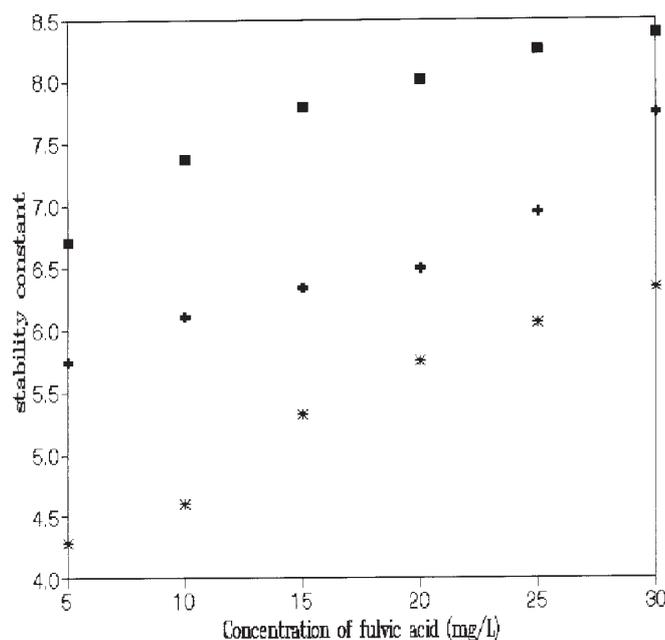
pH-dependent stability constants ( $\beta'$  defined in equation (5), see table II) and  $\gamma$  support this consideration. Figure 2 plots the values obtained for these constants at pH = 6 and the three metal concentrations used (similar results were obtained for other pH values in the range studied here). These stability constants increase with the concentration both of FAH<sub>n</sub> and Cu(II). The former could be a result from an increment in the number of groups of stronger ligand strength available for complexation, due to the increment in the number of FAH<sub>n</sub> molecules in solution. As it is well known, natural FAH<sub>n</sub> polyelectrolytes contain molecules of different sizes, with different functional groups which can interact more or less strongly with metal ions, forming

**Table I.** Complexation equivalent weight (EW), mean number of released H<sup>+</sup> ( $\gamma$ ) and apparent pH-independent stability constant ( $\beta$ ) variation of the complexes formed between Cu(II) ions and FAH<sub>n</sub>.

[Cu(II)] / $M \cdot 10^5$ FAH <sub>n</sub> ( $\text{mg L}^{-1}$ )	1.96				1.18				0.392			
	EW	$\gamma$	$\beta$	$\log \beta$	EW	$\gamma$	$\beta$	$\log \beta$	EW	$\gamma$	$\beta$	$\log \beta$
5	873	1.04	2.98	0.47	1127	0.94	1.26	0.10	1356	0.72	0.90	-0.05
10	1250	1.20	1.49	0.17	1361	1.03	0.84	-0.07	1479	0.83	0.42	-0.38
15	1598	1.35	0.50	-0.30	1726	1.10	0.27	-0.56	1896	1.00	0.21	-0.68
20	2009	1.45	0.21	-0.68	2486	1.21	0.17	-0.76	3001	1.13	0.095	-1.02
25	2645	1.53	0.12	-0.92	3635	1.35	0.070	-1.15	4287	1.23	0.048	-1.32
30	3245	1.60	0.060	-1.22	4316	1.55	0.028	-1.56	5079	1.33	0.023	-1.64

**Table II.** Influence of pH and ligand concentration on the stability constants of the complexes reported in Table I.

	pH = 5			pH = 6			pH = 7			pH = 8		
[FAH <sub>n</sub> ] (mg L <sup>-1</sup> ) \ [Cu(II)]M (.10 <sup>5</sup> )	1.96	1.18	0.392	1.96	1.18	0.392	1.96	1.18	0.392	1.96	1.18	0.392
5	5.7	4.8	3.6	6.7	5.7	4.3	7.8	6.7	5.0	8.8	7.6	5.7
10	6.2	5.1	3.8	7.4	6.1	4.6	8.6	7.1	5.4	9.8	8.2	6.3
15	6.5	5.2	4.3	7.8	6.3	5.3	9.2	7.5	6.3	10.5	8.6	7.3
20	6.6	5.3	4.6	8.0	6.5	5.8	9.5	7.7	6.9	10.9	8.9	8.0
25	6.7	5.6	4.8	8.3	7.0	6.1	9.8	8.3	7.3	11.3	9.7	8.5
30	6.8	6.2	5.0	8.4	7.7	6.3	10.0	9.3	7.7	11.6	10.8	9.0



**Figure 2.** Change of the pH-dependent stability constant (as log) of complexes with different Cu(II) - to fulvic acid concentration ratio. Experimental conditions: pH = 6, Cu(II) added ■  $1.96 \cdot 10^{-5}M$ , ●  $1.18 \cdot 10^{-5}M$ , \*  $0.392 \cdot 10^{-5}M$ .

complexes with different stability constants. Therefore, macroscopic equilibrium constants, like the apparent constants reported here, are average values of the microscopic constants for the interaction between Cu(II) and the FAH<sub>n</sub> molecules and their different coordinating groups. Hence, their value will depend on the nature of the group and the strength of the bond formed. If the concentration of available stronger groups is increased by the increment in the number of FAH<sub>n</sub> molecules, the metal ions will preferably bond to these groups, and the average apparent stability constant also rises. The increase of Cu(II) ion concentration also increases the extent of the coordination. As the complexation process implies the interchange of H<sup>+</sup> and Cu(II) ions (with the release of different quantities of protons, equation (1)), the values of  $\gamma$  must grow with the FAH<sub>n</sub>

concentration. The values obtained here show that tendency, and reinforce the explanation outlined before.

A similar conclusion can be reached when the influence of pH on  $\beta'$  constants is analyzed. An increase of  $\beta'$  (presented in their logarithmic form in table II) with the pH is observed, which is explained by a more extensive deprotonation of functional groups, and, consequently, the use of more coordination sites to which the metal ion can be bound. Moreover, the tendency to higher values of the stability constants at higher FAH<sub>n</sub> concentration also supports the interpretation above.

As a final conclusion, the consistence of the present results between themselves and with those published before [12] shows that: 1) ASV can be used as experimental technique for the implementation of the pH-independent methodology introduced before with Cu(II) ion selective electrode potentiometry as measuring technique; and 2) ASV provides an adequate alternative to study the interaction of this type of ions and humic substances like fulvic acids.

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