Interactions between copper and organic macromolecules: determination of conditional complexation constants

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Interactions between copper and organic macromolecules: determination of conditional complexation constants

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Abstract – This study presents an original method of estimating the complexant properties of organic macromolecules with a metal. The complexant capacities and conditional constants of complexation with copper of humic-like acids extracted from the leachate of a sanitary landfill were determined. These macromolecules, with high molecular weight, compete with HEDP (1-hydroxyethane-1,1'-diphosphoric acid), a small molecule, to complex copper; this constitutes the principle of the method. HEDP-copper complexes and free metal species are selectively separated from macromolecule-copper complexes by ultrafiltration (YM 1; 1000 Da). An experimental design was carried out to minimise the number of experiments and to provide a model that related total copper concentration in the filtrate to the main variables of the system (macromolecules and HEDP concentrations and pH). The experimental data are compared to the equilibrium model, which enables the complexation capacities of the macromolecules with copper to be estimated.

complexation / organic macromolecules / humic acids / copper / ultrafiltration / leachate

Résumé – Interactions entre le cuivre et les macromolécules organiques : détermination des constantes conditionnelles de complexation. Cette étude présente une méthode originale d'estimation des propriétés de complexation des macromolécules organiques avec un métal. Les capacités et les constantes conditionnelles de complexation entre le cuivre et des macromolécules de type acides humiques (extraites de lixiviat de décharge d'ordures ménagères) ont été déterminées. Le principe consiste à mettre en compétition vis-à-vis du cuivre deux complexants, d'une part, les macromolécules de poids moléculaire élevé, d'autre part l'HEDP (acide 1-hydroxyéthane-1,1'-diphosphorique) une molécule de petite taille. Les complexes HEDP-cuivre et les espèces métalliques libres sont séparés sélectivement des complexes macromolécules-cuivre par ultrafiltration (YM 1 ; 1000 Da). Un plan d'expérience a été utilisé afin de minimiser le nombre d'expériences et fournir un modèle qui permet de relier la concentration totale en cuivre du filtrat aux principales variables du système (concentrations en macromolécules, en HEDP et pH). Les données expérimentales sont comparées au modèle théorique (établi à partir des équilibres chimiques), ce qui permet d'estimer les capacités de complexation des macromolécules avec le cuivre.

complexation / macromolécules organiques / acides humiques / cuivre / ultrafiltration / lixiviat

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1. Introduction

Organic macromolecules play an important role in the transport, the bioavailability and the solubility of heavy metals [3]. Interactions of organic macromolecules such as humic and fulvic acids with heavy metals have been widely studied [10, 19]. A number of techniques have been used to study the complexation with metal (exchange resins, gel chromatography, dialysis, ultrafiltration, polarography, potentiometry, ISE, fluorescence quenching). All these techniques lead to the determination of capacities and conditional constants of complexation between the ligand and the metal. Among these techniques, ultrafiltration, a non-destructive technique that doesn't need the addition of chemical reactants, is an interesting one.

Many authors use ultrafiltration to determine the class of size of the complexed metal, and to measure the lability of the humic substances-metal complex [1]. Lee [13], Staub and Buffle [18] and Ephraim [7] used ultrafiltration to determine complexant capacities and conditional constants of complexation.

This paper describes a method based on ultrafiltration to determine conditional constants of complexation of macromolecules with copper.

2. Materials and methods

2.1. Origins of the macromolecules

The macromolecules used in this study were extracted from an anaerobic sanitary landfill. The leachate was collected in the Crézin landfill (near Limoges, France) which was opened in 1974. Household waste was dumped there until 1989 as was ordinary industrial waste until it was closed in 1997. The waste can be considered as being well-stabilised (methanogenic-phase) from physico-chemical parameters of the leachate (BDO_5/COD, COD, pH).

The method used to extract macromolecules is the one developed by Mejbri et al. [15] on the basis of the method first proposed by Thurman and Malcolm [20]. The extraction method relies on the adsorption of humic acids onto Amberlite resins (XAD-7) and their variation in solubility with pH. This method enabled the isolation of the so-called "humic acids".

Humic acids were characterised by elemental analysis, NMR[13]C, UV and IR spectroscopy, apparent molecular weight and spectrofluorescence. The results showed similarities between these substances and humic acids of aquatic origins (unpublished data). It also brought to the fore the high proportion of the high molecular weight fraction; humic acids (designated as HA in the present study) represent 35% of the leachate DOC.

The distribution of humic acids by apparent molecular weight, using ultrafiltration, was determined by the measurement of the dissolved organic carbon (DOC) in every fraction. Figure 1 shows a wide distribution, which proves the large heterogeneity of the molecules that were extracted from the leachate.

2.2. Principle and conditions

The affinity of copper for humic acids is compared to that which it has for a small complexing molecule of known properties: HEDP. The separation of organic matter/copper complexes and other forms of copper (free copper and HEDP acids/copper complexes) is carried out by ultrafiltration through a 1 kDa membrane.

The total copper concentration in the ultrafiltrate is measured by atomic absorption spectrometry and is denoted as Cu*. It corresponds to the sum of all the species of copper, free and complexed, and is a function of the concentrations of humic acids, HEDP and pH.

A series of experiments is realised for different concentrations of HA and HEDP and different pHs. In this way, an experimental model which matches the concentration of copper in the ultra-filtrate to the concentrations of HA, HEDP and pH in the experimental domain is explored. Equally, the concentration of total copper can be expressed theoretically from the equilibrium reactions of the different species and more particularly as a function of the complexation constants of Cu-HEDP and Cu-HA.
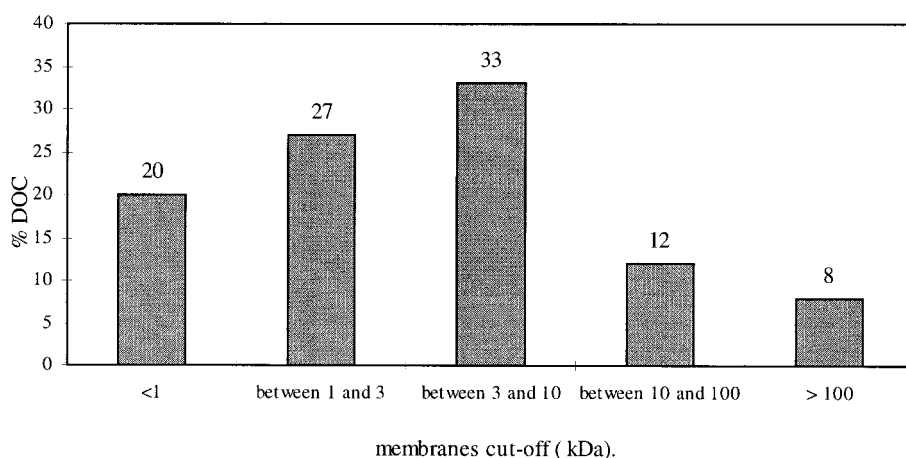
Figure 1. Apparent molecular weight from humic like acids extracted from leachate determined by ultrafiltration. (pH = 7.5; $NaNO_3$ = 0.1 M).

2.2.1. Verification of the absence of interactions between HEDP and Humic-type macromolecules

There is very little data on this subject in existence. Deluchat et al. [5] noted no influence of fulvic acids on the protonation of HEDP. Equally, the study on the influence of fulvic acids on complexation of HEDP with copper shows no synergy or inhibition between these two ligands but only that their complexing capacities are additional [5]. These results lead one to assume that there is no interaction between fulvic acids and HEDP.

Various trials were carried out to check these results on the chosen humic acids:

- The zeta potential measurements (pH and ionic strength both constant) of 200 mg·L^{-1} solutions of humic acids containing increasing concentrations of HEDP showed no significant changes in their values.

- The UV spectra of these solutions was also taken between 220 and 300 nm, and no modification of the spectra of the HA solution was observed (only the HEDP does not absorb for these wavelengths).

2.2.2. Determination of the ligand/copper stoichiometric quantities

It is essential to elucidate the stoichiometry of the ligand/metal in order to study the competition between the two complexing agents.

As for the HEDP-copper complex the stoichiometry is 1:1 [6].

For the organic macromolecules the stoichiometry, or complexing capacity, is determined experimentally by two methods. The concentrations are expressed in gC·L^{-1}, as it is not possible to determine the molar masses of the macromolecules studied.

Zetametry:

Increasing quantities of copper are added to a 200 mg·L^{-1} solution of humic acids. The pH is kept constant by adding quantities of nitric acid (10%) and NaOH (1 M). The ionic strength is fixed at 0.01 M by $NaNO_3$. The zeta potential of the different samples is measured.

The zeta potential increases with an increase in copper concentration (cf. Fig. 2).

For a copper concentration of 10^{-4} M the zeta potential no longer varies. Above this value the humic acids no longer complex thus their zeta potential is no longer modified. A complexation capacity of 5×10^{-7} mol per gram i.e. about 1 mmol·gC^{-1} is thus obtained.

Ultrafiltration:

Lee [13] showed that the stoichiometry of the complexing reaction can be determined from the concentrations of the free copper in the ultra-filtrate from solutions with different ligand/metal concentrations then graphing them as a function of the concentration of the complexed metal. The same reasoning can be applied to finding the concentration
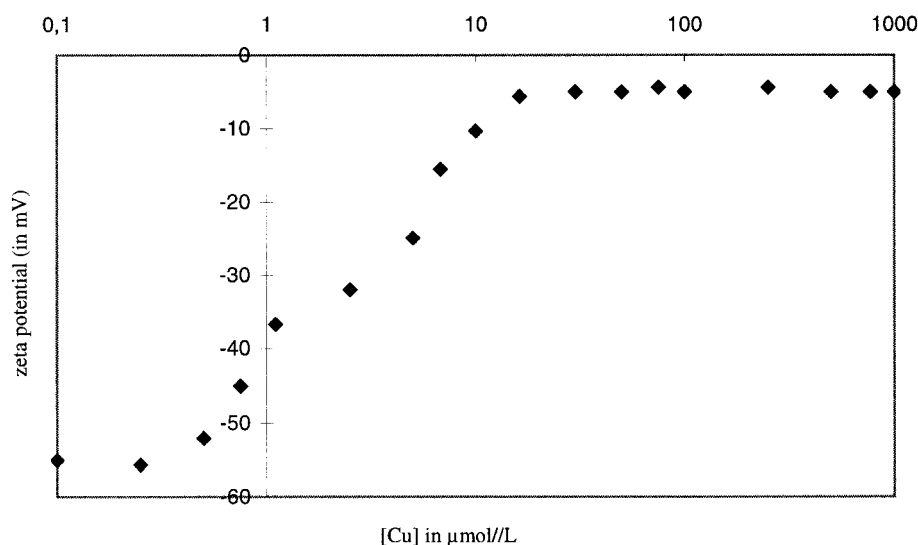
Figure 2. Evolution of the zeta potential as a function of copper concentration.

of the free metal in the ultra-filtrate as a function of the quantity of complexing agent added.

From Figure 3 it can be seen that above an HA concentration of 100 mg·L^{-1}, 20% of the total copper passes through the membrane. This value corresponds to 20% of the COD of the humic acids not retained during ultra-filtration (Fig. 1). It can be assumed that the copper that traverses the membrane is complexed with HA and that the solution is homogenous. This fraction of HA that passes the membrane is taken into account in the calculations that allow the equilibrium model to be established.

Under these experimental conditions a complexing capacity of 10^{-5} M of copper for 20 mg·L^{-1} of HA i.e. around 1 mmol Cu·gC^{-1} is obtained. The humic acids studied exhibit, in fact, 9.3 milli-equivalents of total acid functions per gram of carbon in witch 8.5 are carboxylic groups. This corresponds to 1 mmol of copper for 9.1 milli-equivalents of total acid functions. The values obtained by this method are very close to those obtained by zetametry and also agree with the literature values. Matthes et al. [14] accept that the SHs complex between 0.5 and 2 mmol of heavy metals per gram.
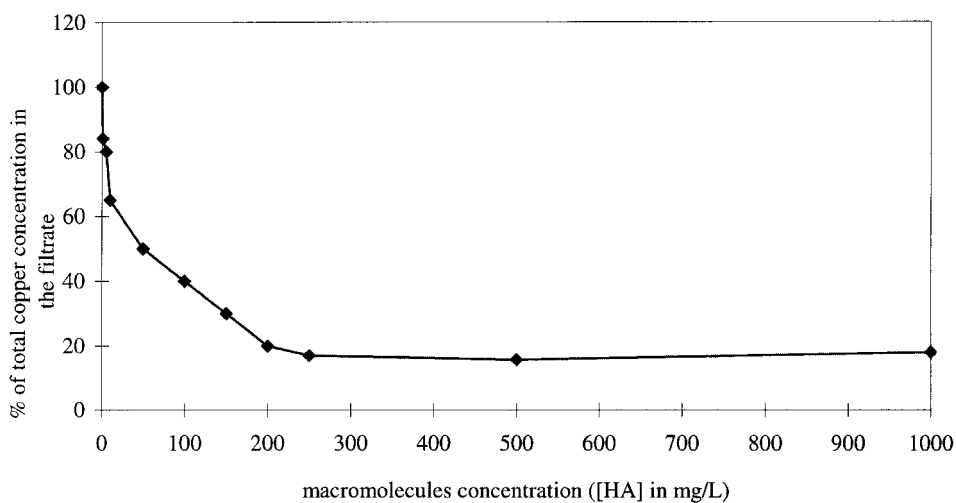

Figure 3. Determination of the complexant capacities of the macromolecules: copper concentration in the filtrate as function of the [HA]. (pH = 6; I = 0.1 M; [Cu] = 10^{-4} M).

Kinetic trials were carried out to verify that the ultra-filtration was done when all species were at equilibrium. The absence of adsorption of copper by the container walls was also checked.

2.3. Materials

The different solutions were ultrafiltered using a membrane cut-off of 1 kDa (YM 1; Amicon), these membranes are composed of regenerated cellulose, they are considered as being non-ionic and are often used to fractionate humic substances [4, 17]. A Millipore cell of 400 mL was used. Ultrafiltrations were performed under pressurised nitrogen. No more than 10% of the total volume was ultrafiltered in order to avoid complex dissociation [8].

The copper concentrations were determined in each fraction by graphite furnace atomic absorption spectrophotometry (Varian spectra AA 800, Zeeman background correction). In order to avoid matrix effects, some matrix modifiers were used $(Pd(NO_3)_2, ADPH, NH_4NO_3)$. The analytic range is between 7–30 ppb and the accuracy is $\pm 5\%$.

2.4. Experimental design

In recent studies on the complexation of copper with organic molecules, experimental designs appear to be largely used (e.g. [11, 12]). The first step was to identify all the parameters which were likely to have an influence on the response Cu*(copper in the filtrate). We found eight parameters:

– humic acid concentration

– HEDP concentration

– copper concentration

– stirring speed

– order in which reactants were introduced

– pH

– pressure

– ionic strength.

We chose to study the following three parameters:

the macromolecule concentration (noted [HA]), the HEDP concentration (noted [HEDP]) and the pH.

Other parameters were fixed for all the experiments. Ionic strength was fixed at 0.1 M (NaNO_3), (approximately the value of the ionic strength of the leachate). Stirring speed was fixed at 500 RPM in order to avoid the polarisation of the membrane. The order of introduction of the different species was also fixed; the two complexants were introduced simultaneously and, after 10 minutes stirring, the copper(II) solution was added. The pressure of the ultrafiltration was fixed at 100 kPa (nitrogen). The copper concentration was fixed at 10^{-5} mol·L^{-1}.

The next step was the choice of the most appropriate experimental design. In order to model the response a Doehlert lattice was used. This method has been extensively explained [9]. Another advantage of this experimental design was that the interactions between the different parameters was analysed for a relatively large number of combinations of the levels of the selected variables.

For the macromolecules, seven levels were chosen for the concentration. The range of concentrations was determined after the determination of the complexant capacities of the macromolecules.

For the HEDP, five levels were selected. We chose concentrations around the stoichiometry corresponding to 1:1 between HEDP and the copper. Only 3 pH values were chosen. The range was narrow (between 5 and 7); actually, pHs higher than 7 were avoided to prevent copper hydroxide formation and, for a pH lower than 5, precipitation of the humic acids was observed.

Table I sums up the ranges and the numbers of chosen levels for each variable.

Table I. Ranges and number of levels that have been chosen for the experimental design.

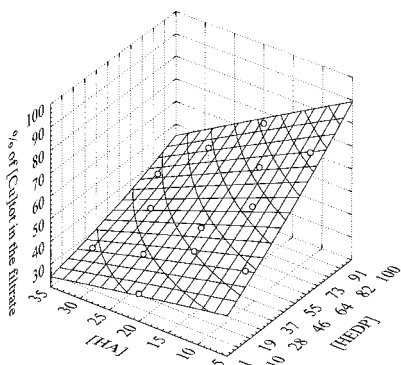| [HA]	7 levels	5 to 35 mg·L^{-1}
[HEDP]	5 levels	10^{-6} to 10^{-4} mol·L^{-1}
PH	3 levels	5; 6; 7

Figure 4. Evolution of the % of Cu in the filtrate as a function of [HA] and [HEDP]; the pH was fixed at 6.

3. Results and discussion

3.1. Experimental results

Estimation of the interactions between the three variables as well as the model was obtained from the different trials (13). No phenomena of precipitation was noted during experiments. The expression obtained from the model related the % of the copper in the filtrate to the three variables in the experimental domain. So, it was possible to represent on a 3D graphic the response as a function of two of the three variables. In Figure 4, pH was fixed at 6. It can be observed that the copper percentage in the filtrate increases when the macromolecule concentration decreases and when the HEDP concentration increases. The experimental Cu* can be expressed by:

Cu* = 39.171 + 0.5 × [HEDP] – 0.395 × [HA] + 7.474e^{-4} × [HEDP]^2 – 0.011 × [HEDP] × [HA] –7.637e^{-4} × [HA]^2.

One could expect this kind of graphic variation. The experimental model indicates that 93% of the points are significant. Complementary trials were carried out in order to validate this model.

3.2. An equilibrium model

The total copper concentration in the filtrate can also be expressed using an equilibrium model.

Table II. List of possible reactions the different species.

Copper and hydroxydes		
(1) $Cu^{2+} + 2\,OH^-$	$= Cu(OH)_2$	$K_{S1} = Cu^{2+}.(OH^-)^2$
(2) $Cu^{2+} + OH^-$	$= CuOH^+$	$K_1 = CuOH^+/Cu^{2+}.OH^-$
(3) $CuOH^+ + OH^-$	$= Cu(OH)_2$	$K_2 = Cu(OH)_2 / Cu^{2+}.(OH^-)^2$

HA acidities		
(4) $A^{2-} + H^+$	$= HA^-$	$Ka_1 = H^+.A^{2-}/HA^-$
(5) $HA^- + H^+$	$= H_2A$	$Ka_2 = H^+.HA^-/H_2A$

HEDP acidities		
(6) $Y^{4-} + H^+$	$= HY^{3-}$	$Ka_3 = Y^{4-}.H^+/HY^{3-}$
(7) $HY^{3-} + H^+$	$= H_2Y^{2-}$	$Ka_4 = HY^{3-}.H^+/H_2Y^{2-}$
(8) $H_2Y^{2-} + H^+$	$= H_3Y^-$	$Ka_5 = H_2Y^{2-}.H^+/H_3Y^-$
(9) $H_3Y^- + H^+$	$= H_4Y$	$Ka_6 = H_3Y^-.H^+/H_4Y$

Complexation and precipitation of HA-copper		
(10) $HA^- + Cu^{2+}$	$= HA\text{-}Cu^+$	$K_3 = HA\text{-}Cu^+/HA^-.Cu^{2+}$
(11) $2\,HA^- + Cu^{2+}$	$= H_2A\text{-}Cu$	$K_4 = H_2A\text{-}Cu / (HA^-)^2.Cu^{2+}$
(12) $A^{2-} + Cu^{2+}$	$= A\text{-}Cu$	$K_5 = A\text{-}Cu / A^{2-}.Cu^{2+}$
(13) $2\,HA^- + Cu^{2+}$	$= H_2\underline{A\text{-}Cu}$	$Ks_2 = Cu^{2+}.(HA^-)^2$
(14) $A^{2-} + Cu^{2+}$	$= \underline{A\text{-}Cu}$	$Ks_3 = Cu^{2+}.A^{2-}$

HEDP-copper complexation		
(15) $Y^{4-} + Cu^{2+}$	$= CuY^{2-}$	$K_6 = CuY^{2-}/Y^{4-}.Cu^{2+}$
(16) $Y^{4-} + Cu^{2+} + H^+$	$= CuHY^-$	$B = CuHY^- /Y^{4-}.Cu^{2+}.H^+$

HEDP-HA interaction		
(17) HA + HEDP	= HA-HEDP	unknown

Firstly, all the possible chemical reactions of this system (Cu^{2+}, HEDP , HA) were written: 17 different reactions (see Tab. II). Among these reactions, some can be put aside.

First of all the reactions involving copper hydroxides can be ignored (reactions 1, 2 and 3) due to the very low concentration of copper, the presence of two complexing agents in the solution and a maximum pH of 7. The second dissociation of the humic acids (reaction 4) can also be ignored due to its high pKa (a pKa of 9.0 was obtained by titration). Reaction 12 is highly unlikely to occur as the A^{2-} species is present in such small quantities between pH 5 and 7 and the complexation constants of the groups involved (phenolic) are much smaller than those of more acidic groups. Precipitation of neutral complexes (13 and 14) was not noted at these concentrations, as was shown by the DOC measurement (Figs. 1 and 3). Reaction 17 which would represent an interaction between the two

complexing agents can also be ignored as no inter-action was noted.

With reactions retained for the model, after some simplifications, it was possible to express K_{HA-Cu} with:

$Cu* = [Cu^{2+}] + [CuY^{2-}] + [CuHY^-] + 0.2 [HA-Cu^+]$,

$Cu* = [Cu^{2+}]\{1 + K_6 [Y^{4-}] + B [Y^{4-}] [H^+] + K_3 0.2 [AH^-]\}$,

and

$CuT = [Cu^{2+}] + [CuY^{2-}] + [CuHY^-] + [HA-Cu^+]$.

$$K_{HA-Cu} = \frac{([Cu_T] - Cu*) (1+[Y^{4-}] (K_6 - B[H^+]))}{[AH^-] (Cu* - 0.2 [Cu_T])}$$

where the factor 0.2 comes from the 20% of HA able to pass the membrane (see Fig. 3).

$[AH^-] = \dfrac{[AH_T]}{1 + 10^{(pka_2-pH)}}$, AH_T is the total humic acid concentration,

$$[Y^{4-}] = \frac{[Y_T]}{1 + 10^{(pka_3-pH)} + 10^{(pka_3+(pka_4-2pH))} + 10^{(pka_3+(pka_4+(pka_5-3pH)))}}$$

and Y_T is the total HEDP concentration.

The constants used in the calculations are those determined by Deluchat et al. [6] for the acidity constants of HEDP and HEDP-copper complexation constants. The acidity constants used for the humic acids were determined experimentally. The values of the constants used are reported in Table III.

For each point of the experimental domain, it became possible to calculate a value of K_{HA-Cu}; the % of Cu in the filtrate at this point was calculated using the experimental model. Table IV shows some results: the experimental values of Cu*, the Cu* values calculated with the experimental model and the values of the constants determined with the previous expression of K_{HA-Cu}.

A value for K_{HA-Cu} could be determined in all the experimental domains. The frequency of apparition of K_{HA-Cu} according to the value of K_{HA-Cu} is represented in Figure 5. Two groups of values can be distinguished; the first group has an average K_{HA-Cu} value of about 2, the second of about 9. It can be noted that the values of K_{HA-Cu} are between 1 and

Table III. Values of constants.

reaction	constants values	
1	pK_{s1}	18.80
2	pK_1	–6.10
3	PK_2	–11.20
5	pK_{a2}	5.0
6	pK_{a3}	2.7
7	pK_{a4}	6.9
8	pK_{a5}	11.0
15	pK_6	–12.0
16	pB	–17.3

15 with the macromolecules' concentration expressed in mg·L^{-1}. It corresponds to K_{HA-Cu} between 4×10^5 and 60×10^5, with their concentrations expressed in meq of acid groups per litre. The values of the constant that were found with this method are comparable to constants determined by fluorescence quenching on the same material (not yet published) ($K_{HA-Cu} = 1.03 \times 10^5$ at pH 6). We developed a second model that took reactions 1–3 into account in order to check our results. The new values of K_{HA-Cu} were close and no significant difference was noted. We can infer that the shape of the distribution is not due to a too simple equilibrium model. We also checked that the uncertainty in the values of the constants used in the equilibrium

Table IV. Experimental and calculated results.

[HA] mg·L^{-1}	[HEDP] µmol·L^{-1}	pH	% Cu*	% Cu calculated	$K_{HA/Cu}$
20.00	50.50	6.00	45.8	47.0	8.12
20.00	100.00	6.00	66.2	66.4	7.81
20.00	1.00	6.00	28.7	31.2	0.77
32.99	76.25	6.00	39.4	40.1	11.19
7.01	24.75	6.00	48.6	47.3	10.67
7.01	76.25	6.00	72.8	73.0	10.62
32.99	24.75	6.00	29.6	29.2	7.49
24.33	76.25	6.82	45.3	44.2	10.10
15.66	24.75	5.18	51.9	53.0	0.19
15.66	76.25	5.18	70.0	68.7	0.31
28.65	50.50	5.18	56.2	56.3	0.34
24.33	24.75	6.82	39.5	38.6	7.54
11.34	50.50	6.82	59.6	59.9	10.33

Total volume: 50 mL; filtrate volume 5 mL; total copper concentrations 10^{-5} mol·L^{-1}.
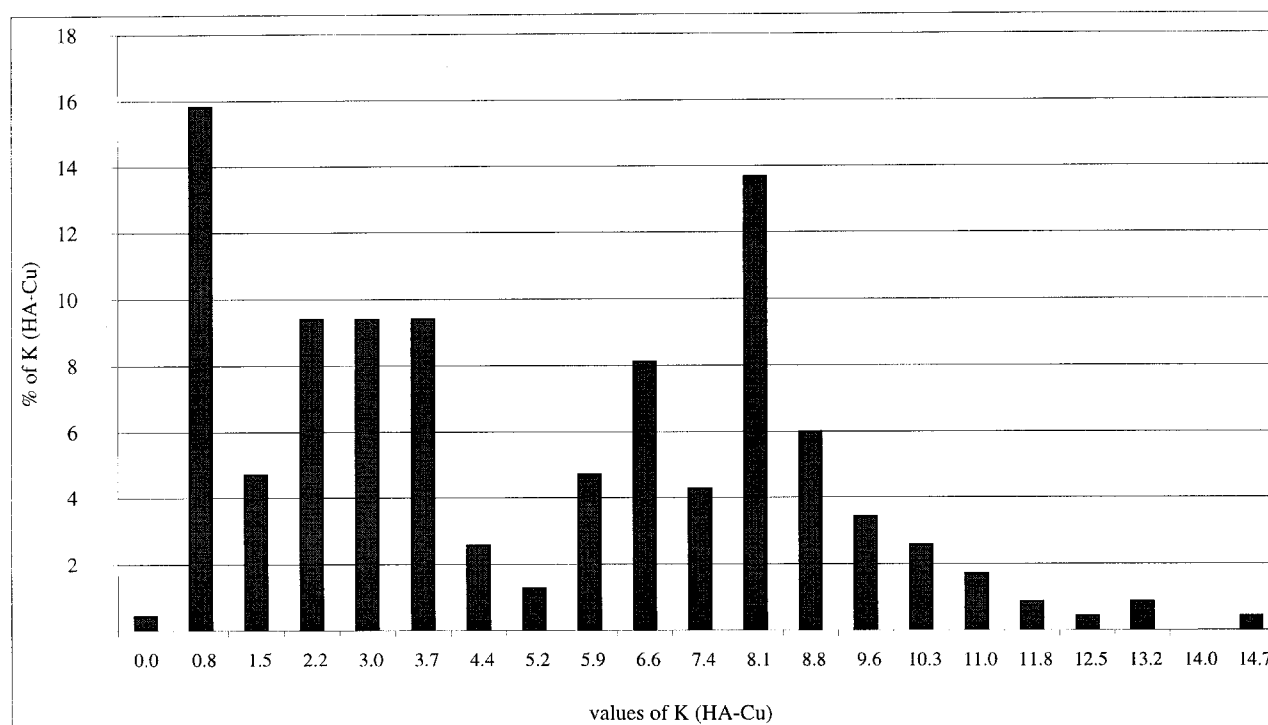
Figure 5. Percentage of apparition of $K_{HA\text{-}Cu}$ as a function of $K_{HA\text{-}Cu}$ values – the macromolecules's concentrations have been expressed in $mg \cdot L^{-1}$.

model do not have a significant effect on the determination of the values of $K_{HA\text{-}Cu}$. The shape of the $K_{HA\text{-}Cu}$ distribution seems therefore to be exclusively linked to the complexant behaviour of the complex macromolecules-copper and is probably due to two main complexing sites.

This method can be considered as an improvement on Lee's method [13] which consists of the determination of the conditional constant of complexation between fulvic acids and copper by the measurement of the free copper in the filtrate. This method avoids the measurement of free copper (always considered difficult) and allows for lower concentrations of copper (between 10^{-7} and 10^{-5} M) and complexants. The determination of any conditional constant in the experimental domain may be obtained from this model. The very low proportion of free copper is ensured by the use of the competition between complexants which decreases the hazard of complex dissociation during the ultrafiltration.

4. Conclusion

The intent of this paper was to present a method for the determination of a conditional constant of complexation between macromolecules and metal. In this example, macromolecules of humic acids extracted from the leachate of a sanitary landfill were used; the selected metal was copper. Modelling of the total copper concentration in the filtrate as a function of the three parameters (humic acids concentration, HEDP concentration and pH) was made possible by the experimental design (Doehlert lattice). Using an equilibrium model based on chemical reactions of the different species, it was possible to determine any value of $K_{HA\text{-}Cu}$ in the experimental domain, using the total copper concentration in the filtrate (calculated with the experimental model), the [HA], the [HEDP],

constants of complexation ($K_{HEDP-Cu}$) and acidity constants. We noted two different groups of constant values that correspond to 8×10^5 and 36×10^5. These constant values are comparable to constants determined by fluorescence quenching. It is possible that these two constants correspond to the two main different sites of complexation of the humic acids: carboxylic and phenolic. This method presents some advantages (i) only the total copper in the filtrate has to be measured, (ii) the copper concentration may be low, (iii) the experimental conditions can be chosen closer to the conditions that are found in the environment, (iv) the absence of free copper due to the competition of the complexant avoids the dissociation of the complex during ultrafiltration already noted by other authors [8].

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