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The effect of pH, ion strength and reactant content on the complexation of Cu^{2+} by various natural organic ligands from water and soil in Hong Kong [☆]

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Abstract

The complexation constants for copper associated with different natural organic ligands, including dissolved organic carbon (DOC) extracted from water, water soluble organic carbon (WSOC), fulvic acid (FA) and humic acid (HA) from soil, were determined and then compared based on discrete single site model. Both ion-selective electrode (ISE) and anodic stripping voltammetry were used to determine the content of free copper ions, while the relative number of complexation sites was estimated using a fluorescence quenching (FQ) at the same time. ISE proved to be the most applicable technique when the concentration of copper was above $10^{-7} \text{ mol l}^{-1}$. The $\log k$ values for two WSOC sample sites (Song Ziyuan and Xin Niangtan) were 4.64 and 4.66; higher than both the DOC and HA values yet lower than the FA values, which were unusually high due to unavoidable pollution from the cation exchange resin used during the purification process. Binding affinities between the copper ions and the organic ligands obtained from streams in Yong Wei (DOC) and Song Ziyuan (WSOC) were influenced by pH, ion strength, and reactant concentration. Values for $\log k$ increased with increases in pH (ion strength of 0.1 N). At pH 4, the $\log k$ values decreased with increases in the supporting electrolyte concentration and total copper ion additions.

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Keywords: Natural organic ligands; Copper; Complexation; Stability constants; Complex capacity

1. Introduction

It is well established that the transport, bioavailability, fate and toxicity of metal ions depends upon their physical and chemical forms. Copper is an essential trace nutrient to plant but also can be toxic at high concentrations. Its toxicity is determined by cupric ion activity but not the total copper concentration. Many

natural organic ligands in soil, water and sediment could complex with copper, affect its activity and then its retention and mobility in soil and waters (Piccolo, 1996; Nierop et al., 2002). Many Geochemical speciation models have been utilized to estimate the free form of metal as the toxicity index, in which some type of thermodynamic equilibrium constants, mostly the discrete model parameters $\log k$ and complex capacity (CC), are determined to describe the bind strength and total number of binding sites (Wood, 1996).

To obtain these binding parameters and to derive quantitative information, a number of techniques have been used, e.g., potentiometric titration (Buffle et al., 1977; Bresnahan et al., 1978; Gamble et al., 1980; Avdeef and Stuting, 1983), voltammetry (Buffle et al., 1987;

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Filella et al., 1990; Waller and Pickering, 1990; Rozan and Benoit, 1999; Carballeira et al., 2000), fluorescence spectroscopy (Saar and Weber, 1980; Ryan and Weber, 1982; Senesi, 1990b), ion exchange (Arakani and Stevenson, 1972; Tipping et al., 1991), gel filtration (Mantoura and Riley, 1975), dialysis (Wilson and Kinney, 1977; Tipping et al., 1988), ultrafiltration (Caceci, 1985; Ephraim, 1992), electron spin resonance spectroscopy (Templeton and Chasteen, 1980; Senesi, 1990a) etc. to determine the concentration of free metal or ligand binding sites. The methods of analysis of the complexation will affect the stability constant. Moreover, the experimental condition such as temperature, pH, ionic strength of the solution, concentration of metal and ligand will also influence the binding parameters (Witter et al., 1998; Filella and Town, 2000; Johan et al., 2001; Bryan et al., 2002; Joaquim et al., 2002). The stability constants depend on so many factors that they are conditional stability constants and are valid only for the experimental conditions under which they were determined.

Another important factor influencing the conditional stability constants is the sources of the nature organic ligands and the extraction or isolation procedure employed. The variations in composition and structure of ligand can lead to variation in the characteristic of nature organic ligands binding with metal. The binding of copper to dissolved organic carbon (DOC) in natural water and humus substance or their fractions (humic acids (HA), fulvic acids (FA)) extracted from soil has been studied extensively (Krajnc et al., 1995; Witter et al., 1998; Filella and Town, 2000; Town and Filella, 2000; Johan et al., 2001; Joaquim et al., 2002; Nierop et al., 2002). Water soluble organic carbon (WSOC) is the most mobile fraction and thus potentially facilitates metal transport through soils in the form of organic complexes. It is of special importance for copper since all divalent cations form the strongest complexes with humus substance. However, information about the binding of copper to WSOC is scarce.

The primary objective of this study was to compare the complexation constants for copper associated with various natural organic ligands (including FA, HA, WSOC from soil and DOC from water) and the influence imposed by experimental conditions (including pH, ionic strength and reactant concentration). A compari-

son was made first among different analysis techniques (ion-selective electrode (ISE), anodic stripping voltammetry (ASV) and fluorescence quenching (FQ)) to select the most available method finally and to be used in the consequent study.

2. Experimental design

2.1. Sample preparation

Soil samples were collected from the A1 horizon (0–10 cm) at Xin Niantan and Song Ziyuan in Hong Kong, China. They were air dried and sifted through a 2 mm sieve following removal of root material and a hand breakup of aggregates. The physico-chemical properties of the soil sample are listed in Table 1. The WSOC was obtained by extracting 20 g of soil with 50 ml distilled and deionized water. Both FA and HA were extracted using 0.1 N NaOH and shaken for 24 h at room temperature. HA was recovered by precipitation with HCl. The detailed processes of extraction and purification followed currently recognized procedures (Tan, 1996). The DOC content in the water came from small streams in Song Ziyuan (Nos. 1 and 2), Xin Niantan (No. 3), Yong Wei (No. 4), Liu Shuixiang (No. 5), and Chong Ji (No. 6) in Hong Kong, China. All the material was filtrated through 0.45 μm membrane and the total organic carbon (TOC), total copper concentration, pH, and alkalinity identified (Table 2).

2.2. Complexation titration

2.2.1. Ion-selective electrodes

A 10 ml solution containing a known amount of natural organic carbon was added to a 50 ml volumetric flask. A measured amount of supporting electrolyte was then added to give the desired ionic strength (0.1 or 0.05 N NaNO_3). The pH was adjusted to a constant value (4.00 ± 0.02 , 5.00 ± 0.02 , 6.00 ± 0.02) by addition of NaOH and HNO_3 and the sample was brought to a volume of 50 ml with deionized water and transferred to a 100 ml beaker. The standard Cu solution was then added in a stepwise process to the beaker and the free copper ions measured using ISE.

Table 1
Physico-chemical properties of the soil samples from Xin Niantan

Density (g/cm^3)	Water (%)	pH_w^a	Size distribution (%)					WSOC mg C/g
			>0.05 mm	0.01–0.05 mm	0.005–0.01 mm	0.001–0.005 mm	<0.001 mm	
2.55	11.71	4.25	72	5	4	9	10	0.27

^a pH_w : (soil:water = 1:1).

Table 2
TOC, background of copper content, pH and alkalinity of the samples

	No.	TOC, mg l ⁻¹	Cu, mg l ⁻¹	pH	Alkalinity, mmol l ⁻¹
DOC	1	0.84	0.013	7.38	0.32
	2	1.72	0.014	7.81	0.60
	3	1.24	0.015	7.60	0.32
	4	2.84	0.010	8.00	0.64
	5	2.34	0.007	7.24	0.20
	6	2.40	0.011	6.89	0.20
WSOC	1	13.78	0.047	6.43	0.08
	3	35.83	0.060	5.76	0.16
FA	1	293.00	0.027	3.32	–
	3	541.00	0.024	3.43	–
HA	1	112.50	0.027	2.44	–
	3	266.30	0.046	2.34	–

2.2.2. Anodic stripping voltammetry

A 25 ml solution containing a known amount of natural organic carbon was added to a reaction cell. Next, 0.5 ml 5 N NaNO₃ was added to adjust the ionic strength to 0.1 N. The pH was maintained at a constant value (4.00 ± 0.02 , 5.00 ± 0.02 , 6.00 ± 0.02) by addition of an HAc–NaAc buffer and the whole volume brought to 25 ml with deionized water. The free copper ion concentration was determined using ASV following gradual additions of Cu.

2.2.3. Fluorescence quenching

A 2.5 ml solution with the same natural organic ligands and supporting electrolyte content mentioned above was put in an absorption cell and the fluorescence intensity determined by FQ following the gradual addition of copper to the solution.

2.3. Analytical procedures

A SpectrAA30 Atomic Absorption Spectrophotometer was used to analyze the total copper concentrations. Free copper activity was determined by a cupric ion-selective electrode (Cu-ISE, Model 9429, from Orion Research) and a double junction reference electrode (Model 900200, from Orion Research). The pH was measured using a pH combination electrode Model 710 A, also from Orion Research.

The free copper ions were measured by ASV from Metrohm 693 VA. The specific settings used for the differential pulse mode were deposit potential, –600 mV; deposit time, 60 s; sweep, –600 to 100 mV; sweep rate, 20 mV/s; pulse height, 50 mV; and drop size, 0.32 mm².

Because the active group exhibits fluorescence that is quenched upon binding with a copper ion, FQ provided another alternative for complexes between the natural organic carbon and copper ions. A Model F-4500 Flu-

orescence spectrophotometer was used to obtain fluorescence data. The fluorescence excitation and emission wavelengths were 350 and 450 nm.

In order to study the differences in binding affinity and capacity of different natural organic ligands and the influence of experimental conditions, a single site discrete model was used to calculate the average binding constant and complex capacity (Jun et al., 2002).

All reagents used in the study were of analytical grade. All the plastic and glassware were soaked in 10% HNO₃ for at least 48 h before use, then rinsed with distilled and deionized water.

3. Results and discussion

3.1. Determination of copper ion and ligand

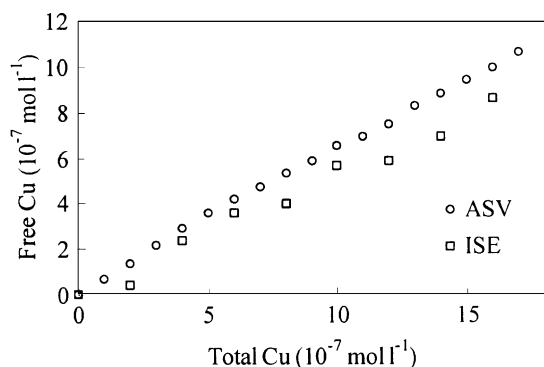
Several different methods were used to measure the content of free copper ions or free ligand sites in the equilibrium solution. The titration processes were conducted for different levels of pH, reactant content, and background electrolyte concentration as listed in Table 3.

The free copper ion concentration was determined using both the ISE and ASV methods. Fig. 1 shows the overlap in data obtained by both methods. The squares represent the enlargement of the forepart of the titration curve based on ISE while the dots represent the whole curve according to ASV. The copper ion concentrations determined by ASV are all higher than those by produced using ISE, primarily due to the dissociation of complexed copper ions to ligands in the membrane layer of electrode during the deposition time (Tao et al., 1987). And it is important to remember that the buffer (HAc–NaAc) that was added to the equilibrium solution contained acetate that could form complexes with copper.

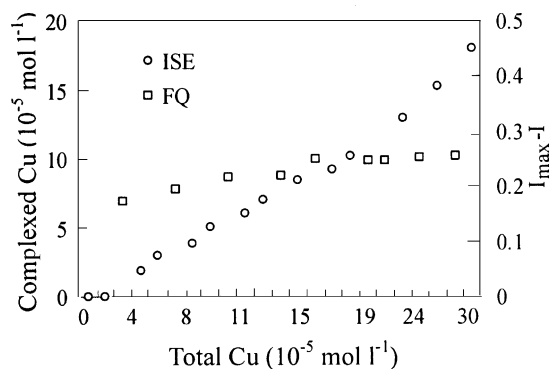
Table 3

Binding parameters obtained by different methods (NaNO₃: 0.1 N)

<i>ISE</i>						
pH	4	5	6	6	6	6
TOC/mg l ⁻¹	0.24	0.24	0.24	0.24	0.48	0.72
Cu added/mol l ⁻¹	10 ⁻⁷ –10 ⁻⁵	10 ⁻⁷ –10 ⁻⁵	10 ⁻⁷ –10 ⁻⁵	10 ⁻⁵ –10 ⁻³	10 ⁻⁵ –10 ⁻³	10 ⁻⁵ –10 ⁻³
log <i>k</i>	4.36	6.20	6.47	2.29	2.71	3.44
CC (Cu/C)/mol g ⁻¹	1.52	0.20	0.21	26.39	37.27	52.71
<i>ASV</i>						
pH	4	5	6			
TOC/mg l ⁻¹	0.24	0.24	0.24			
Cu added/mol l ⁻¹	10 ⁻⁷ –10 ⁻⁶	10 ⁻⁷ –10 ⁻⁶	10 ⁻⁷ –10 ⁻⁶			
log <i>k</i>	2.66	6.03	6.75			
CC (Cu/C)/mol g ⁻¹	63.14	0.02	0.01			
<i>FQ^a</i>						
TOC/mg l ⁻¹	0.24	0.72	0.24	0.72		
Cu added/mol l ⁻¹	10 ⁻⁴ –10 ⁻³	10 ⁻⁴ –10 ⁻³	10 ⁻⁵ –10 ⁻⁴	10 ⁻⁵ –10 ⁻⁴		
log <i>k</i>	3.52	3.62	4.52	4.71		
CC (Cu/C)/mol g ⁻¹	0.11	0.09	0.04	0.06		

^a pH = 6.75.Fig. 1. Titration curves of dissolved organic carbon (pH=4, NaNO₃: 0.1 N, TOC: 0.24 mg l⁻¹) obtained by ISE (○) and ASV (□).

Because pH is impossible to monitor and keep constant during the fluorometry titration process, the log *k* values measured by FQ at natural pH levels (initial value = 6.75), were higher than those produced using ISE (total Cu from 10⁻⁵ to 10⁻³ mol l⁻¹) at lower pH levels (4, 5, and 6). A comparison of fluorescence and ISE results for Cu²⁺-natural organic carbon complexes appears in Fig. 2. The x-axis variable is the concentration of total copper ions added. The y-axis variable for ISE titration (left) is complexed copper concentration and the y-axis variable for the fluorescence titration (right) is the fluorescence intensity quenched, or the relative amount of bound ligand which should ideally be directly proportional. In fact, the fluorescence of organic molecules was diminished when bound with heavy-metal ions, although the results gained here don't coincide too closely with

Fig. 2. Comparison of Cu²⁺ titrations into dissolved organic carbon (pH=6, NaNO₃: 0.1 N, TOC: 0.72 mg l⁻¹) as performed by ISE (○, left-hand scale) and by FQ (□, right-hand scale).

those obtained by other researchers (Ryan and Weber, 1982). The slope of the former curve in the initial stage is higher than for the later. One reason for this may stem from the intrinsic chemical heterogeneity of the macromolecular which contain different function groups with different physico-chemical properties and other factors may affect the quantitative interpretation of quenching interaction such as the concentration of the samples and the potential formation of polynuclear complexes (*M_nL_m*) in which the metal ions act as the central group. The FQ method assumes that the quenching is only due to the formation of 1:1 stoichiometry complexes but divalent copper ions may possibly combine with two separate binding sites on the same molecule, forming a more stable internal ring structure (Joaquim et al., 1998). As

such, it was not possible to obtain binding parameters through the proportionality constant calculated using the relationship between the percentage quenched and complexed copper. Conversion coefficient was introduced in order to determine the binding constants.

All of the binding parameters obtained using various methods have been listed in Table 3. Due to their various added copper contents, the binding constants measured using ASV are higher than those obtained by FQ, while the complex capacity (CC) appear to have an adverse trend. Different concentration ranges of total Cu (10^{-7} – 10^{-5} mol l⁻¹ and 10^{-5} – 10^{-3} mol l⁻¹) were designed in ISE titration analysis. The results indicate that a definite rising trend for total Cu leads to a lowering of the stability constant. Under similar copper addition concentrations, the log *k* determined by ASV (total Cu from 10^{-7} to 10^{-6} mol l⁻¹) was close to that obtained by ISE (total Cu from 10^{-7} to 10^{-5} mol l⁻¹). It is important to remember that the parameters mentioned above are difficult to compare directly given the high degree of correlation among them, i.e. values of log *k* and CC have a negative correlation. As such, the decrease in one parameter can be readily compensated for by an increase in the other while fitting the data to roughly the same degree (Brown et al., 1999). Consequently, although the data are fitted well, the parameter estimates are not robust. The CC is deficient and unable to accurately represent the actual ligand sites.

The results indicate that ISE, ASV, and FQ can be used to study the complexation of copper ions by organic ligands. The difference in calculated parameter values is primarily due to the variation of added copper concentrations. When the concentration of copper is above 10^{-7} mol l⁻¹, ISE is the most applicable technique since this method allows for direct measurement of copper concentration, avoiding the dissociation of weak organic complexes and disturbances associated with the addition of buffers. In the subsequent study, ISE was used exclusively.

3.2. Influence of experimental conditions

Stability constants influenced by pH, ionic strength, and the concentration of reactants are reported as conditional constants. The results of this study on the WSOC values from Song Ziyuan and DOC values from Yong Wei are similar to those obtained by other researchers in this research field (Wood, 1996; Witter et al., 1998; Pandey et al., 2000; Johan et al., 2001; Bryan et al., 2002; Joaquim et al., 2002).

3.2.1. pH

A number of titrations were carried out at pH values of 4.00 ± 0.02 , 5.00 ± 0.02 , and 6.00 ± 0.02 , respectively. As expected, the log *k* values increased with increases in pH, primarily as a result of dissociation in the acidic

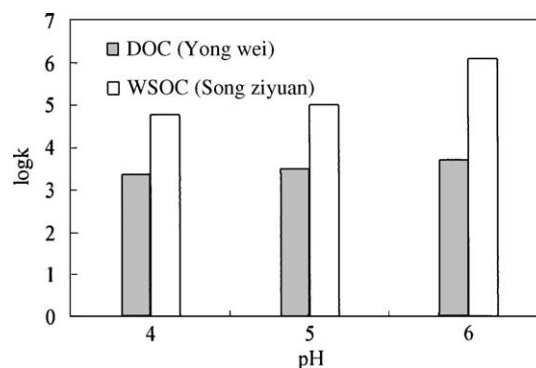


Fig. 3. Conditional stability constant under different pH values (Cu added: 10^{-5} – 10^{-3} mol l⁻¹; NaNO₃: 0.1 N; DOC: 0.284 mg l⁻¹; WSOC: 0.276 mg l⁻¹).

functional groups. The negative charge on the molecules increased to produce an electric field attracting the copper ions and lowering the level of competition of protons for copper ions; leading to increase in log *k* (Fig. 3) while the CC did not change much (DOC: 1.666, 1.453, 1.578 mol g⁻¹C; WSOC: 0.079, 0.082, 0.064 mol g⁻¹C respectively). Many other researchers observed the same phenomenon in their studies (Stevenson et al., 1993; Bryan et al., 2002; Joaquim et al., 2002).

3.2.2. Ion strength

The binding ability of natural organic carbon for Cu is believed to increase with decreases in ionic strength. Same result was obtained in this study (Fig. 4). Complexation at one site will decrease the tendency of a neighboring functional group to complex with another metal ion electrostatically and increasing counter ion condensation in the diffuse double layer of the macromolecule will also weaken the affinity of the binding site (Stevenson et al., 1993; Bryan et al., 2002), so the binding ability was lower at high ionic strength.

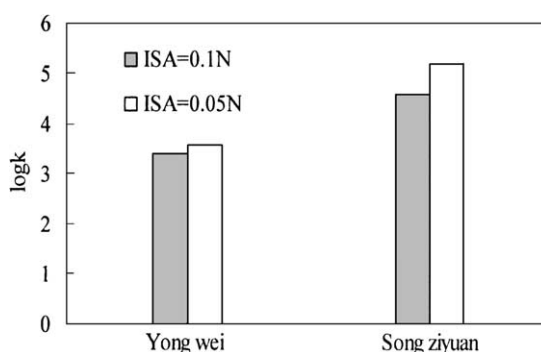


Fig. 4. Influence of ionic strength on Conditional stability constant (Cu added: 10^{-5} – 10^{-3} mol l⁻¹; pH = 4; DOC: 0.284 mg l⁻¹; WSOC: 0.276 mg l⁻¹).

3.2.3. Reactant concentration

With regard to comparisons of different analytical techniques, we have drawn the conclusion that reactant concentration has an effect on the binding constant. In this part of the work, ISE was used solely for changes in the reactant concentration of copper ions and natural organic carbon. The ligand function groups are mixture of ligands, rather than a single well-defined complexing agent and exhibit as approximate continuous distribution of thermodynamic stability constants. At low metal loadings, the strongest binding sites will be satisfied first, followed by successively weaker binding sites. Thus, the intensity of metal binding changes as a function of metal or ligand content. Otherwise, binding at one site will affect binding at all subsequent sites (Wood, 1996; Filella and Town, 2000). As shown in Table 4, the binding constants descend while the copper ion content rises. At low copper content levels, those sites forming the most stable complexes reacted first and appear to have a strong affinity for the metal ions. As the stronger sites became saturated, binding at the weaker sites became an increasingly important factor in lowering the average stability constant. Another issue for consideration is that configurational changes in the macromolecule may accompany changes in pH or reactant concentrations and neutral salts. The macromolecular ligands behave like “spherocolloids” at high reactant concentrations, low pH levels, or at high concentrations of electrolyte. On the other hand, they behave like flexible linear colloids at low ligand concentrations, high pH levels, or at low ionic strengths lowering its binding capability with copper (Stevenson, 1994).

3.3. Binding parameters of the various samples

It has been recognized that the introduction of natural organic ligands into thermodynamic models is not yet realistic, since the chemical nature of the naturally occurring organic molecules is very complex and still rather poorly described (Wood, 1996). All of the natural organic ligand samples from the different sources used

here were polydisperse mixtures containing different functional groups—carboxylic, phenolic, amino, sulphydryl and quinone—that may be present and act as binding sites, each of which exhibits a characteristic chemical affinity for heavy metals. The variations in composition and structure can lead to variations in the binding property.

Humus substances are structurally similar, but differ in that the FA fraction tends to have lower molecular weight and higher oxygen content relative to HA. DOC and WSOC are water soluble and have the semblable behavior as FA.

In this study, binding parameters of DOC, WSOC, FA, HA from various sources were measured by ISE at a pH level of 4.00 ± 0.02 , a copper ion titration concentration range from 10^{-5} mol to 10^{-3} mol l⁻¹, and electrolyte concentration of 0.1 N NaNO₃. The results are shown in Table 5, from which we conclude that the affinity of the ligand for copper followed the order: FA > WSOC > DOC = HA. WSOC was extracted from soil freshly and contain more relatively higher molecular weight fractions with stronger binding sites (carboxyl, phenolic etc.). A majority of DOC in water derives from soil as allochthonous input but some of them has decomposed to lower weight molecular with weaker binding site (amino, sulphydryl etc.) in water. So, the log *k* for the WSOC concentrations from the two sample sites (4.66 and 4.64 respectively) appear to be higher than those obtained for DOC (3.28–4.13). Their CC values are 0.079 and 0.023 mol g⁻¹C, much lower than the DOC values (1.058–1.666 mol g⁻¹C). HA's stability constant was also lower than that for WSOC, but access to DOC may be influenced by the content of the ligand site. Most of the log *k* values shown in Table 5 fall within the ranges reported by other researchers (Krajnc et al., 1995; Witter et al., 1998; Filella and Town, 2000; Town and Filella, 2000; Johan et al., 2001; Joaquim et al., 2002; Nierop et al., 2002), except for the unusually high stability constants of two FA samples. This may have been caused by unavoidable pollution from the cation exchange resin during the purification process. As such,

Table 4
log *k* values influenced by the content of the reactant (pH = 4; NaNO₃: 0.1 N)

	Cu added/mol l ⁻¹	TOC/mg l ⁻¹	log <i>k</i>	CC (Cu/C)/mol g ⁻¹
DOC (No. 4)	10 ⁻⁴ –10 ⁻²	0.28	2.75	14.10
		0.57	2.20	10.99
		0.85	2.10	4.734
		0.28	3.38	1.666
	10 ⁻⁵ –10 ⁻³	0.57	3.43	0.856
		0.85	3.38	0.738
WSOC (No. 1)	10 ⁻⁵ –10 ⁻³	0.28	4.66	0.079
		0.55	4.41	0.055
		0.83	4.61	0.075

Table 5

Stability constants of different samples (Cu added: 10^{-5} – 10^{-3} mol l⁻¹; pH = 4; NaNO₃: 0.1 N)

Samples		log <i>k</i>	CC (Cu/C)/mol g ⁻¹	TOC/mg l ⁻¹
DOC	No. 1	4.03	1.634	0.25
	No. 2	4.13	1.058	0.69
	No. 3	3.96	1.648	0.25
	No. 4	3.38	1.666	0.28
	No. 5	4.14	1.386	0.23
WSOC	No. 1	4.66	0.079	0.28
	No. 3	4.64	0.023	0.72
FA	No. 1	5.30	0.012	2.25
	No. 3	10.01	0.006	5.33
HA	No. 1	3.99	0.101	5.86
	No. 3	4.49	0.048	10.82

the CCs of the FAs were all excessively low because of correlation among the parameters.

The complex capability (CC) of DOC was the highest, and all of the sample CC values from Song Ziyuan were higher than those from Xin Niantan. This may have been caused by the difference between the samples and the initial content of the binding site during the titration. If the negative relation between the stability constant and the CC is considered, it can be inferred that under the same reactant concentrations, the CC of all samples may be in the same range.

4. Conclusion

ISE, ASV, and FQ were all used to study the complexation of copper ions by organic ligands. The calculated parameters varied due to changes in added copper concentrations. Of the various techniques, ISE was the most applicable as long as the concentration of copper was above 10^{-7} mol l⁻¹. It was then used in all subsequent studies to directly measure the copper concentration and avoid the dissociation of weak organic complexes as well as disturbances from the addition of buffers. The binding affinities of the organic ligands from Yong Wei and Song Ziyuan for copper ions were influenced by pH, ion strength, and reactant concentration. Values for log *k* increased with the increases in pH. At pH 4, log *k* decreased with increases in the supporting electrolyte concentration and the total amount of copper ions added.

The binding constants of copper for DOC, WSOC, FA and HA from the various sources were calculated using discrete single site model. The log *k* values for the two WSOC samples were higher than those for DOC and HA, and lower than that for FA, that may be influenced by the initial content of the binding site during the titration and negative relation between the stability constant and the CC.

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