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Ultraviolet absorbance titration for determining stability constants of humic substances with Cu(II) and Hg(II)

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ABSTRACT

We describe an ultraviolet (UV) absorbance titration method that can be used to determine complexing capacities (C_L) and conditional stability constants (log K) of humic substances (HSs) with metal ions such as Cu(II) and Hg(II). Two fulvic acids (FA) and one humic acid (HA) were used for this study. UV absorbance of HSs gradually increased with the addition of Cu(II) or Hg(II) after blank correction, and these increases followed the theoretical 1:1 (ligand:metal ion) binding model. The results from the absorbance titration calculation for HSs with Cu(II) and Hg(II) compared well with those from fluorescence quenching titration. The titration of the model compound L-tyrosine with Cu(II) proved the validity of this method, and the K and C_L were within 2.3% and 7.4% of the fluorescence quenching titration. The results suggest that the UV absorbance titration can be used to study the binding capacities of HSs and/or dissolved organic matter (DOM) with trace metals. The advantages and disadvantages of the absorbance titration method were also discussed.

1. Introduction

Dissolved organic matter (DOM) is a complicated mixture of organic macromolecules with a variety of building blocks and functional groups, and it is ubiquitous in water, soils and sediments. Humic substances (HSs) are the major component of DOM, and account for up to 50–80% of DOM, in terms of dissolved organic carbon (DOC), in natural waters [1,2]. According to its solubility, HSs can be separated into fulvic acid (FA, soluble at all pHs), humic acid (HA, soluble in alkaline media and insoluble at pH 1), and humin (insoluble at all pHs) [3]. DOM played a key role in influencing the solubility, mobility, bioavailability and toxicity of trace metals in aquatic environments because of its strong binding with metal ions [4–7]. For example, DOM mainly controlled copper speciation in both ocean and freshwater [8], and it was also reported that DOM played a key role in the biogeochemical cycling of mercury in aquatic environments [6,7,9].

The interaction between DOM and metal ions has been intensively studied with many analytical methods, e.g., anodic stripping voltammetry, ion selective electrode potentiometry, equilibrium dialysis, ultrafiltration and fluorescence quenching titration [6,7,10–16]. UV (ultraviolet) absorbance titration method has only been applied to calculate the binding constants between pure chemical compounds, e.g., (−)-epigallocathechin gallate and nanomolybdomanganate (IV) anion, with metal ions such as Al(III), Mg(II) and Li(I) [17,18]. The absorbance titration method measures the effect

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of metal ion binding on organic ligand directly and allows the calculation of the binding constants using theoretic fitting models. The intrinsic absorbance increased or decreased due to the binding of compounds with metal ions. However, it is unknown whether this technique could be applied to the naturally occurring DOM or HSs, which generally do not have characteristic peaks in their absorbance spectra.

This study proves the validity of UV absorbance titration method in investigating the interaction between HSs and metal ions, e. g., Cu(II) and Hg(II). The assumption of 1:1 stoichiometry and nonlinear fitting model equation allow the calculation of conditional stability constants ($\log K$) and complexing capacities (C_L). In addition, the results will also be compared with those from fluorescence quenching titration.

2. Theoretical fitting models

The 1:1 stoichiometric model has been successfully applied to study the binding abilities between HSs and metals such as Al, Cu and Hg [6,7,10]. Therefore, the reaction between HSs (L) and metal ion (M) can be quantitatively described by

$$M + L \leftrightarrow ML \quad (1)$$

The corresponding conditional stability constant (K) at a certain experimental condition can be represented by

$$K = \frac{[ML]}{[L][M]} \quad (2)$$

The Ryan–Weber nonlinear equation (Eq. (3)) was widely used in studying DOM binding with metal ions in fluorescence quenching titration method [10,19].

$$F = \frac{F_{end} - 100}{2KC_L}\left[(KC_L + KC_M + 1) - \sqrt{(KC_L + KC_M + 1)^2 - 4K^2C_LC_M}\right] + 100 \quad (3)$$

F_0 (equal to 100), F and F_{end} are the fluorescence intensity without metal ion added, with metal ion added, and the lower limiting fluorescence intensities after metal ion titration, respectively. Similarly, the nonlinear equation can be used in the absorbance titration calculation, and the measured UV absorbance intensity and the total concentration of metal ion added can be written as:

$$I = \frac{I_{end} - 100}{2KC_L}[(KC_L + KC_M + 1) - \sqrt{(KC_L + KC_M + 1)^2 - 4K^2C_LC_M}] + 100 \quad (4)$$

where I is UV absorbance intensity with metal ion present. The UV absorbance intensity without metal ion added was set on 100 units. I_{end} is the limit of UV absorbance intensity after all the ligands were bound. C_L and C_M are the total concentration of ligand and metal ion, respectively. Both K and C_L can be calculated in Eqs (3) and (4) using Matlab™ 6.1 (The MathWorks Inc., MA, USA).

3. Experimental

3.1. Reagents and chemicals

All chemicals were AR grade unless mentioned otherwise. All solutions including 0.20 and 0.018 mol L^{-1} $CuSO_4{\cdot}5H_2O$, 0.01 and 0.05 mol L^{-1} of $HgCl_2$, 0.1 mol L^{-1} of NaOH, and 0.1 mol L^{-1} of $HClO_4$ were prepared using Milli-Q water (18.2 MΩ cm, Millipore Corp., MA, USA). The concentrations of both $CuSO_4$ and $HgCl_2$ titrants were standardized with an EDTA solution [20].

3.2. Samples and preparation

Two fulvic acid samples were isolated from a landfill leachate (LLFA) and lake surface waters (LSFA), respectively by the standard IHSS isolation method (http://www.ihss.gatech.edu). The landfill leachate was collected in Guiyang City, China in April 2005. The lake surface water was collected in Lake Baihua, Southwestern China Plateau in March 2004, DOC and pH were 3.46 mg L^{-1} and 8.0, respectively. Humic acid was Amherst humic acid, and its isolation method and characteristics were reported in previous studies [21,22]. FA and HA sample were purified with XAD-8 resin (Rohm and Haas Corp., Bellefonte, PA, USA) [23]. Then FA and HA solutions were adjusted to DOC = 5.0 mg L^{-1}, and ionic strength of 0.1 mol L^{-1} $KClO_4$. L-tyrosine (Biological Grade) was used as a model compound in the experiments [10]. All solutions were re-filtered through 0.45 μm glass microfiber membranes (pre-combusted at 500 °C for 5 h, Whatman Corp., Maidstone, UK) before titration experiments.

3.3. Apparatus

UV absorbance was measured with a UNICO UV-2000 spectrophotometer (Unico Corp., Shanghai, China) equipped with a 3- or 5-cm quartz cell. Fluorescence spectra were recorded with a fluorescence spectrophotometer (Hitachi Corp., Model F-4500, Tokyo, Japan), which was corrected according to the manufacturer's instruction. All experiments were maintained at a temperature of 25 ± 0.5 °C in a water bath. pH was measured using a pH meter (Orion 818, Orion Corp., WI, USA). DOC concentrations were measured by a high temperature catalytic oxidation method (High TOC/N II, Elementar Analysensysteme GmbH, Hanau, Germany) with potassium hydrogen phthalate as the standard [24].

3.4. UV absorbance and fluorescence spectra of tyrosine and HSs

Fig. 1 shows the absorbance spectra of tyrosine and HSs solutions with and without the addition of Cu(II) after blank correction. For tyrosine, there was a major peak at 276 nm (Fig. 1a). With the gradual addition of Cu(II), the absorbance peak wavelength of tyrosine remained constant. However,

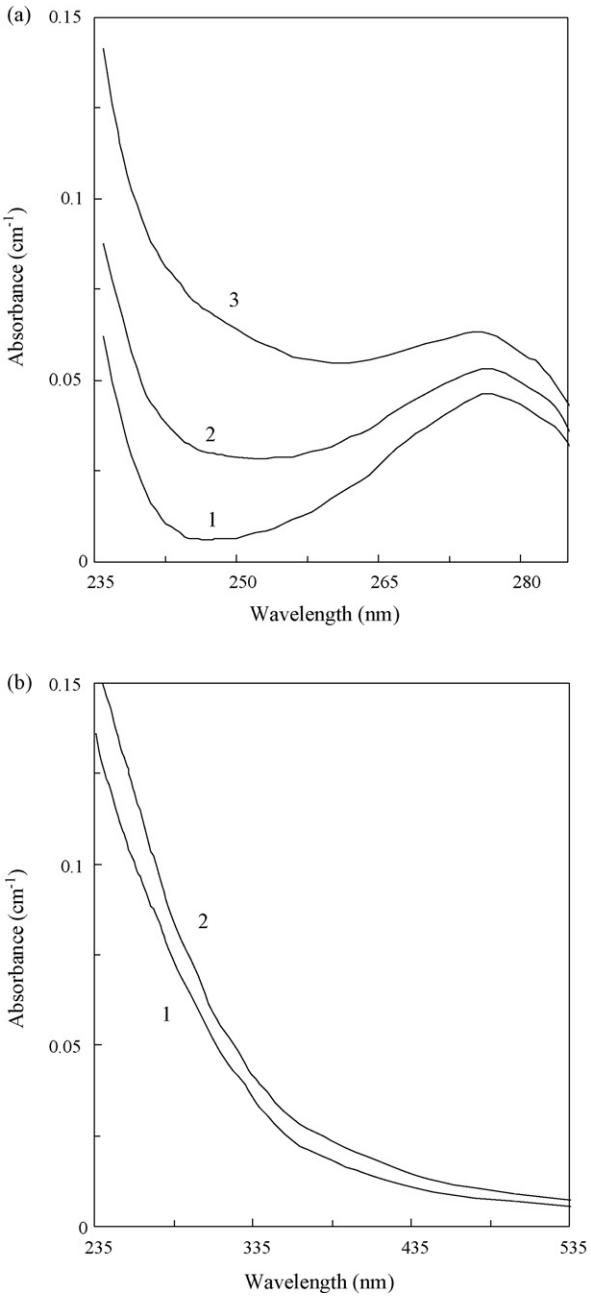

Fig. 1 – The absorbance spectra of compounds with and without Cu(II) after blank-subtracted. (a) L-tyrosine with Cu(II) (1: 0, 2: 12 μmol L^{-1}, 3: 50 μmol L^{-1}) and (b) LLFA with Cu(II) (1: 0, 2: 300 μmol L^{-1} at pH 6.0, 0.1 mol L^{-1} $KClO_4$).

there were no obvious peak maxima in the absorbance spectra for all HS solutions, and absorbance gradually decreased as wavelength increased (Fig. 1b), which is consistent with many previous reports and is attributed to the large number of chromophores and complex structures of DOM [25,26]. The wavelengths at around 250 and 280 nm were often used to study the spectral properties of DOM [27,28]. In this study, wavelengths at 246, 254 and 276 nm were chosen to monitor the spectral changes of both tyrosine and HSs solutions during the titration experiments.

Table 1 – The equilibrium parameters calculated from fluorescence quenching titration in 0.1 mol L^{-1} $KClO_4$ medium at 25 °C

Metal ions	Samples	PH	Wavelengths (nm)[a]	log K	F_{end}
Cu(II)	Tyrosine	6.0	277/296	4.76 ± 0.02	0.7
	LLFA	6.0	308/428	4.31 ± 0.03	46.9
	LLFA	7.0	304/428	4.95 ± 0.05	50.9
	LSFA	6.0	304/418	4.45 ± 0.03	48.2
Hg(II)	LLFA	4.0	306/430	3.60 ± 0.04	79
		6.0	304/426	4.57 ± 0.02	61.3
	HA	6.0	268/516	4.94 ± 0.01	32.9

F_{end}: fluorescence intensity at the end of the titration experiments; a: the fluorescence peak wavelengths of tyrosine and HSs.; LLFA: fulvic acid from a landfill leachate; LSFA: fulvic acid from lake surface water; HA: an Amherst humic acid

Table 1 shows the excitation/emission (Ex/Em) wavelengths applied in the fluorescence quenching titration experiments of the HSs. For tyrosine, the fluorescence intensity at Ex/Em 277/296 nm was used to monitor the fluorescence intensity changes during the titration experiments [10]. Rayleigh scattering was related to the number of particles in solution [7,10], and its intensity was simultaneously obtained at Ex/Em 400/400 nm to monitor possible precipitate formation during the titration experiments. Both fluorescence and UV absorbance spectra were blank-subtracted during the titration experiments. Fluorescence intensity was expressed in an arbitrary unit. Three independent experiments were carried out, and results were reported as their average.

3.5. *Cu(II)–HS and Hg(II)–HS experiments*

The basic kinetic experiments showed that Cu(II)–HS and Hg(II)–HS reactions reached equilibrium within 15 min and 24 h, respectively, which is consistent with prior reports [6,7,10]. The reaction between Hg(II) and HSs is relatively slower than that between Cu(II) and HSs, which is probably attributed to the static conditions and the slow replacement of Hg-binding OH^- by organic ligand [6]. Equilibrium time was set 15 min and 24 h, respectively, for Cu(II) and Hg(II) titration experiments.

The 100 mL solutions of tyrosine and FAs were used in Cu(II) titration with 0.1 mol L^{-1} in $KClO_4$ as the blank control. The titration experiments were conducted as follows: (1) pH adjustments were performed to the desired value within ±0.05 units using KOH and/or $HClO_4$, (2) adequate stirring was maintained for 15 min with a magnetic stir bar, (3) both UV absorbance and fluorescence intensity were monitored simultaneously, and (4) a certain volume of Cu(II) titrant was added and the process was repeated.

For Hg(II) titration, a gradual series of Hg(II) solutions were added to HS and 0.1 mol L^{-1} in $KClO_4$ (as a blank control), respectively. All mixed solutions were shaken, and stored in the dark for about 24 h at 25 °C after pH adjustment. UV absorbance and fluorescence intensity were then measured, respectively.

4. Results and discussion

4.1. *Comparison of absorbance and fluorescence quenching titration*

After the blank correction, the absorbance of both tyrosine and HSs gradually increased during initial titration, however, the absorbance did not increase significantly for concentrations of metal ions higher than 100 μmol L^{-1} (Fig. 2). This is consistent with previous reports that UV absorbance of organic chemicals is enhanced by the addition of metal ions [17,18]. The

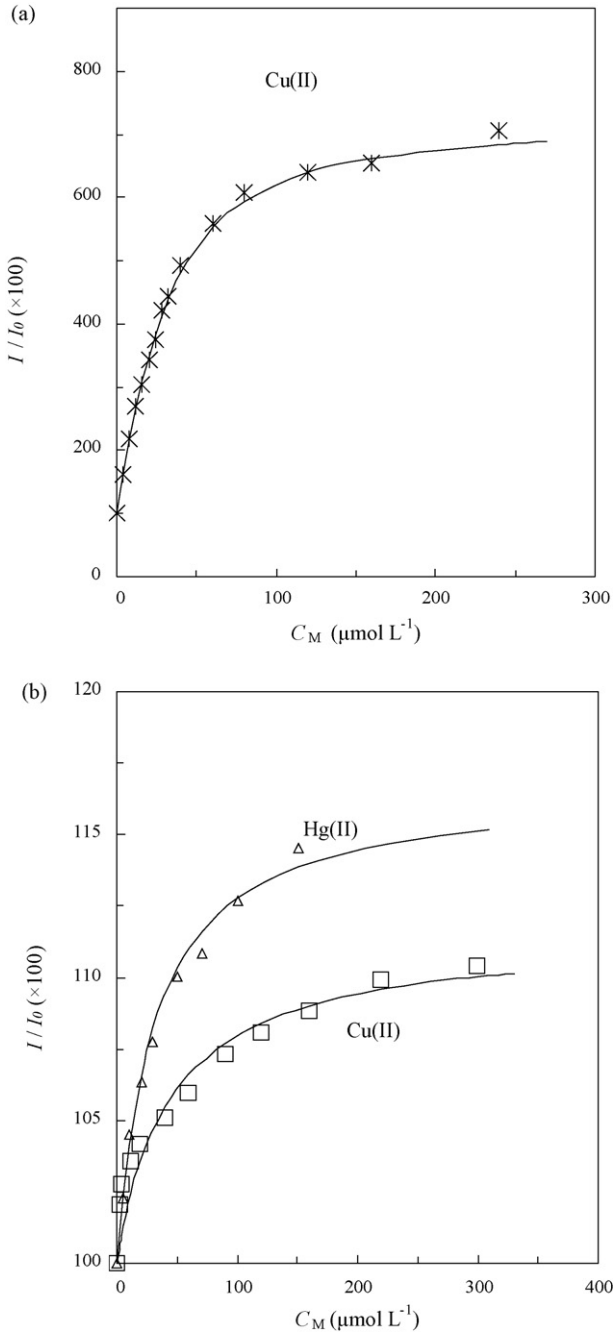

Fig. 2 – The absorbance titration curves in 0.1 mol L^{-1} $KClO_4$ at 25 °C and pH 6.0 for (a) tyrosine titration with Cu(II) and (b) LLFA titration with Cu(II) and Hg(II).

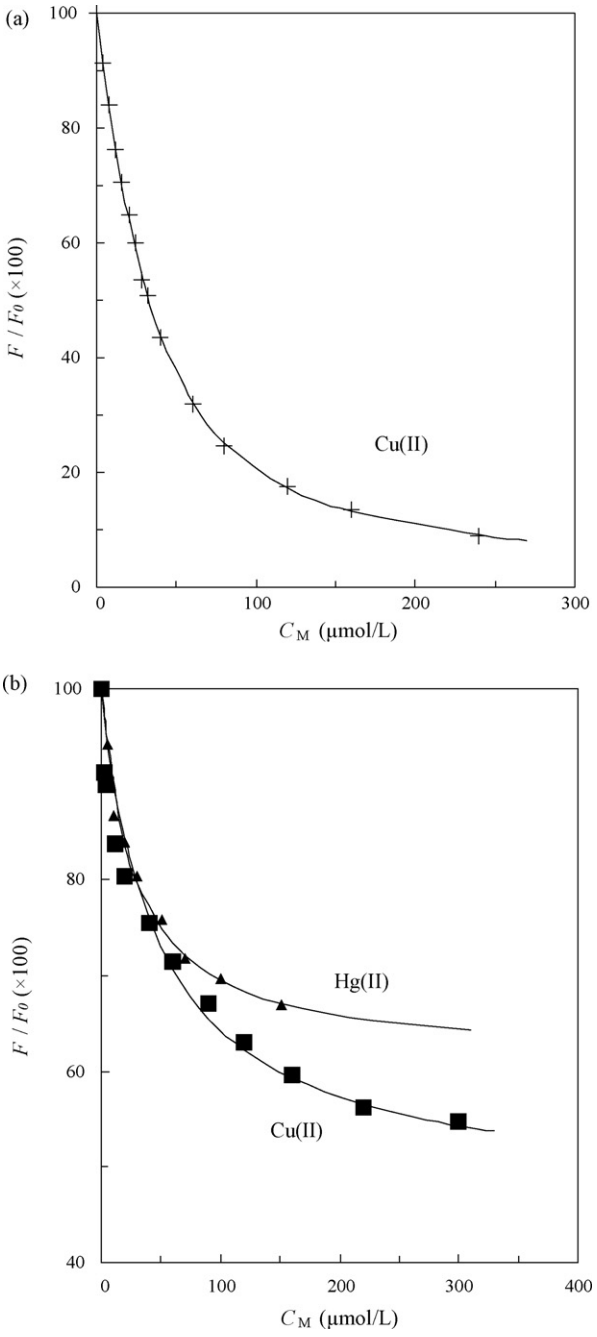

Fig. 3 – Fluorescence quenching titration curves in 0.1 mol L^{-1} $KClO_4$ at 25 °C and pH 6.0 for (a) tyrosine titration with Cu(II) and (b) LLFA titration with Cu(II) and Hg(II).

increase trend in absorbance of tyrosine and FA titration with Cu(II) was the quickest at 246 nm, and the slowest at 276 nm. The fluorescence intensity of both tyrosine and HSs gradually decreased during initial titration, however, the fluorescence intensity did not decrease significantly for concentrations of metal ions higher than 100 μmol L^{-1} (Fig. 3). This is a common phenomenon during fluorescence quenching titration of HSs with metal ions, and agrees with many previous studies [7,10]. Rapid increase in absorbance and decrease in fluorescence intensity of tyrosine titration with Cu(II) were observed

in comparison to those of HS titration with Cu(II) and Hg(II) (Figs. 2 and 3). That is, metal ions influenced the tyrosine more efficiently than complex HSs. Absorbance of LLFA titration with Hg(II) increased more quickly than that with Cu(II) (Fig. 2b), while fluorescence intensity decreased more slowly than that with Cu(II) (Fig. 3b), indicating different complexing capacities of Cu(II) and Hg(II) with LLFA.

There were significant and negative correlations between absorbance and fluorescence intensity during the titration ($R^2 = 0.99$, $p < 0.001$) (Fig. 4). It was reported that HS binding with metal ions may be related to some functional groups containing lone pairs and π bonds electrons such as —COOH, —OH, and aromatic structures [16,29], these possible complexations increased dislocation of electrons, thus resulting in the increase of absorbance. On the other hand, the intrinsic fluorescence of HSs quenched by metal ions, e. g., Hg(II) and Cu(II) could be attributed to changes of the electronic polarization of both the metal ion and the binding site in HS molecules and effects of paramagnetic metal ions [6]. Fluorescence quenching titration was a powerful method to investigate complexing capacities, stability constants, and the possible mechanisms of DOM binding with trace metal ions. The simultaneous changes in absorbance and fluorescence intensities observed in this study suggest that the formation of complexes between HSs and metal ions has occurred during the titration experiments. More importantly, this indicates that DOM absorbance can be influenced by metal ions.

4.2. *Conditional stability constants and complexing capacities*

Similar to the data treatment for fluorescence quenching titration method [10,19], nonlinear fitting model (Eq. (4)) was applied to UV absorbance titration. The calculated stability constants and complexing capacities are shown in Table 2.

4.2.1. *Conditional stability constants and complexing capacities of tyrosine with Cu(II)*

With the absorbance titration method, C_L values were calculated to be 23.9, 26.1 and 25.3 μmol L^{-1} measured at wavelengths of 246, 254 and 276 nm, respectively. These values are close to the experimental concentration (26.0 μmol L^{-1}), and also close to the value simultaneously obtained with fluorescence quenching titration method (24.3 μmol L^{-1}). log$K$ values were 4.75, 4.77, and 4.77 measured at wavelengths of 246, 254 and 276 nm, respectively. The results are consistent with those simultaneously and independently determined using fluorescence quenching titration (4.76, Table 1), the first conditional stability constant calculated from the thermodynamic stability constant and acid dissociation constants (4.77, the theoretical value) [30], and that measured by Ryan and Weber using fluorescence quenching titration (4.76) [10]. The relative standard deviation was less than 1.5% and 5.6% for log$K$ and C_L, respectively, in triplicate titration experiments.

4.2.2. *Conditional stability constants of HSs with Cu(II) and Hg(II)*

The log$K$ values calculated by the absorbance titration method ranged from 4.34 to 5.06 for Cu(II) and FA. This is close to log$K$ for soil-derived FAs (4.7–5.5) [10], that for anthropogenic FAs (4.2–4.5) [19], and that for HAs from pig slurry and soils calculated using fluorescence method (4.7–5.3) [16]. log$K$ values calculated with absorbance titration method ranged from 3.64 to 4.85 for Hg(II) and HSs. This is also similar to that for soil-derived HSs determined using an iodide selective electrode (4.7) [31], for natural and leached DOM (4.1–5.3) [6], and that for stream waters measured using fluorescence method (4.3–5.2) [7]. The log$K$ values calculated by absorbance agree

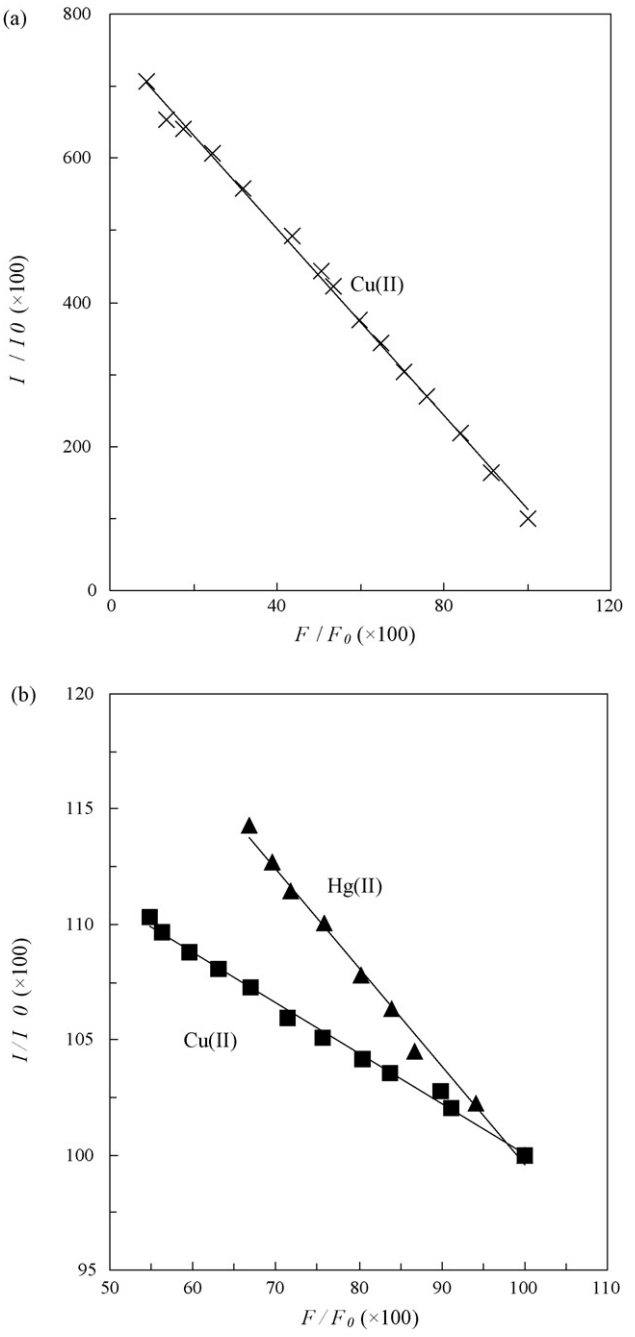

Fig. 4 – The UV absorbance vs. fluorescence intensity during the titration (in 0.1 mol L^{-1} KClO4 at 25 °C and pH 6.0) (a) tyrosine titration with Cu(II) and (b) LLFA titration with Cu(II) and Hg(II).

Table 2 – The equilibrium parameters calculated by UV absorbance titration in 0.1 mol L^{-1} $KClO_4$ medium at 25 °C

Metal ions	Samples	pH	Wavelengths (nm)	log K	I_{end}
Cu(II)	Tyrosine	6.0	246	4.75 ± 0.07	1300
			254	4.77 ± 0.04	725
			276	4.77 ± 0.05	147
	LLFA	6.0	246	4.41 ± 0.06	110
			254	4.34 ± 0.03	111
			276	4.45 ± 0.05	107
	LLFA	7.0	246	5.06 ± 0.06	107
			254	4.88 ± 0.06	110
			276	4.92 ± 0.08	110
	LSFA	6.0	246	4.37 ± 0.08	111
			254	4.46 ± 0.05	112
			276	4.46 ± 0.04	110
Hg(II)	LLFA	4.0	254	3.64 ± 0.02	116
		6.0	254	4.51 ± 0.06	117
	HA	6.0	254	4.85 ± 0.02	117

I_{end}: UV absorbance intensity at the end of the titration experiments; LLFA: fulvic acid from a landfill leachate; LSFA: fulvic acid from lake surface water; HA: an Amherst humic acid.

well with those by simultaneous fluorescence quenching titration, and the relative errors were less than 3.2% and 1.8% for Cu(II) and Hg(II) titration, respectively. In addition, log K from the absorbance titration had higher standard deviations than that from fluorescence titration, except for LLFA titration with Hg(II) at pH 4.0 (Tables 1 and 2), which is consistent with the high sensitivity of fluorescence technique. log K values increased 0.6 and 0.9 units for LLFA–Cu(II) (pH 6.0–7.0) and LLFA–Hg(II) (pH 4.0–6.0), respectively. This increase may be attributed to the protonation of the binding sites at low pH [7,10].

Unlike the results obtained for tyrosine titration with Cu(II), C_L values were extremely low for HSs with both absorbance and fluorescence quenching titration methods (less than 10^{-10} mol L^{-1}, not shown in Tables 1 and 2). The high deviation of C_L value may be caused by the low concentrations of the binding sites and/or the conditional stability constants, which is in agreement with the conclusion of Esteves da silva et al., who determined the complexing abilities of anthropogenic fulvic acids with Cu(II), Fe(III) and UO_2(II) using fluorescence quenching method [19]. The C_L variation did not obviously influence the value of log K, I_{end} and F_{end} with Matlab program, which also agrees with Esteves da silva's report [19].

The I_{end} values were 1300, 725 and 147 for tyrosine with Cu(II) calculation with UV absorbance titration at wavelengths of 245, 254, and 276 nm, respectively. For HS, the I_{end} values ranged from 107 to 117 during the titration with Cu(II) and Hg(II), respectively. The I_{end} values were relatively higher for tyrosine with Cu(II) than those of HSs with Cu(II) and Hg(II). This may be due to different structures and binding site concentrations of the model compound and the natural HSs.

Fig. 5 shows the results of Rayleigh scattering change during tyrosine and LLFA titration at pH 6.0. The scattering intensity increased gradually, but the increase was not doubled during the titration. This indicates that complexation of HS and metal ion was the major process during the experiments [10].

4.3. *The advantage and disadvantage of UV absorbance titration*

"Strong" complexing organic ligands with lower concentrations and "weak" ligands with higher concentrations were commonly observed in natural waters [24,32]. High metal ion concentrations (up to micromolar levels) are usually applied in those methods, being therefore, expected a quick saturation of the strong binding sites and that the majority of metal ions become bound to the weak sites, thus lowering the conditional

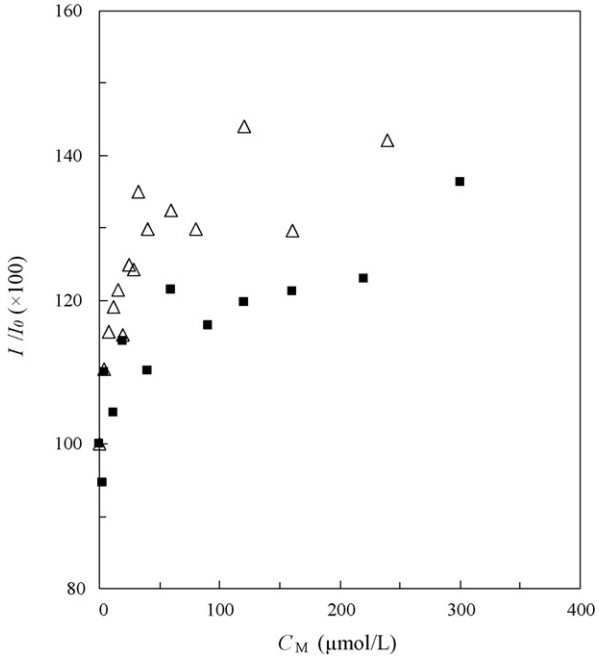

Fig. 5 – Rayleigh scattering at Ex/Em 400/400 nm in 0.1 mol L^{-1} $KClO_4$, at 25 °C for tyrosine (△) and LLFA (■) with Cu(II) at pH 6.0.

constants. Like fluorescence quenching titration method, log K determined by the absorbance titration can probably be considered as overall averaged constants [24]. Although Hg(II) and Cu(II) are soft and bordline Lewis acid, the log K values ranged 3–6 for DOM of different origins detected with UV absorbance and fluorescence titration methods, and no strong ligands were reported [6,7,10,16,19,31]. A modified data treatment process was applied to identify the "strong" and "weak" ligands of DOM during fluorescence quenching titration [32]. However, the spectroscopic sensitivity is still a limitation during the initial stage of titration, especially metal ions at relatively low concentrations.

The anodic stripping voltammetry, ion selective electrode potentiometry, equilibrium dialysis, and ultrafiltration methods were commonly used to measure the binding capacity of DOM with metal ions [6,7,10–16]. Most of these methods estimated the stability constant by the difference of total and free metal ion concentration. However, the UV absorbance and fluorescence quenching titration methods measure the effect of metal ion binding on the ligand of HSs, and do not need to measure metal ion. Generally, it seems that both UV absorbance and fluorescence quenching titration methods had similar sensitivity within the spectroscopic range for the log K calculation (Tables 1 and 2). One major advantage of the absorbance method is that it does not require expensive instruments. The UV absorbance titration offers a quick scan for the log K calculation for various HSs and metal ions, and therefore, should be considered as a good complement technique to other analytical methods.

5. Conclusions

UV absorbance titration method was used for the first time to study the binding abilities between HSs and metal ions. The absorbance titration calculation for HSs with Cu(II) and Hg(II) compared well with those from fluorescence quenching titration. Titration of model compound tyrosine with Cu(II) proved the validity of this method, and the C_L and K value errors were less than 7.4% and 2.3%, respectively. The log K calculated with absorbance titration method ranged from 4.34 to 5.06 and 3.64 to 4.85 for Cu(II)–HS and Hg(II)–HS, respectively, and the relative errors were less than 3.2% as compared to log K value obtained using fluorescence quenching titration.

UV absorbance titration method measured the conditional stability constants in terms of the difference of free and bound ligands, it is relatively rapid and simple, and should be an alternative to other methods, e.g., anodic stripping voltammetry, ion selective electrode potentiometry and equilibrium dialysis. Further research would focus on its applications to other trace metal ions and DOM or HSs of different sources.

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