**The copper complexation ability of a synthetic humic-like acid formed by an abiotic humification process and the effect of experimental factors on its copper complexation ability**

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**Abstract**

Humic acids have an important impact on the distribution, toxicity and bioavailability of hazardous metals in the environment. In this study, a synthetic humic-like acid (SHLA) was prepared by an abiotic humification process using catechol and glycine as humic precursors and a MnO2 catalyst. The effect of physico-chemical conditions (ionic strength from 0.01M to 0.5M NaNO3, pH from 4 to 8, temperature from 25 °C to 45 °C and humic acid concentration from 5 to 100mg/L) on the complexation ability of SHLA for Cu2+ were investigated. A commercial humic acid (CHA, CAS: 1415-93-6) from Sigma-Aldrich was also studied for comparison. The results showed that for pH 4 to 8, the conditional stability constants (log K) of SHLA and CHA were in the range 5.63-8.62 and 4.87-6.23, respectively and complexation capacities (CC) were 1.34-2.61 mmol/g and 1.42-2.31 mmol/g, respectively. The Cu complexation ability of SHLA was higher than that of the CHA due to its higher number of acidic functional groups (SHLA: 19.19 mmol/g; CHA: 3.87 mmol/g), extent of humification and aromaticity (AL/AR: 0.333 (SHLA); 1.554 (CHA)), and O-alkyl functional groups (SHLA:15.56%; CHA:3.45%). The log K and complexation efficiency (fraction of metal bound to SHLA) of SHLA were higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration. Overall, SHLA was a good and promising complexation agent for copper in both soil washing of copper contaminated soil and the treatment of copper-containing wastewater .

**Keywords:** Abiotic humification; synthetic humic-like acid; copper; complexation

1. **Introduction**

Hazardous metal pollution is currently a serious environmental issue worldwide (Lesmana et al., 2009; Li et al., 2014). Human activities have introduced numerous potential hazardous trace elements into the environment. Elevated concentrations of metals in soils and water originate from many sources, including atmospheric deposition, sewage irrigation, intensive use of sewage sludge and compost, tanneries, mining activities, paper industries and use of pesticides and fertilizers (Fu and Wang, 2011; Li et al., 2014; Shi et al., 2009; Su, 2014). Unlike most organic contaminants, metals are of great concern because they are non-biodegradable and can accumulate in living organisms, posing a great threat to both human health and the environment (Lesmana et al., 2009). Hazardous metals of great concern in the treatment of water and soil include zinc, copper, cadmium, nickel, chromium and lead (Li et al., 2014; Tang et al., 2014). Among them, copper does essential work in animal metabolism. However, copper is very toxic at high concentrations, and can cause gastrointestinal problems, kidney damage, cramps, hair loss, convulsions, anemia, hypoglycemia, and even death (Fu and Wang, 2011; Tang et al., 2014). Permitted levels of Cu in soils and water vary across regulators, with land and water use, with soil and water chemistry and with protection targets (e.g. EPA, 2002, 2007; NRM, 2017; Recatalá et al., 2012) such that it is not meaningful to cite a single value for an acceptable soil or water Cu concentration. However, in all legislative regions across the globe examples can be found where Cu concentrations in soils and waters are above permitted levels and need to be reduced.

Although synthetic chelating agents such as DTPA and EDTA have been proposed as remedial agents given their ability to complex metals, these compounds can have adverse environmental and health effects (e.g. Peng et al., 2009). Humic substances (HS) are organic compounds that occur naturally in soils, waters and sediments. Humic acid (HA) is a major component of humic substances (Stevenson, 1994). HA has long been recognized to strongly complex metal ions and thus have an important impact on the distribution, toxicity and bioavailability of hazardous metals in the environment (He et al., 2016; Perminova and Hatfield, 2005; Sposito and Weber, 1986; Tang et al., 2014; Yang et al., 2016). The strong complexation ability with metal ions is usually attributed to the large number of oxygen-containing functional groups present in HA, including carboxyl, phenol, hydroxyl, enol and carbonyl structures of various types (Perminova and Hatfield, 2005). Among them, carboxylic group and phenolic-OH group are of particular importance for metal ion binding (Boguta et al., 2016; He et al., 2016; Vidali et al., 2011; Xu et al., 2016). The use of humic acids as complexation agents has been considered in soil remediation and water treatment; in polymer enhanced ultrafiltration (PEUF) metals are complexed and the resulting solutions filtered through membranes which have a pore size too small to permit passage of the complexed metals resulting in a cleaned solution (Perminova and Hatfield, 2005; Fu and Wang, 2011). The potential of PEUF methods has led to a large number of studies that show that humic acid can be used to form stable complexes with Cu2+, Zn2+,Cr2+, Cd2+, Fe3+, and Pb2+ (Dudare and Klavins, 2013; Fang et al., 2015; Fuentes et al., 2013; Gustafsson et al., 2014; Hernández et al., 2006; Pandey et al., 2000; Plaza et al., 2005a). Among these metals, the stability constant between copper and humic acid is highest (Pandey et al., 2000).

HAs used in most of the reported studies are natural humic acids extracted from soil, peat or leonardite, and have poorly defined structural characteristics (Chen et al., 2015; Fuentes et al., 2013; He et al., 2016; Plaza et al., 2005a, 2005b; Xu et al., 2016; Zherebtsov et al., 2015). It has been reported that materials containing metallic oxide (e.g., MnO2, Fe2O3, and Al2O3) can catalyze and enhance the transformation of humic precursors (e.g. amino acids, sugars, and quinones) to humic substances; this process is called abiotic humification (Fukuchi et al., 2012; Fukushima et al., 2009a; Huang, 1995, 2000; Jokic et al., 2004; Nishimoto et al., 2013; Qi et al., 2012a, 2012b; Wang et al., 2000). To some extent the composition of humic acid can be controlled in abiotic humification reactions. Changing the species of humic precursors or catalyst used can alter the aromaticity and functional groups of the HA produced (Chen et al., 2010; Fukuchi et al., 2012; Hardie et al., 2009; Jokic et al., 2004). For example, the aliphatic structure content of SHLA can be increased when using Fe-loaded zeolite as the catalyst, compared with an unloaded-zeolite catalyst (Fukuchi et al., 2012) and by adding more catechol as a precursor, the N content can be reduced (Zhang et al., 2015). Controlling the humification processes raises the possibility of manipulating the composition of HAs to increase their complexation capacity for use in remediation. Recently, it has been reported that a humic-like substance associated with bentonite and synthesized by abiotic humification can be used to adsorb pentachlorophenol (Fukushima et al., 2014). However, studies on how to use synthetic HAs as metal complexing agents are still rare.

In this study, a synthetic humic-like acid (SHLA) was prepared via abiotic humification using catechol and glycine as humic precursors in the presence of a MnO2 catalyst. These were chosen because they have been used in previous studies on the abiotic synthesis of humic acid (Hardie et al., 2009; Jokic et al., 2004; Zhang et al., 2015). Cu2+ was chosen as the target metal. The complexation efficiency, conditional stability constant, complexation capacity and chemical characteristics of the SHLA were determined, and compared to a commercial humic acid available from Sigma-Aldrich. The effects of pH (4 - 8), ionic strength (0.01 - 0.5M NaNO3), temperature (25 – 45 °C ), and the concentration (5 - 100 mg/L) of SHLA on complexation between Cu2+ and the SHLA were investigated.

1. **Materials and Methods**

**2.1 Materials**

Catechol (C6H6O2, >99% purity), glycine (C2H5NO2, >99% purity), glucose (C6H12O6, >99% purity), thimerosal (C9H9HgNaO2S, >97% purity), manganese oxide (MnO2, >99% purity), commercial humic acid (CHA, technical grade, CAS: 1415-93-6) and copper nitrate trihydrate (Cu(NO3)2·3H2O, >99% purity) were purchased from Sigma-Aldrich. Sodium phosphate dibasic dihydrate (Na2HPO4·2H2O, >99% purity), sodium phosphate monobasic dihydrate (NaH2PO4·H2O, >99% purity), sodium nitrate (NaNO3, >99% purity) and barium hydroxide octahydrate (Ba(OH)2·8H2O, >98% purity) were purchased from ACROS Organics. Calcium acetate monohydrate (C4H6CaO4·H2O, >99% purity) was purchased from Alfa Aesar. Sodium hydrogen carbonate (NaHCO3, >99% purity) was purchased from Fisher Chemical. Ultra-pure water was prepared by PURELAB Classic ultra-pure system (ELGA, UK) from deionized water.

**2.2** **Preparation of a synthetic humic-like acid by abiotic humification**

To ensure that the humification was abiotic, sterile conditions were maintained throughout the experiments. Pure water and glassware were autoclaved (121 °C for 27 minutes, 0.12 MPa). 1L autoclaved phosphate buffer (pH 7, 0.2 M Na2HPO4 / NaH2PO4 autoclaved at 121 °C for 27 minutes, 0.12 MPa) containing thimerosal (0.02% w/v) was prepared in a 3L beaker, to which 25 g of MnO2 was added. Then catechol and glycine were added to give concentrations of 1 M for each of the two chemicals and the mixture stirred at 45 °C for 240 h in the dark on a heated magnetic stir plate (IKA, Germany). After incubation, SHLA was extracted from the dark brown solution and purified using the standard method recommended by the International Humic Substances Society (IHSS) (Swift., 1996). Details of the extraction and purification process of SHLA are given in the Supplementary materials.

**2.3 Characterization of synthetic humic-like acid (SLHA) and CHA**

**2.3.1 Elemental analysis**

The elemental composition (C, H, N) of the SHLA and CHA were determined using a vario MICRO cube type elemental analyzer (Elementar, Germany). The percentage of oxygen was calculated by subtracting the sum of C, H, and N from 100. Peat (NJV942, from LGC company) and organic sediment (B2150, from Elemental Microanalysis, National Institute of Standards and Technology (N.I.S.T.), U.S. Department of Commerce) standards were used as reference materials and to calculate analytical accuracy. The accuracy for C, N and H analysis was 86%-94%, 99%-103% and 86%, respectively. Sulphanilic acid (from Merck Company) was used as a drift corrector and to calculate precision. The precision, based on the standard deviation of 7 analyses carried out over the course of the analytical run for C, N and H were 0.16%, 0.54% and 0.22%, respectively. Before analyses, powdered SHLA and CHA were dehydrated under -0.1Mpa for 24 h at room temperature.

**2.3.2 FTIR**

FTIR spectra of the SHLA and CHA were obtained using an Alpha FT-IR Spectrometer (Bruker, Germany). Spectra were recorded from 4000 to 400 cm-1 with a resolution of 4 cm-1.

**2.3.3 Acid functional groups**

Total acidity and carboxylic group content were measured using the barium hydroxide and calcium acetate methods. The phenolic-OH content was calculated as the difference between total acidity and carboxylic group content (Schnitzer and Kahn, 1972).

**2.3.4 Ratio of the absorbances at 465 and 665 nm (E4/E6)**

The UV spectra of the SHLA and CHA were determined using a Lambda 25 UV/Vis Spectrophotometer (PerkinElmer, USA). Absorbance at 465 and 665 nm (E4 and E6) was recorded on solutions of 20 mg of each HA dissolved in 100 ml of 0.05 M NaHCO3, with the pH adjusted to 8 with NaOH and HCl. The E4/E6 ratio gives an indication of the degree of humification and the molecular weight of the humic acid was calculated as the ratio of the absorbances at 465 and 665 nm (Chen et al., 1977; He et al., 2016; Zalba et al, 2016).

**2.3.5 Solid-state CP-MAS13 C NMR**

Solid-state CP-MAS 13C NMR spectra of the SHLA and CHA were measured using an HD 400 spectrometer (Bruker, Germany) using the following conditions: spectrometer frequency, 100.64 MHz; contact time, 2 ms; pulse delay, 2.0 s; line broadening, 300 Hz; scans numbers, 800. The spectra were integrated into 6 chemical-shift regions: 0–45 ppm, 45–110 ppm, 110–140 ppm, 140–160 ppm, 160–185 ppm, and 185-215 ppm. The integrated areas were determined using Topspin 3.5 software. The aromaticity and AL/AR ratios (i.e. the ratio of alkyl to aromatic carbons) were calculated using equations (1) and (2) (Qi et al., 2012a):



where AAlk and AAr represent the peak integration values for alkyl and aromatic carbons, respectively.

**2.4 Determination of conditional stability constants (log *K*), complexing capacities (*CC*) and complexation efficiency**

For the complexation experiments, 100 mg SHLA or CHA were dissolved in 50 ml of 1 M NaOH, and then 50 ml of 1 M HNO3 was added. The HA solution was then diluted with ultrapure water to a volume of 1L. 50 ml of the CHA and SHLA HA solution and 50 ml of Cu(NO3)2 (2 μM, 10 μM, 20 μM, 60μM, 100 μM, 140 μM, 200 μM, 250 μM and 300 μM) in 0.2 M NaNO3 were mixed. This produced solutions at nine Cu2+ concentrations (1 μM, 5 μM, 10 μM, 30μM, 50 μM, 70 μM, 100 μM, 125 μM and 150 μM) each with an HA concentration of 50 mg/L in a background electrolyte of 0.1 M NaNO3. Experiments were performed at 25 °C and an initial pH of 4, 6, 7 and 8, obtained by addition of 0.1M NaOH or 0.1M HNO3.

Complexation experiments at different ionic strengths, used 100 mL of solution of different concentrations of Cu2+ (1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 125 μM and 150 μM) and 50 mg/L of humic acids at 25 °C and pH=6. The initial ionic strength in solution was set to 0.01M NaNO3, 0.05 M NaNO3, 0.1 M NaNO3 and 0.5M NaNO3.

Complexation experiments at different HA concentrations, used 100 mL of solutions of different concentrations of Cu2+ (1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 125 μM and 150 μM) at 25 °C, 0.1 M NaNO3 and pH=6. The initial humic acid concentration in solution was set to 5 mg/L, 50 mg/L and 100 mg/L. Complexation experiments at different temperatures, used 100 mL of solutions of different concentrations of Cu2+ (1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 125 μM and 150 μM) and 50 mg/L of humic acids at 0.1 M NaNO3 and pH=6. The initial temperature in solution was set to 25 °C, 35 °C and 45 °C.

All solutions were shaken orbitally in a water bath for 24 hours at 150 rpm and then free Cu2+ concentrations were measured using a Cu-ion selective electrode (Orion, model 9629 BNWP). The initial Cu2+ concentrations were determined on control, HA-free solutions. All the measurements were performed in triplicate. Prior to experiment, the electrode was calibrated using serially diluted Cu2+ stock solutions at the working ionic strength, following the procedures of the ISE manual (Thermo Scientific Orion, 2008). Calibration was repeated every hour, and direct electrode measurements were reproducible within ±4%.

The 1:1 stoichiometric model has been successfully applied to study the binding abilities between HA and Cu (Ružić, 1982; Tipping, 2002), and the stability constants (log *K*) and complexation capacities (*CC*) obtained represent the average properties of the ligands contained in the humic structures (Fuentes et al., 2013; Plaza et al., 2005; Ružić, 1982). Therefore, the reaction between HA and Cu (M) can be quantitatively described by equation (3).



The conditional stability constant (*K*) which represents the average binding ability was calculated as:



The mass balance equations for the ligand are



where Ct is the apparent complexation capacity (Plaza et al., 2005a).

[MHA] is calculated by mass balance as:



where M0 is the initial Cu2+ concentration and M is free Cu2+ concentration after 24h. Combining equations (4), (5) and (6) and rearranged into a linear form gives equation (7):



Linear regression analysis was performed for 1/(M0-[M]) and 1/[M] allowing K and Ct to be calculated (Ružić, 1982).

Complexation efficiency at different copper concentrations was calculated as:



where M0 is the initial Cu2+ concentration and M is free Cu2+ concentration after 24h.

**2.5 Thermodynamic study**

Log *K* values at 25℃, 35℃ and 45℃ were used to determine the thermodynamic parameters. The thermodynamic parameters (free energy change (*ΔG*), the entropy change (*ΔS*) and enthalpy change (*ΔH*)) were calculated following the method of Garcia-Valls and Hatton (Garcia-Valls and Hatton, 2003).





where *R* (8.314 J/(mol·K) is the ideal gas constant, *T* (K) is the absolute temperature, and *K* is the conditional stability constant.

**2.6 Statistical analysis**

The Shapiro-Wilk test was used to assess whether the data were subject to normal distribution. T-tests were used to determine whether there was a significant difference in complexation ability between SHLA and CHA. The non-parametric Friedman test was used to assess whether the log *K*, *CC* and complexation efficiency of SHLA changed significantly as a result of changes in pH, ionic strength, temperature and humic acid concentration. The level of significance between the data was set to P < 0.05. Data analysis was carried out using SPSS 23.0 (IBM, USA).

**3. Results and Discussion**

**3.1 Characterization and Cu complexation ability of synthetic humic-like acid (SHLA) and commercial humic acid (CHA)**

**3.1.1 Characterization of synthetic humic-like acid and CHA**

The SHLA and CHA were characterized by determining the elemental composition, acidic functional group content and E4/E6, and by recording FTIR and 13C NMR spectra. Compared with CHA, SHLA had a higher concentration of acidic functional groups, and higher degrees of humification and aromatization.

(a) Elemental composition

The elemental composition and atomic ratios of SHLA and CHA are summarized in Table 1.

Table 1. Elemental composition, acidic functional group content and E4/E6 of the SHLA and CHA (n=3; mean value ± standard deviation).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | N/% | C/% | H/% | O/% | N/C | H/C | O/C | COOH  (mmol/g) | Phenolic-OH  (mmol/g) | Total acidity  (mmol/g) | | E4/E6 |
| SHLA | 4.61±0.02 | 53.81±0.07 | 2.97±0.06 | 38.61±0.09 | 0.086±0.000 | 0.055±0.001 | 0.717±0.002 | 4.59±0.34 | 14.61±4.45 | 19.19±4.79 | 3.18±0.00 | |
| CHA | 0.94±0.02 | 46.29±0.06 | 2.99±0.09 | 49.78±0.08 | 0.020±0.000 | 0.064±0.002 | 1.075±0.003 | 1.22±0.34 | 2.65±0.34 | 3.87±0.68 | 4.32±0.01 | |

Compared with CHA, SHLA had larger C and N contents and a greater N/C ratio which generally indicates a higher degree of humification (Mahieu et al., 2000; Qi et al., 2012a; Tu et al., 2017). The O/C ratio of SHLA (0.717) was lower than that of CHA (1.075), suggesting that CHA had more oxygen-containing functional groups (Fukushima et al., 2014). The lower H/C ratio of SHLA (0.055) indicates that it has more aromatic structures than CHA (0.064) (Qi et al., 2012a).

(b) Acidic functional group content and E4/E6

Table 1 shows the acidic functional group content and E4/E6 ratio of the SHLA and CHA. SHLA contained 3.8 times as many carboxylic acids groups and 5.5 times as many phenolic hydroxyl groups as CHA. Thus, SHLA has a higher concentration of acidic functional groups than CHA, suggesting that SHLA has more hydrophilic properties than CHA (Nagasawa et al., 2016). These results, together with the higher O/C ratio of CHA indicate that the oxygen-containing functional groups in CHA may be based on ketone, ethers, aldehydes, quinones or esters (Fukushima et al., 2014).

The E4/E6 ratio is related to the degree of condensation of the aromatic carbon network, the degree of humification and the molecular weight of the humic acid (Chen et al., 1977; He et al., 2016; Zalba et al., 2016). SHLA had a lower value of E4/E6 value than CHA (3.18 vs 4.32) indicating a more aromatic structure, a higher degree of humification and a larger molecular weight (Chen et al., 1977; He et al., 2016; Zalba et al., 2016), consistent with the elemental analysis.

(d) FTIR

The FTIR spectra of SHLA and CHA are shown in Figure S1. Several difference are apparent. Assignment of the absorption bands in Figure S1 is given in Table 2. The absence of a peak at 1710 cm-1 for CHA is consistent with its lower carboxylic acid group content.

(f) Solid-state CP-MAS13 C NMR

The Solid-state CP-MAS 13C NMR spectrums of SHLA and CHA are shown in Figure S2 and show differences. Peak assignments for the spectra are given in Table 3. The compositions of the different carbon species, AL/AR ratio and aromaticity for SHLA and CHA were calculated and the results are summarized in Table 3. The percentages of both phenolic carbon (140-160 ppm) and carboxylic carbon (160-185 ppm) of SHLA were higher than those of CHA, consistent with the determination of acidic functional group contents. The alkyl carbon content of CHA (52.23%) was much higher than that of SHLA (6.67%), indicating that CHA has more aliphatic characteristics. The higher AL/AR ratio and lower aromaticity of SHLA relative to CHA indicate that SHLA has a lower degree of unsaturation (Qi et al., 2012a; Tu et al., 2017). The above results are consistent with the lower H/C ratio of SHLA determined by elemental analysis.

Table 2. Assignments of FTIR absorption bands present in the spectra for SHLA and CHA (Fukushima et al., 2009a, 2009b; Hardie et al., 2009; Rodrigues et al., 2009; Qi et al., 2012a)

|  |  |  |  |
| --- | --- | --- | --- |
| SHLA | | CHA | |
| Wavenumber/cm-1 | Assignment | Wavenumber/cm-1 | Assignment |
| 2925 | C-H stretching of aliphatic structures | 2921 | C-H stretching of aliphatic structures |
| 1715 | C=O stretching of carbonyl groups |  |  |
| 1592 | C=C ring stretching, symmetric C–O stretch of COO- and N–H | 1572 | C=C ring stretching, symmetric C–O stretch of COO- and N–H |
| 1439 | O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH3 and C-O stretch of COO- |  |  |
|  |  | 1375 | symmetric bending vibration of CH3 |
| 1175 | C–O stretching and C–O–H deformation of alcohols, phenols and ethers |  |  |
|  |  | 1008 | C-O stretching vibration in alcohols, phenols and ethers |

Table 3. Interpretation of the 13C NMR spectra of SHLA and CHA (Fernández-Gómez et al., 2015; Nagasawa et al., 2016)

|  |  |  |  |
| --- | --- | --- | --- |
| SHLA | | CHA | |
| Chemical shift /ppm | Assignment | Chemical shift /ppm | Assignment |
| 33 | Methylene carbon in long aliphatic chains | 29 | Methylene carbon in long aliphatic chains |
| 45 | Methyl and methylene groups in aliphatic rings and chains |  |  |
|  |  | 76 | Aliphatic alcohols and esters from carbohydrates and flavonoids |
| 118 | Protonated aromatic carbon, and/or aromatic carbon ortho to oxygen-substituted aromatic carbon |  |  |
|  |  | 130 | Aromatic carbon bound to H |
| 145 | Phenolic carbon |  |  |
| 171 and 183 | Carboxyl carbon | 176 | Carboxyl carbon |

**3.1.2 The Cu complexation ability of synthetic humic-like acid**

Figure 1 shows the conditional stability constants (log *K*) and complexation capacity of SHLA and CHA at pH values of 4 – 8 for HA concentrations of 50 mg/L, a temperature of 25 °C and an ionic strength of 0.1M.



Figure 1. Mean conditional stability constant (log *K*) and complexation capacity (*CC*) of SHLA and CHA (mean values, n=3 ± standard deviation).

The log *K* values of SHLA were significantly higher than those of CHA (p<0.05). The complexation capacities of SHLA were higher at pH 6 and pH 7 (p<0.05) and similar at pH 4 and pH 8, compared with CHA. The stronger Cu2+ complexation ability of SHLA was due to its composition. Carboxylic and phenolic functional groups play a significant role in binding metals (Alvarez-Puebla et al., 2004; Baken et al., 2011; Kautenburger et al., 2014; Zherebtsov et al., 2015) as do acidic functional groups (Tipping, 2002; Baker and Khalili, 2005), and all are present at higher concentrations in SHLA than CHA (Table 1). Although both SHLA and CHA contain a higher concentration of carboxylic groups than phenolic groups (Table 1), our current data do not allow us to state which of these groups is more important in the complexation reaction. The larger log *K* values of SHLA are related to the higher aromaticity and higher degree of humification of the SHLA relative to the CHA. It has been demonstrated that extremely stable, Cu2+-bearing, salicylate-like and catechol-like ring structures can be formed by an aromatic COOH and adjoining phenolic-OH or two adjoining aromatic COOH. (Fuentes et al., 2013; Hernández et al., 2006; Xu et al., 2016). Fuentes et al. report that the log *K* of Cu is positively correlated to numbers of O-alkyl groups (Fuentes et al., 2013). The NMR data indicated that the O-alkyl carbon content of SHLA was 12% higher than that of CHA.

Synthetic organic chelating agents (e.g. EDTA, DTPA, etc) and natural organic acids (e.g. oxalic acid, citric acid, and tartaric acid, etc) can also be used as metal chelate agents (Alkorta et al., 2004; Yuan et al., 2007). The sequence of log *KCu* is: DTPA (20.5-21.9) > EDTA (18.8-19.4) > SHLA (5.6-8.6) > citric acid (5.2-7.3) > oxalic acid (4.5-5.1) > tartaric acid (3.2-3.4) (Baumann, 1974; Buffle et al., 1977; Playle et al., 1993; Martell and Smith., 1974, Yuan et al., 2007).

Though synthetic chelate agents have much higher metal complexation efficiency than humic acids, synthetic chelate agents such as EDTA and DTPA can lead to adverse health and environmental effects, which restricts their use (Peng et al., 2009). Humic substances are important components of soil organic matter (Stevenson, 1994) and if SHLAs were used in soil washing, any residual SHLA left in the soil could contribute to soil organic matter. Thus the use of SHLAs has the potential to be environmentally friendly compared to using compounds such as DTPA or EDTA though clearly before their widespread use their application would need to be assessed for any possible toxic side effects. Previous research on natural humic acids from various sources (i.e. peat, soil, leonardite, etc) indicates that log *KCu* values ranged from 4.58 to 5.36 at similar reaction conditions to those considered here (Fuentes et al., 2013; Paradelo et al., 2012; Plaza et al., 2005a). The higher log *KCu* of the SHLA (5.63-8.62) suggests that it can be a reasonable substitute for natural humic acids as a metal chelating agent.

**3.2 The effect of experimental factors on the complexation of Cu2+ on synthetic humic-like acids**

The complexation ability of humic acid can be influenced by pH, ionic strength, temperature, and the concentration of humic acids (Tipping, 2002). In our tests the effect of these factors on the complexation of Cu2+ by SHLA was similar to the effects reported on natural humic acids.

**3.2.1 The effect of pH**

In this study, pH was set to 4, 6, 7 and 8 to investigate the effect of pH on complexation of Cu2+ by SHLA at an SHLA concentration of 50 mg/L, temperature of 25 ℃ and ionic strength of 0.1M (Figure 2)

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Figure 2. The effect of pH and ionic strength on complexation of Cu2+ and SHLA (a & b: log *K*; c & d: complexation capacity; e & f: complexation efficiency; SHLA concentration of 50 mg/L and temperature of 25 °C; mean values, n=3 ± standard deviation).

The complexation efficiency and log *K* increased with pH (p<0.05). This trend is consistent with studies of natural humic acid (Christl, 2012; Vidali et al., 2011; Xu et al., 2016). Proton concentration is higher at lower pH and protons will compete for binding sites with Cu2+ (Tipping, 2002; Christl, 2012). Also, at lower pH conditions the acidic functional groups of SHLA will be less deprotonated, providing fewer binding sites for Cu (Cao et al., 2004; Fang et al., 2015; Lippold et al., 2007). By increasing the pH, the negative charge of SHLA will increase as carboxylic groups and phenolic OH groups deprotonate; this enhances the electrostatic interaction between Cu2+ and the SHLA (Cao et al., 2004; Vidali et al., 2011). The complexation capacity increased from pH 4 to 6 (p<0.05), and decreased from pH 7 to 8 (p<0.05). The decrease at pH 8 might be caused by changes in the speciation of Cu due to increases in the concentration of OH- (Liu et al., 2014).

**3.2.2 The effect of ionic strength**

Experiments were conducted at ionic strengths of 0.01 M, 0.05 M, 0.1 M and 0.5 M to investigate the effect of ionic strength on complexation of Cu2+ by SHLA at the SHLA concentration of 50 mg/L, temperature of 25 ℃ and pH of 6 (Figure 2).

When ionic strength increased from 0.01M to 0.5M, both the complexation efficiency and log *K* decreased sharply (p<0.05). Complexation capacity decreased (p<0.05) when ionic strength increased from 0.01M to 0.05 M and did not change much when ionic strength increased from 0.05 M to 0.5 M. The results are consistent with other studies on natural humic acids (Tipping, 2002; Xu et al., 2016). Ionic strength can affect the electric potential and spatial structure of SHLA thereby influencing the complexation ability of SHLA (Fang et al., 2015). As ionic strength increases, counter ion condensation in the HA’s diffuse double layer increases as well, which attenuates the ability of binding sites to bind Cu2+ (Cao et al., 2004; Tipping, 2002; Vidali et al., 2011). At the same time the electrostatic repulsion between adjacent functional groups can be lowered causing the humic acid to fold up on itself to form a more compact shape (Chin and Gschwend, 1991). This reduces the diffusion of Cu2+ to binding sites (Fang et al., 2015).

**3.2.3 The effect of temperature**

Experiments were conducted at 25 ℃, 35 ℃ and 45 ℃ to investigate the effect of temperature on complexation of Cu2+ by SHLA at an SHLA concentration of 50 mg/L, ionic strength of 0.1M and pH of 6 (Figure 3).

**D:\百度云同步盘\Experiment部分\Journal paper-chapter2\Applied Geo\FIGURE4-7)\FIGURE4-7).tif**

Figure 3. The effect of temperature and SHLA’s concentration on complexation of Cu2+ and SHLA (a & b: log *K*; c & d: complexation capacity; e & f : complexation efficiency; ion strength of 0.1M and pH of 6; n=3 for each condition; error bar indicates the standard deviation).

The complexation efficiency (from 87.8% to 92.5%, p<0.05), log *K* (from 6.39 to 6.65, p<0.05) and complexation capacity (from 2.61 mmol/g to 2.72 mmol/g, p = 0.097) increased with increasing temperature.

Thermodynamic parameters for the Cu2+-SHLA complexation reaction are shown in Table 4. The positive *ΔH* indicated that complexation reaction between Cu2+ and SHLA was endothermic, which would result in the trend seen in our experiments and was consistent with previous studies (Baker and Khalili, 2003, 2005). The negative *ΔG* suggested that the Cu2+-SHLA complexation reaction was spontaneous. With increasing temperature from 25℃ to 45 ℃, *ΔG°* decreased from -4.60 kJ/mol to -5.01 kJ/mol, indicating that higher temperatures favoured complexation.

Table 4 Thermodynamic parameters for the complexation reaction between Cu2+ and SHLA.

|  |  |  |  |
| --- | --- | --- | --- |
| *T (K)* | *ΔG*(kJ/mol) | *ΔS*  (kJ/(mol·K) | *ΔH*  (kJ/mol) |
| 298.15 | -4.5976 | 0.0207 | 1.5670 |
| 308.15 | -4.7876 |  |  |
| 318.15 | -5.0114 |  |  |

**3.2.4 The effect of concentration of synthetic humic-like acid**

SHLA concentrations of 5 mg/L, 50 mg/L and 100 mg/L were used to investigate the effect of SHLA concentration on the complexation of Cu2+ by SHLA at 25 ℃, an ionic strength of 0.1M and pH of 6 (Figure 3).

The complexation efficiency and log *K* (from 5.80 to 7.34) increased with increasing SHLA concentration; the complexation capacity (from 2.79 mmol/g to 1.53 mmol/g) had the opposite trend. Increasing the concentration of SHLA increased the total number of binding sites available, thereby enhancing the complexation efficiency as more Cu2+ was able to be complexed. In the same fashion, the Cu2+ bound to SHLA per unit humic acid decreases due to the excess binding sites (Vidali et al., 2011).

**4. Conclusions**

In this study, a synthetic humic-like acid (SHLA) was prepared by an abiotic humification reaction between catechol and glycine in the presence of a MnO2 catalyst. The complexation ability of SHLA for Cu2+ and the influence of solution physico-chemical factors on the complexation reaction (ionic strength, pH, temperature and humic acid concentration) were investigated. The Cu complexation ability of the SHLA was compared to that of a commercial humic acid (CHA) from Sigma-Aldrich. The results showed that the SHLA had a stronger complexation ability than CHA and most natural humic acids, which was attributed to the higher levels of acidic functional groups, degree of humification , aromaticity, and higher O-alkyl groups content of the SHLA. The log *K* and complexation efficiency of SHLA were higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration, and the effects of solution physico-chemical factors on the complexation reaction were similar to natural humic acids. In conclusion, SHLA was a good and promising complexing agent for copper in both soil washing of copper contaminated soil and polymer enhanced ultrafiltration of copper-containing wastewater.

The composition and structure of the SHLA could be further manipulated by changing factors during the synthesizing process, such as the species of precursors, the ratio of precursors, the duration of synthesizing process and temperature, etc (Jokic et al., 2004; Zhang et al., 2015). This provides a feasible way to produce an SHLA with a better copper complexation ability. Besides, to reduce the cost of raw materials, waste containing polyphenols, sugars, amino acids or metal oxides can be considered as precursors or catalyst for abiotic humification process in the future study. For example, Brunetti et al show that SHLA can be produced from olive oil mill wastewater catalyzed by MnO2 (Brunetti et al., 2008). And some waste residue, like steel slag and municipal solid waste incineration residue, can be used as catalyst to enhance abiotic humification process (Kim and Osako, 2004; Nishimoto et al., 2013; Qi et al., 2012a)

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**Supplementary Materials**

Details of the HA extraction process and the FTIR spectra (Figure S1) and solid-state CP-MAS 13C NMR spectrums (Figure S2) are shown in supplementary materials.

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