JOURNAL OF ENVIRONMENTAL SCIENCES 27 (2015) 188–196

Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Effect of environmental factors on the complexation of iron and humic acid

Kai Fang, Dongxing Yuan*, Lei Zhang, Lifeng Feng, Yaojin Chen, Yuzhou Wang

State Key Laboratory of Marine Environmental Science, College of the Environment and Ecology, Xiamen University, Xiamen 361102, China.
E-mail: justmist@126.com

ARTICLE INFO

Article history:
Received 26 February 2014
Revised 16 June 2014
Accepted 16 June 2014
Available online 13 November 2014

Keywords:
Iron
Humic acid
Complexation
Size exclusion chromatography
Graphite furnace atomic absorption spectrometry

ABSTRACT

A method of size exclusion chromatography coupled with ultraviolet spectrophotometry and off-line graphite furnace atomic absorption spectrometry was developed to assess the complexation properties of iron (Fe) and humic acid (HA) in a water environment. The factors affecting the complexation of Fe and HA, such as ionic strength, pH, temperature and UV radiation, were investigated. The Fe–HA complex residence time was also studied. Experimental results showed that pH could influence the deprotonation of HA and hydrolysis of Fe, and thus affected the complexation of Fe and HA. The complexation was greatly disrupted by the presence of NaCl. Temperature had some influence on the complexation. The yield of Fe–HA complexes showed a small decrease at high levels of UV radiation, but the effect of UV radiation on Fe–HA complex formation at natural levels could be neglected. It took about 10 hr for the complexation to reach equilibrium, and the Fe–HA complex residence time was about 20 hr. Complexation of Fe and HA reached a maximum level under the conditions of pH 6, very low ionic strength, in the dark and at a water temperature of about 25°C, for 10 hr. It was suggested that the Fe–HA complex could form mainly in freshwater bodies and reach high levels in the warm season with mild sunlight radiation. With changing environmental parameters, such as at lower temperature in winter or higher pH and ionic strength in an estuary, the concentration of the Fe–HA complex would decrease.

Introduction

Since the demonstration of the "iron hypothesis" (Martin, 1990), iron species in natural waters have been attracting great attention due to their significant effects on oceanic primary production, nitrogen fixation and even global carbon cycles (Cooper et al., 1996; Martin and Fitzwater, 1988; Mills et al., 2004; Moore et al., 2009; Watson et al., 2000). Previous studies have found that nearly 99% of dissolved Fe is associated with dissolved organic matter (DOM), to form complicated complexes that are ubiquitous in aquatic environments and play a key role in Fe speciation (van den Berg, 1995; Hutchins et al., 1999; Macrellis et al., 2001; Nagai et al., 2004; Nierop et al., 2002; Rottmann and Heumann, 1994).

Humic substances (HS) account for around 15%–90% of the DOM in aquatic environments (McDonald et al., 2007; Obernosterer and Herndl, 2000; Thurman and Malcolm, 1981), and become the major candidates for Fe complexation due to their multifunctional groups (Garcia-Mina, 2006; Laglera et al., 2007, 2011; Senesi and Calderoni, 1988). The association between Fe and HS has been attributed to metal-bridged complexation (Batchelli et al., 2010; Bolea et al., 2006; Jones et al., 1993; Sharma et al., 2010). The formed Fe–HS complex not only controls the distribution of Fe and plays a key role in the

---

* *Corresponding author*. E-mail: yuandx@xmu.edu.cn (D. Yuan)
E-mail addresses: justmist@126.com (K. Fang), 460203861@qq.com (L. Zhang), 635847814@qq.com (L. Feng), chenyaojin05xd@163.com (Y. Chen), 199205061@qq.com (Y. Wang).

http://dx.doi.org/10.1016/j.jes.2014.06.039

transport and fate of Fe in natural waters (Kitayama et al., 2009; Tipping et al., 2002), but also affects the existence of species and transformation of some pollutants, such as glyphosate and arsenic (Piccolo et al., 1992; Liu et al., 2011). Environmental factors have great influence on the complexation of Fe and HS and thus affect the concentration of the Fe–HS complex in natural water. Therefore, the study of environmental effects on complexation has become an especially important research issue. However, reports are few in the literature, and most of the research still consists of studying the properties of Fe–HS complexes and has not focused on the influence of environmental factors on the complexation process.

Nowadays, competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV) is the most popular method for the study of the Fe–HS complex (Batchelli et al., 2010; Laglera et al., 2007, 2011; Laglera and van den Berg, 2009; Yang and van den Berg, 2009). However, CLE-ACSV can only indirectly reflect the properties of the complex because an artificial iron ligand is needed in the detection. Some discrepancy of results could occur with different artificial iron ligands and could be induced by interaction between artificial iron ligands and HS (Laglera et al., 2011). The factors affecting the complexation process such as pH and ionic strength are still difficult to investigate using electrochemical methods.

In recent years, ultraviolet/visible (UV/Vis) spectrophotometry has been used in Fe and HS complexation research (Lippold et al., 2007; Weishaar et al., 2003). Previous studies led to the finding that the addition of Fe increases the UV/Vis absorbance of HS solution, which is attributed to Fe and HS complexation (Pullin et al., 2007; Weishaar et al., 2003). However, it is almost impossible to study the complexation of Fe and HS with only UV/Vis spectrophotometry since uncomplexed HS produces a large UV/Vis absorbance (Lippold et al., 2007). The spectral increment caused by complexation is faint compared with the absorbance of the matrix HS. Thus, the separation of the Fe–HS complex from uncomplexed Fe and HS is greatly needed. The Fe–HS complex has a larger molecular weight compared with uncomplexed Fe and HS (Everett et al., 1999; Jones et al., 1993; Maurice et al., 2002), therefore, size exclusion chromatography (SEC), in which analytes are separated based on hydrodynamic molecule size (Huber et al., 2011; Zhou et al., 2000), is one of the choices available for separation. With the application of SEC, UV/Vis should be able to detect different HS species. Furthermore, if a graphite furnace atomic absorption spectrophotometer (GFAAS) is employed, the technique can be used to differentiate complexed Fe from uncomplexed Fe by analyzing the eluate from the SEC column.

The purpose of this work was to simulate environmental factors such as ionic strength, pH, temperature and UV radiation to study their effects on complexation of Fe and HS in nature. With its abundant functional groups, humic acid (HA) was used to represent HS to complex with Fe in this study. SEC–UV was applied to separate the Fe–HA complex from the uncomplexed Fe and HA, and GFAAS was adopted to detect Fe in the SEC eluate.

1. Materials and methods

1.1. *Chemicals and solutions*

ACS grade $FeCl_3 \cdot 6H_2O$, NaCl and NaOH were purchased from Sigma-Aldrich (St. Louis, MO, USA). HNO_3 (65%, G.R.) and HCl (39%, G.R.) were from Merck (Darmstadt, Germany) and KunshanJincheng Chemical Reagent (Kunshan, China). $Mg(NO_3)_2 \cdot 2H_2O$ (graphite furnace AAS grade) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

The HA used in this study, which is often used for the purpose of method calibration and as a model compound for DOM speciation research (Chin et al., 1994; Góra et al., 2012; Reiller et al., 2006; Rodríguez and Núñez, 2011), was supplied by Sigma-Aldrich (St. Louis, MO, USA). The HA was extensively purified monthly using the following procedure: (1) The HA powder was dissolved in 0.01 mol/L NaOH solution and stirred for 10 hr for complete dissolution. The HA solution was centrifuged at 5000 r/min for 30 min and the residue was discarded, and this procedure was repeated three times to remove the associated insoluble humin and inorganic solids completely. (2) The solution was acidified to pH 1 with 3 mol/L HCl solution and left overnight to precipitate HA. The supernatant was removed after centrifugation at 10,000 r/min for 40 min to eliminate acid-soluble fulvic acid and metals. The residue was resuspended in HCl solution (pH 1) and centrifugated (10,000 r/min, 40 min), and then the supernatant discarded again. (3) The resulting low-metal, low-ash HA was dissolved in HCl solution (pH 1.7) and stored in the dark at 4°C until use. The unpurified and purified HA solutions were subjected to analysis by inductively coupled plasma mass spectroscopy (Agilent 7700×). The analytical results indicated that approximately 92% Fe and 60%–95% Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb had been removed through the purification procedure.

Ultra pure water (resistivity 18.2 MΩ/cm) obtained from a Millipore Purification Water System (Millipore Co., MA, USA) was used for the preparation of all solutions. HA working solution with dissolved organic carbon (DOC) of 5 mmol C/L was prepared by diluting the purified HA solution daily. A standard stock solution of 100 mmol/L Fe was prepared from $FeCl_3 \cdot 6H_2O$ in acidified ultra pure water (HCl, pH 1.7) daily. The working solution of Fe at the desired concentration was prepared by diluting the standard stock solution with acidified ultra pure water (HCl, pH 1.7).

All containers, except those especially mentioned, were made of low density polyethylene (LDPE) and subjected to a rigorous cleaning by soaking in 4 mol/L HCl solution for a week, then in 2 mol/L HCl solution for another week, finally in acidified ultra pure water (pH 1.7), and then thoroughly washed with ultra pure water before use. Transparent polyethylene terephthalate (PET) containers were used instead of semi-transparent LDPE bottles for the UV radiation study.

1.2. *Preparation of Fe–HA complex solution*

The Fe–HA complex solution was prepared as follows: 30 mL HA working solution was mixed with 100 μL Fe working solution at the desired concentration in order to achieve a final solution having 16.11 μmol/L Fe. The pH of the solution was immediately adjusted to 6 with HCl and NaOH solutions. No NaCl or other ionic compounds were added to the solution. The solution was protected from light and placed at room temperature (ca. 25°C) for 10 hr.

1.3. *Analyses*

All samples were filtered with 0.22 μm polyether sulfone (PES) membranes before analysis using SEC, which was performed with a liquid chromatography system equipped with an LC-20ADXR pump (Shimadzu, Kyoto, Japan), an SIL-20AXR auto sampler (Shimadzu, Kyoto, Japan), an SPD-M20A diode array detector (Shimadzu, Kyoto, Japan), a CBM-20A communications bus module (Shimadzu, Kyoto, Japan) and controlled by Shimadzu LC solution software (Shimadzu, Kyoto, Japan). A