A study on resonance Rayleigh scattering spectra of Humic Acid (HA)

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Abstract   This paper deals with the influences of pH, acidity and ionic intensity of the solutions on the resonance Rayleigh scattering spectra and fluorescence spectra of humic acid. When the pH value is low and the acidity and ionic intensity are high, the resonance Rayleigh spectra and fluorescence spectra both show a tendency of increasing, though the former's intensity is much higher. In combination with the transmission electron microscope data, the factors leading to the occurrence and enhancement of the resonance Rayleigh scattering spectra of humic acid were explored. It is considered that particle enlargement caused by aggregation, the increase of heterogeneity, the increase of hydrophobility, the formation of interface, etc., are the factors leading to the occurrence and enhancement of the resonance Rayleigh scattering spectra of humic acid. As the intensity of the resonance Rayleigh scattering spectra of humic acid is much higher, resonance Rayleigh scattering spectroscopy can be used as a newly developed spectrum technology, which is more sensitive and simpler, to study humic acid and its complicated behaviors.

Key words   humic acid; resonance Rayleigh; scattering spectra; fluorescence spectra; transmission electron microscopy

1 Introduction

Humic Acid (HA) is ubiquitous in the soils, waterbodies and sediments of the Earth's environment and it is very complicated in texture. In addition, it has a diversity of sources and forming modes, thus leading to the lack of information about its concrete texture and properties. HA and its inherent coordinating groups can interact with secondary minerals, metallic ions, organic pollutants by way of adsorption and complexation (Song Jianzhong et al., 2008; Feng Xiaojuan et al., 2005; Fan Wenhong and Chen Jun, 2006; Lou Tao et al., 2004), which may play an important role in their environmental geochemical cycling processes such as their concentrations and chemical forms, transport and transformation and fates.

It is hard to conduct research on HA because of the complicity of HA itself or the soil or water environment in which HA is present. So, systematic research needs to be carried out on HA from different angles. Therefore, almost all kinds of approaches and technological methods have been employed in research on HA, for example, chemical method, electrochemical method, spectroscopy, chromatography, ultrafiltration, ion exchange and so on. The dominating approaches are spectroscopy and electrochemical techniques, mainly including ultraviolet-visible spectrophotometry, Fourier infrared spectroscopy, nuclear magnetic resonance spectroscopy, fluorescence spectroscopy and electron spin resonance spectroscopy, ion-selective electrodes, anodic stripping voltammetry and so on (Hofrichter and Steinbuchel, 2004; Fu Pingqing et al., 2004). Research on new technology and new methodology will help deepening our understanding of the behaviors and evolutionary rules of HA and its environments from different angles. Resonance Rayleigh-scattering or resonance light-scattering (RRS or RLS) is a kind of molecular spectral technology. Pasternack et al. (1993, 1995) adopted this technology for the first time in research on the aggregation of porphyrins on DNA and that of chromophores. Almost at the same time, Liu Shaopu et al. (1995) applied this technology to the study of small molecular compounds of heavy metals at home. At present time, this technology has been widely applied to the analysis and measurement of protein (Yang Rui and Liu Shaopu, 2001), DNA (Liu Shaopu and Long Xiufeng, 2002), and drugs (Sun Shuting et al., 2007) and research on their spectral characteristics, but no research work has been made on HA, even up to now.

In this paper the RRS technology is used to study

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the RRS characteristics of Elliott Soil 1S102H-HA and the influences of pH and ionic strength on it in the solutions. In combination with the fluorescence spectroscopic and transmission electron microscopic characteristics of HA the authors explored the reasons for the occurrence and intensification of RRS spectra of HA under chemical conditions of different solutions, i.e., the connections with the different properties and behaviors of HA. Exploratory research has been conducted on the application of RRS technology to the study of HA and its environments.

2 Experimental samples, instruments and experimental methods

2.1 Preparation of stock solution

A certain amount of HA standard sample was weighed and taken (sample No. Elliott Soil 1S102H, purchased from the International Humic Substances Society (IHSS), dissolved with a small amount of NaOH, then filtered with the GF/F glass fiber membrane (Whatman, UK) burnt at 450℃ for 5 hours, and again filtered with hydrographic fiber membrane (0.45 μm). The pH value was adjusted to about 8, followed by the step of constant volume with ultrapure water. The prepared solution was stored in a 4℃ refrigerator for future use. Other chemical reagents are analytically pure and the water is ultrapure water unless special instructions are given.

2.2 Parameters for the experimental instruments and experimental methods

Fluorescence spectrum analyzer (Hitachi-2500, made in Japan): Synchronous scanning was conducted at Δλ=0, yielding the RRS spectra, with the largest RRS wavelength being 365 nm. The largest excitation wavelength obtained from fluorescence spectrum scanning was 260 nm, the largest emission wavelength was 520 nm. At the largest emission wavelength the RRS and fluorescence strength of the samples were measured.

The transmission electron microscope (JEM-2000FXII) was used for microcosmic morphological analysis. Copper net was dipped with a minor amount of the solution and then dried in air. In case that the accelerating voltage was 160 kV and the morphological textures and sizes of HA molecules were observed.

A PHS-3C precision-type acidometer was used to determine the pH values of all solutions.

3 Results and discussion

In consideration of the fact that the pH values of soils in the concrete environment are mostly within the range of 4.5–8.5 (Zhang Hui, 2006) and those of water environments on the Earth's surface are within the range of 6–9 (GB3838-2002, The Surface Water Quality Standard of the People's Republic of China). The pH value of 7 was taken in the case of investigating the influence of ionic strength on RRS spectra. In the case of studying the influences of pH and acidity on RRS spectra the results of research acquired by Yang Jiewen et al. (2002) were taken as the reference data. In combination with Fig. 3 such a range of ionic strengths as to be 0–0.2 mol/L NaCl, i.e., the ionic strength is 0.1 mol/L NaCl, was selected in consideration of the fact that within this range the ionic strength has only a slight influence on RRS spectra.

3.1 Influences of the pH values of the solutions on the resonance Rayleigh scattering spectra and fluorescence spectra of HA

Shown in Figs. 1 and 2 are the variations in intensities of RRS and fluorescence spectra of HA with pH, respectively. As can be seen from Fig. 1, the maximum wavelengths of RRS and fluorescence spectra of HA basically remain unchanged with the variation of pH, although some variation is observed in their spectrum shape. The difference in spectrum shape between the RRS and fluorescence spectra of HA reflects the different physicochemical properties or forms of materials in the solutions. Figure 2 also shows that the intensities of both RRS and fluorescence spectra are relatively high at pH<4, but they tend to diminish drastically with increasing pH. At pH=4–12, their intensities tend to become stable.

3.2 Influence of ionic strength on the resonance Rayleigh scattering spectra and fluorescence spectra of HA

By taking the NaCl solution of a certain concentration as the regulator of ionic strength. As shown in Fig. 3, at pH=7 the ionic strength has similar influences on the RRS and fluorescence spectra of HA. There is almost no influence in case that the ionic strength varies within the range of 0–0.2 mol/L, but in case that the ionic strength exceeds 0.2 mol/L, the intensities of RRS, in particular, and fluorescence spectra of HA begin to increase with ionic strength.

3.3 Factors leading to the intensification of resonance Rayleigh scattering spectra of HA

The main factors leading to the intensification of RRS spectra are the aggregation of chromophores or long-distance assemblaging, electrostatic attraction and hydrophobic force, as well as the formation of nano-particles of larger sizes and supramolecules (Liu
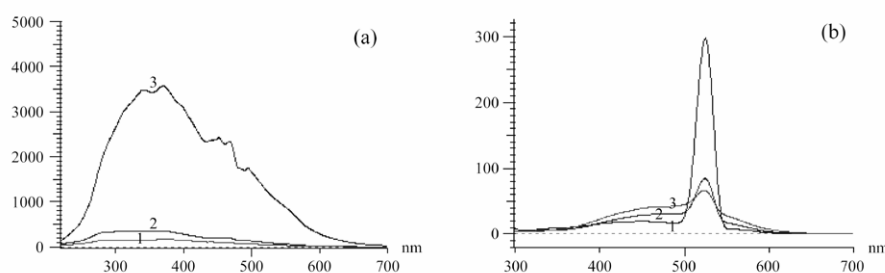
Fig. 1. The RRS and fluorescence spectra of HA (10 mg/L HA; I=0.1 mol/L NaCl). 1–3 refer to pH=7, 4, 2, respectively; (a) RRS of HA; (b) fluorescence spectra of HA.
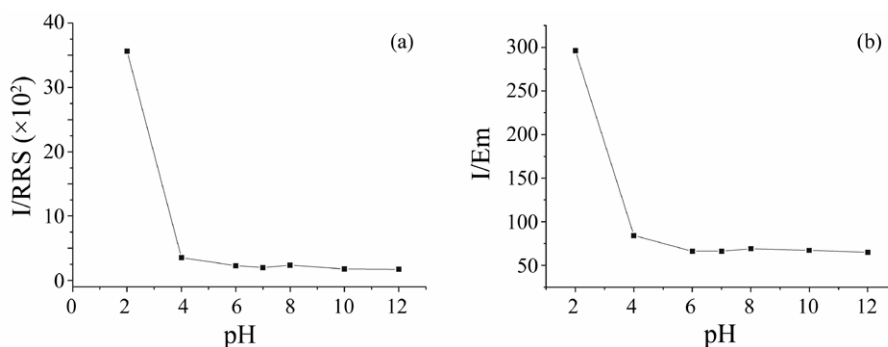

Fig. 2. RRS and fluorescence spectrum intensity curves at different pH values (10 mg/L HA; I=0.1 mol/L NaCl). (a) RRS spectrum intensity of HA; (b) fluorescence spectrum intensity of HA.

Shaopu, 2003). The factors leading to the intensification of RRS spectra in different systems may be different, and sometimes, it would also be caused by several different reasons (Liu Shaopu et al., 1999).
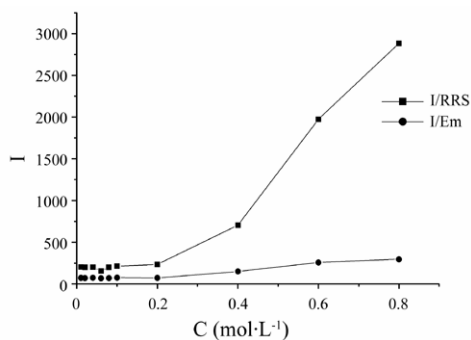

Fig. 3. Influences of Na$^+$ ionic strength on RRS and fluorescence spectrum intensities (10 mg/L HA; pH=7).

3.3.1 Influences of pH and acidity

As shown in Figs. 2 and 4, at pH<4 the intensities of RRS and fluorescence spectra of HA solutions are enhanced rapidly and tend to become stable with further increasing concentrations of hydrochloric acid. Under acidic conditions the mechanisms of RRS spectrum intensification of HA are generally consistent with one another. As HA is a kind of weakly acidic naturally-occurring large bio-molecules whose surface is electronegative and which possesses the properties of surfactants. With the increase of solution acidity or the addition of strong acids, many hydrophillic radicals such as —COO$^-$ or —OH will be protonized rapidly, leading to a great decrease in hydrophility. As a result, the whole molecules will become hydrophobic organic molecules. Due to their hydrophobicity, all molecules aggregated because of their Van der Waals' force, the action of hydrogen bonds and lypophobic repelling force, thus making the molecules increase in volume. Meanwhile, hydrophobicity may lead to the formation of a liquid-solid interface between aggregated molecules and water molecules. The formation of such an interface is another important reason for the intensification of scattering (Nan Haijun et al., 2006).

Baigoni et al. (2007) employed such technologies as Untrafiltration, Dnymaic Light Scattering (DLS) and Size Exclusion Liquid Crhomotography to have investigated the aggregation of humus in the solutions and pointed out that HA could be involved in the aggregation of molecules or that of ultramolecular associations. In the study the related electromicroscopic experiments also evidenced the formation of aggregated HA molecules. As can be seen in Fig. 5, as compared with the situation at pH=7 in Fig. 5a, the surface hydration layer of hydrophilic radicals shown under hydrochloric acid medium in Fig. 5b basically disappeared and the hydrophobicity was intensified so

that the radicals aggregated as larger molecules. RRS was intensified rapidly. When the surface hyrophilic radicals had been protonized and thus became saturated, the decrease of pH value or the further increase of acid concentration would not lead to considerable changes in the size of densely aggregated HA molecules and their hydrophobicity. Therefore, the RRS intensity tended to become stable.

3.3.2 Influence of ionic strength

    As can be seen from Fig. 3, only in the case of high ionic strength the RRS of HA will increase rapidly. That is because HA is a kind of naturally-occurring large bimolecular polyprotic weak acid which contains a variety of phenolic hydroxyls and carboxyls and possesses some colloid properties (Zhou Guoping et al., 1991). The electrostatic attraction of a large amount of monovalent strong electrolytes can cause violent hydration, thus leading to flocculation (or aggregation) of hydrophilic radicals of HA (Guo Xiaofeng, 1996). As a result, nano ions of larger sizes or supermolecules were formed, leading to

the intensification of RRS spectra of HA. The same conclusion is drawn from the results of electron microscopic experiment. In the case of low ionic strength, as shown in Fig. 6a, the HA molecules are relatively evenly distributed and they are encircled by water molecules. So, they are present in the form of smaller-sized molecules and their RRS is relatively weak. In the case of high ionic strength, as shown in Fig. 6b, HA molecules begin to shrink and aggregate owing to dehydration. Thus, molecules of larger sizes were formed and their RRS was also enhanced. DNA and HA both belong to weakly acidic biomolecules of large sizes. In their study of salt effect on the conformation of DNA, He Zhongxiao et al. (1989) pointed out that with increasing salt concentrations (ionic strength), DNA molecules would further shrink (aggregate). In addition, salt effect would cause an increase in disaggregation degree of weak electrolytes and a decrease in pH value (Krumgaiz and Deng Danyun, 1989). That would enhance the tendency of protonation of electronegative hydrophilic radicals and intensify their hydrophobicity. That is one of the reasons for the intensification of RRS.
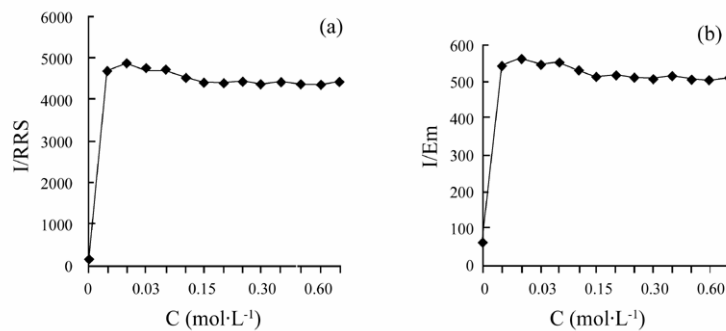

Fig. 4. RRS and fluorescence intensity curves of HA in different concentrations of HCl. (a) RRS intensity of HA; (b) fluorescence intensity of HA.
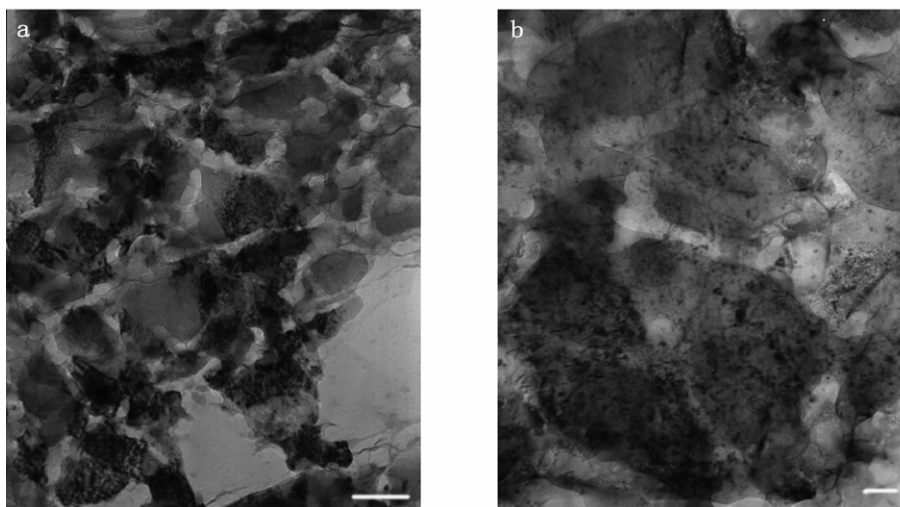

Fig. 5. TEM images of HA at different acidities (10 mg/L HA; I=0.1 mol/L NaCl). a. pH=7 (bar=0.3 μm); b·0.25 mol/L HCl system (bar=80 nm).
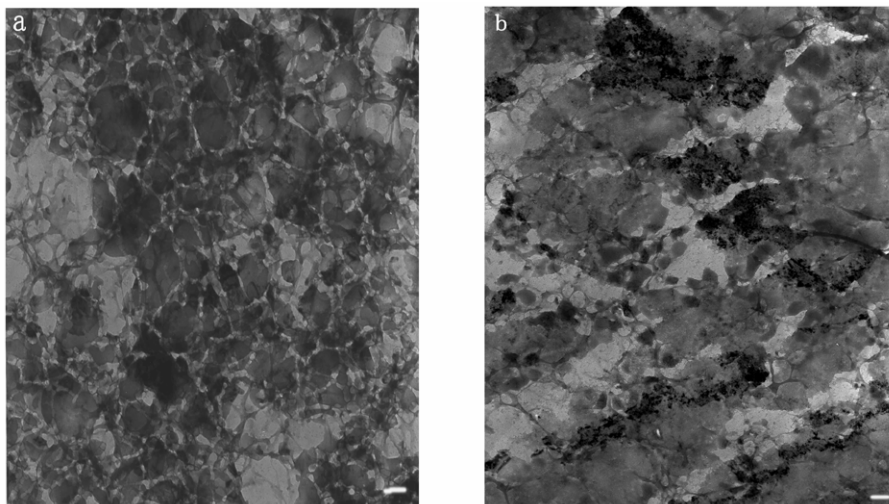
Fig. 6. TEM images of HA at different ionic strengths. I=0.1 mol/L; I=0.6 mol/L; 10 mg/L HA; pH=7; a. I=0.1 mol/L NaCl (bar=0.5 μm); b. I=0.6 mol/L NaCl (bar=0.25 μm).

4 Conclusions

This paper studied the RRS and fluorescence spectra of HA under the influence of solution pH, acidity and ionic strength. When the solution pH decreases progressively and the acidity and ionic strength increase steadily, the variation tendency of RRS is the same as that of fluorescence spectra, i.e., both tending to intensify, but the former's is more obvious. In combination with transmission electron-microscope data, we explored the reasons for the intensification of RRS spectra. It is considered that the aggregation of HA molecules at the conditions of high ionic strength, low pH value and high acidity can cause the particles to become larger and their heterogeneity and hydrophobicity to increase and lead to the formation of interface. All this is the reason for the occurrence and intensification of RRS.

In soil or water environment the high ionic strength means the increase of salinity in soil or water environment, and the decrease of pH value means the acidification of soil or water environment. Based on this study, the dissoluble HA at this time will aggregate to form the particles with hydrophobic interface. In the concrete soil or water environment this means the release of HA from soil solution or water environment. This will greatly influence the environmental biogeochemical cycle. The study can be taken for example, which was carried out by Chin Wei-Chun et al. (1998) on marine snow in high-salinity seawater. The same instrument was employed for the measurement of RRS and fluorescence spectra, though the former's intensity is much higher. All this implies that RRS can be used as a new, more sensitive, much simpler spectrum technology and applied to research on HA—A kind of naturally-occurring biomolecules of large sizes in the environment and its complicated environmental behaviors.

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