Colloid Polym Sci (2009) 287:57–62
DOI 10.1007/s00396-008-1952-5

ORIGINAL CONTRIBUTION

Fluorescence spectroscopy of fulvic acids' interaction with surfactants

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Received: 8 September 2008 / Revised: 8 October 2008 / Accepted: 15 October 2008 / Published online: 5 November 2008

Abstract Fluorescence spectra of two fulvic acid (FA) samples, FA0 from underground water and FA1 from forest soil, were recorded in various surfactant solutions. Alkyltrimethylammonium ions with different alkyl chain lengths induced a decrease in the fluorescence intensity for both FAs at concentrations below the critical micelle concentration (cmc) and an enhancement above the cmc. The intensity minimum thus obtained at the cmc was deeper for surfactants with longer alkyl chains. This effect was attributable to the formation of insoluble FA–surfactant complexes below the cmc and to the solubilization of the complex into micelles above the cmc. Dodecylpyridinium chloride caused a monotonic decrease in the FA fluorescence even far above the cmc. This was attributable to the quenching of FA fluorescence by the positioning of the pyridinium head group near the FA fluorophore. Anionic and nonionic surfactants showed little to no effect on the FA fluorescence.

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Keywords Fulvic acid · Surfactant · Fluorescence spectrum · Fluorescence · Spectroscopy · Complex

Introduction

Humic substances (HSs) are major components of natural organic materials found in organic geological deposits, soil, and almost every aquatic environment. They play an important role in the regulation of natural water systems and are used to improve soils because of their nutritional and colloidal properties. HSs affect the chemistry, cycling, and bioavailability of chemical elements, the transport and degradation of xenobiotic and natural organic chemicals, and the biological productivity of aqueous ecosystems. The properties of HSs depend markedly on the source plant, its location and age, and other environmental factors.

Fulvic acids (FAs), a specific class of dissolved HSs found in the hydrosphere, are often polydispersive, amphiphilic polyelectrolytes [1–4]. Metal–FA complexes are usually soluble in aqueous solutions and can play an important role in the distribution of heavy metals in the environment [5]. The fluorescence excitation and emission spectra of FAs have been utilized for identifying the source plant, its age, and place of origin [6–8]. Fluorescence techniques have also been employed to investigate FA–metal complexes and their stability constants [9–11].

Amphiphilic ligands such as surfactants can form complexes with FAs through hydrophobic and/or ionic interactions [3, 4, 12]. Surfactants are introduced into the environment through natural secretion from aquatic plants or from xenobiotic sources, such as wastewater discharge [13–15] and point-discharge pollution [16]. Natural, plant-derived surfactants have been detected in river water at concentrations sufficiently high to produce persistent foams

[17]. The amphiphilic nature of both surfactants and FAs results in a mutual attraction, particularly when the surfactant and FA are oppositely charged. A mixed system composed of FAs and surfactants shows cooperative and synergetic binding [3, 12]. Knowledge of the chemical interactions between FAs and surfactants is crucial to understanding the fate of xenobiotic compounds in groundwater.

In general, interactions between ionic surfactants and oppositely charged polyelectrolytes are relatively strong and involve cooperative binding events and ionic attraction [18, 19]. Yee et al. [3] demonstrated a quantitative difference in the hydrophobic properties of humic acids and FAs using surfactant-binding curves. However, the fundamental interactions of surfactants with FAs are still unclear. Fluorescence spectra of FAs are complex and depend on the source and history of the sample. In this study, two FA samples with different origin were used: one from underground natural gas well and the other from forest soil. Changes in the fluorescence spectra were compared for two origin-different FAs in the presence of various surfactants. Results revealed that fluorescence measurements using the inherent fluorophores of FA itself can be used for characterization of both FA and FA–surfactant complexes.

Experimental

Materials

Surfactants evaluated were alkyltrimethylammonium bromides with decyl, dodecyl, tetradecyl, and hexadecyl groups (C_nTAB, n=10, 12, 14, 16, respectively; TCI, Tokyo, Japan), dodecylpyridinium chloride (C_{12}PyC; TCI), sodium dodecylbenzenesulfonate (C_{12}BSNa; TCI), sodium dodecanoate (C_{11}COONa; TCI), and hexadecyl-eicosanoxyethylene ($C_{16}E_{20}$; Wako, Osaka, Japan). Surfactants were used without further purification. Pyrene (Sigma-Aldrich, Tokyo, Japan), 1-pyrenylaldehyde (Sigma-Aldrich), and the sodium salt of 8-anilino-1-naphthalenesulfonic acid were used as fluorescent probes for FA–surfactant interactions.

Fulvic acid preparation

FA solutions were supplied from Kanto Tennengas Kaihatsu, Mobara, Japan. A faintly colored, underground water sample was concentrated to 1/30 of the original volume to obtain a yellow solution with the composition given in Table 1. This concentrated solution contained a total of 1,300 mg/L total organic carbon (TOC). Assuming that the dissolved FAs were composed of 50% carbon, the concentration of FA in the concentrated sample was approximately 2,600 mg/L.

Table 1 Ionic components of fulvic acid original solution (FA0)

Cation	Concentration (mg/L)	Anion	Concentration (mg/L)
Na^+	14.4	Cl^-	10.1
NH_4^+	3.8	NO_3^-	N. D.
K^+	1,300	SO_4^{2-}	N. D.
Mg^{2+}	1.0	PO_4^{3-}	N. D.
Ca^{2+}	5.4	I^-	72.3
Total organic carbon	1,300	Estimated FA concentration	2,600

A small amount of 1 M NaCl solution was added to the concentrated sample, which was then repeatedly dialyzed against distilled and deionized (Millipore, Billerica, MA, USA) water until no sodium was evident in flame emission tests and no precipitate was formed upon the addition of $AgNO_3$. This stock solution (FA0) included 1,360 mg/L solid material. No solid materials were observed following digestion in 1 M HCl.

Another FA sample (FA1) was collected from soil in the Kasuya Research Forest at Kyushu University and extracted according to the international standard method IHSS [20]. The purified solid was used to prepare a 1,360-mg/L stock solution. The degradation age of FA1 was inferred to be much younger than FA0 from their origin and their chemical structure might be greatly different.

Measurements

One gram of the FA stock solution was added to 9.0 g of the surfactant solution at various concentrations. The solution pH was 6.6–6.8 for mixtures of FA0 and surfactant and 3.6–3.8 for the mixtures containing FA1. However, C_{11}COONa-mixed solutions changed the solution pH from 8.3 to 10.1, depending on the surfactant concentration (15–225 mM).

Absorption and fluorescence spectra were recorded using a spectrophotometer (UVIDEC 560; Jasco, Tokyo, Japan) and a spectrofluorometer (RF-5000; Shimadzu, Kyoto, Japan), respectively. The excitation wavelength was held at 340 nm for measurements of FA/surfactant mixtures. Faint turbidity was visually observed for some surfactant mixtures and the spectroscopic measurements were carried out for turbid solutions. All measurements were conducted at room temperature.

Results and discussion

FA fluorescence

Many fluorescence-based studies have been conducted to characterize surfactant–polyelectrolyte interactions using

fluorophores that are sensitive to the polarity of the local environment [21–26]. In the beginning, we tried the probing study using pyrene [24, 27–29], 1-pyrenylaldehyde [18, 30], and 8-anilino-1-naphthalenesulfonic acid. The fluorescence spectra of mixed solutions of FA and pyrene at various C_{16}TAC concentrations were measured, but the I_1/I_3 ratio could not be calculated due to the strong intensity of the fluorescence band of the FA, which also interfered in estimating the band shift of 1-pyrenylaldehyde. No new emission band was observed in mixtures of 8-anilino-1-naphthalenesulfonic acid and FA. Therefore, the use of fluorescent probes was rejected and the fluorescence spectrum of FA was measured directly in response to surfactant solutions.

The absorption spectra of FA0 and FA1 exhibited a very broad band that decreased monotonically from the UV to the visible region. At low FA concentrations, a shoulder band was observed at 280 nm. No specific absorption peaks were observed at wavelengths longer than 300 nm for 1,360 mg/L solutions of FA.

Fluorescence emission and excitation spectra were recorded for both FA0 and FA1. Fluorescence maxima appeared at 430 and 460 nm when excited at 340 and 370 nm for FA0 and FA1, respectively. An excitation maximum was observed for FA0 at 348 nm when monitored at an emission wavelength of 438 nm, while the excitation peak for FA1 was observed at 370 nm when monitored at 446 nm. These variations in fluorescence spectra of FA compounds have been recognized among different FA sources [8]. The synchronous-scan spectrum in Fig. 1 shows the differences in the FA fluorescence spectra more clearly. Both the overall shape of the spectra and their respective maxima differed significantly between FA0 and FA1 solutions when recorded at a wavelength difference ($\Delta\lambda$) of 20 nm between the emission and excitation wavelengths (solid curves in Fig. 1). The synchronous-scan spectrum changed significantly at $\Delta\lambda$=50 nm (broken curve in Fig. 1), indicating that FA fluorescence depends on the excitation wavelength.

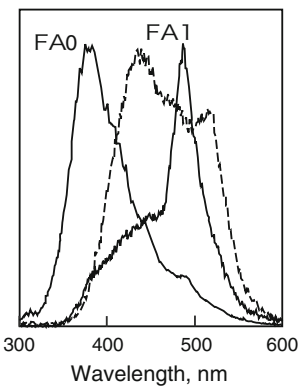

Fig. 1 Synchronous-scan fluorescence spectra of FA0 and FA1 are shown. *Solid curves*: $\Delta\lambda$=50 nm; *broken curve*: $\Delta\lambda$=20 nm

The FA0 fluorescence reached a maximum intensity when excited at 340 nm. A shoulder at 380 nm was observed in the fluorescence spectrum, likely corresponding to the shoulder at 280 nm in the absorption spectrum. The fluorescence maximum redshifts at excitation wavelengths greater than 330 nm. A single fluorophore such as 1-pyrenylaldehyde changed the intensity of the fluorescence but not the location of the maximum when the excitation wavelength was changed. This suggests that the FA moiety itself is composed of different fluorophores subject to different local surroundings. This dependence on the excitation wavelength is characteristic of FAs and other humic acids [8, 31]. Therefore, three-dimensional fluorescence spectrometry, which measures fluorescence intensity as a function of emission and excitation wavelengths, and synchronous scan measurements have been used to characterize the origin of FAs [8, 32–34].

Figure 2 shows the maximum fluorescence intensity as a function of FA0 concentration. The intensity increased with concentration below 270 mg/L and began to decrease above 270 mg/L. However, the emission intensity from the surface of the solution increased monotonically with concentration. Therefore, the observed decrease at high FA0 concentrations was caused by concentration quenching and self-absorption of the fluorescence.

Fluorescence of FA/surfactant mixtures

To account for changes in the fluorescence spectra due to the formation of insoluble complexes and solution turbidity, fluorescence spectra of a very dilute kaolin suspension and a mixture of FA0 and kaolin were obtained. A sharp emission was observed at 363 nm at 340-nm excitation and the intensity grew as the suspension concentration increased. However, only minor changes were observed in

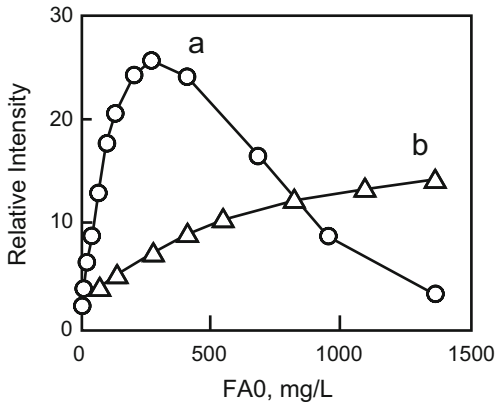

Fig. 2 The fluorescence emission intensity of FA is shown as a function of concentration. The *circles* represent emission from the bulk of the solution; the *triangles* represent emission from the surface of the solution

the FA fluorescence intensity. Thus, the emission peak at 363 nm was deemed appropriate to represent the formation of fine insoluble complexes of FA with surfactants.

Fluorescence spectra of FA with various concentrations of C_{16}TABr are shown in Fig. 3. The fluorescence intensity decreased as the surfactant concentration increased up to 0.92 mM C_{16}TABr (left in Fig. 3a,b) and began to increase at higher concentrations (right in Fig. 3a,b). The surfactant concentration at the minimum fluorescence intensity was close to the critical micelle concentration (cmc; 0.93 mM) [35]. In FA1 mixtures, the sharp bands at 363 nm appeared at low surfactant concentrations and the intensity decreased as the surfactant concentration increased (Fig. 3b). Faint turbidity by file particles was visually observed for the mixtures b, c, and d. This behavior indicates the formation of insoluble FA1–surfactant complexes at low surfactant concentrations that dissociate or become soluble at higher levels of the surfactant. Though no turbidity was visually observed, the band at 363 nm was also observed for FA0 (Fig. 3a), albeit much weaker than in FA1, indicating a weak interaction between FA0 and C_{16}TABr.

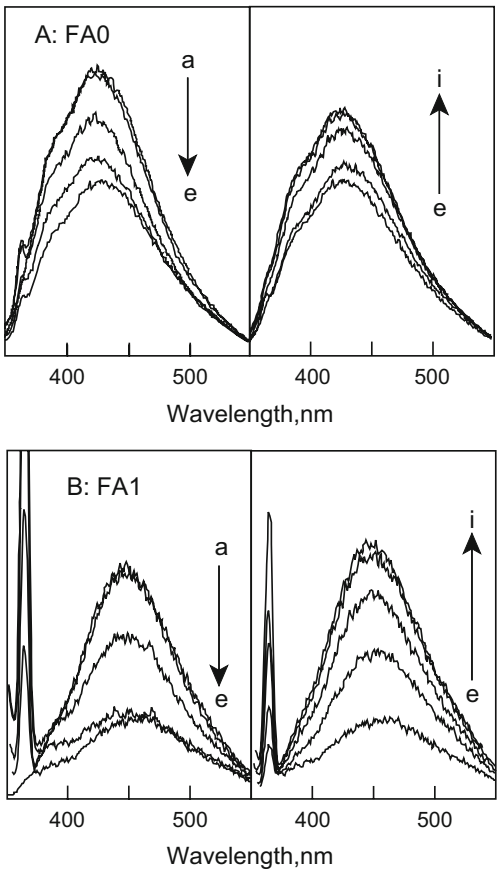

Fig. 3 The fluorescence spectra of FA/C_{16}TABr mixtures are shown as a function of C_{16}TABr concentration (mM): *a* 0, *b* 0.10, *c* 0.25, *d* 0.50, *e* 0.92, *f* 2.0, *g* 4.0, *h* 8.0, *i* 9.0; **a** FA0, **b** FA1

The intensity of the FA fluorescence at maximum was plotted against the ratio of the surfactant concentration to the cmc (*C*/cmc). Figure 4 compares these results with C16TA$^+$ bromide and chloride. Bromide ions often quench fluorescence. Similar behaviors were observed for both C_{16}TABr and C_{16}TACl below the cmc, indicating that the intensity decrease was caused not by fluorescence quenching but by the formation of insoluble complexes. FA sequestered in insoluble complexes with surfactants may not contribute to the solution fluorescence. Above the cmc, where electric repulsions between the FA–surfactant complex and Br ions are minimal, the maximum fluorescence intensity is less for C_{16}TABr than for C_{16}TACl, suggesting that Br ions decrease the quantum yield of the FA fluorophores.

The fluorescence intensity of the FA1/surfactant mixtures was stronger than that of FA1 itself at surfactant levels higher than the cmc. This indicates solubilization of the FA–surfactant complexes in micelles rather than complex dissociation. Nonpolar environments often enhance fluorescence [36] and the fluorophores in FA may be inferred to exist in nonpolar local environments within the FA–surfactant complexes. The lesser change observed in the FA0 fluorescence indicates a weaker interaction between FA0 and C_{16}TA$^+$ surfactants.

Fluorescence spectra of FAs in solutions of alkyltrimethylammonium bromide (C_nTABr) are shown in Fig. 5a,b. An intensity decrease below the cmc and an increase above

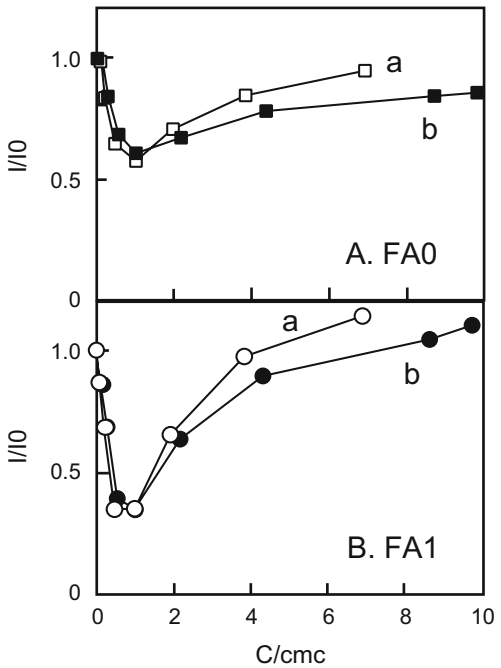

Fig. 4 FA fluorescence intensity is shown as a function of surfactant concentration. **a** FA0, **b** FA1. Surfactant: *a* C_{16}TACl, *b* C_{16}TABr

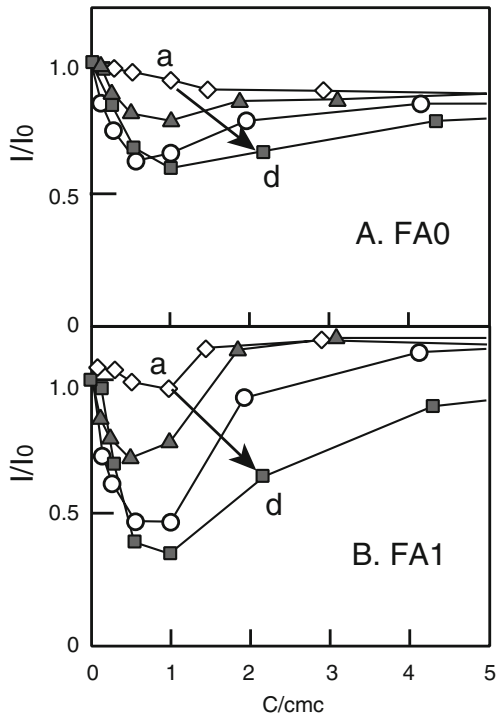

Fig. 5 FA fluorescence intensity is shown as a function of surfactant concentration and alkyl chain length. **a** FA0, **b** FA1. Surfactant: *a* C_{10}TABr, *b* C_{12}TABr, *c* C_{14}TABr, *d* C_{16}TABr

the cmc was observed for both C_{14}TABr and C_{12}TABr. The minima were observed between 0.5 and 1.0 in the ratio of surfactant concentration to the cmc (*C*/cmc). A deeper minimum near the surfactant cmc was seen as the surfactant chain length was increased, indicating the formation of increasingly hydrophobic complexes of FA. The spectra indicate a very weak interaction between C_{10}TABr and FA. However, the weak turbidity peak at 363 nm was still observed in mixtures of FA1 and C_{10}TABr below the cmc, indicating complex formation.

The FA fluorescence intensity decreased markedly in C_{12}PyCl solutions. The turbidity band at 363 nm indicated complex formation, and the fluorescence decreased as the surfactant concentration was increased. Figure 6 compares the fluorescence maxima of FA in C_{12}PyCl and C_{12}TABr solutions. The pyridinium ion is a well-known fluorescence quencher. The different trends seen in Fig. 6 for C_{12}PyCl and C_{12}TABr indicate that C_{12}PyCl quenches the FA fluorescence and that this quenching is effective despite solubilization of the FA–surfactant complexes above the cmc. Both surfactants are cationic and equally hydrophobic (the cmc is 16 mM for C_{12}TABr and 17 mM for C_{12}PyCl). Thus, the cationic surfactants may be safely inferred to bind to the same anionic binding sites in FA such that the fluorophore is located near the anionic site in FA and quenched by C_{12}PyCl. The fluorescence decrease observed below the cmc was likely caused by the formation of

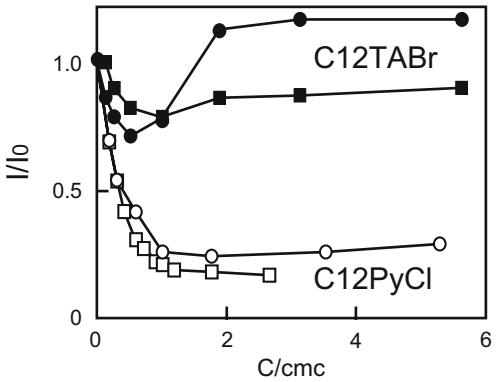

Fig. 6 The fluorescence intensity of FA in a solution of C_{12}TABr (*solid marks*) is compared against that in a solution of C_{12}PyCl (*open marks*). *Squares* represent FA0; *circles* represent FA1

insoluble FA–surfactant complexes. Above the cmc, the complexes were solubilized in micelles resulting in fluorescence recovery in C_nTABr solutions. However, fluorescence emission was still quenched by C_{12}PyCl even above the cmc where the FA–C_{12}PyCl complexes are soluble.

The interactions of FA with anionic surfactants C_{12}BSNa and C_{11}COONa, and a nonionic surfactant $C_{16}E_{16}$, were also evaluated. Only minor effects on FA fluorescence were observed for C_{12}BSNa and $C_{16}E_{16}$, indicating little to no interactions with FA. These results point at strong electric repulsion between FA and anionic surfactants and no contribution of hydrophobic interaction to form a complex of FA with surfactant. However, the fluorescence spectra of FA1/C_{11}COONa mixtures exhibited a turbidity peak at 363 nm and a decrease in FA fluorescence with increasing surfactant concentration (Fig. 7). This may have been caused by surfactant concentration-dependent changes in the solution pH from 6.8 to 10.1 for FA0/C_{11}COONa mixtures and changes from 3.7 to 10.1 for FA1/C_{11}COONa. As a control, the FA fluorescence was measured in solutions at different pH. As the solution pH increased,

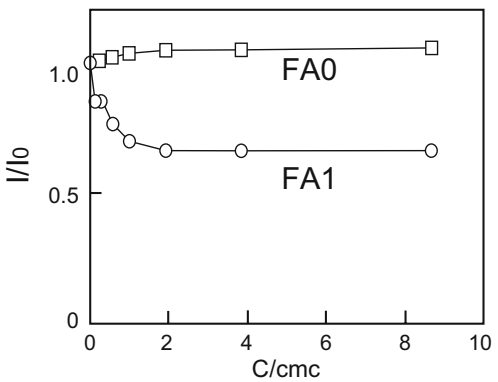

Fig. 7 The FA fluorescence intensity is shown as a function of C_{11}COONa concentration

the fluorescence intensity of FA1 decreased while that of FA0 remained the same. Thus, the observed changes in the fluorescence intensity of FA/C_{11}COONa mixtures were likely caused by changes in the solution pH at different C_{11}COONa concentrations.

Conclusions

Two FA samples, FA0 from underground water and FA1 from forest soil, were employed to study interactions between FAs and surfactants. The use of separate fluorescence probe molecules was unsuccessful due to interference by the fluorescence of FA itself. The fluorescence spectra of both FAs changed with regard to intensity and the wavelength of maximum emission as a function of the excitation wavelength, the solution pH, and the source of FA. FA1 formed insoluble complexes at low concentrations of cationic surfactant, which were solubilized at concentrations above the cmc. Complexes formed with dodecylpyridinium surfactant quenched FA emission, suggesting that the FA fluorophore was located near the anionic group in FA. Nonionic surfactant and sodium dodecylbenzenesulfonate showed no effect on FA fluorescence. Sodium dodecanoate affected the fluorescence intensity of FA1 due to changes in the solution pH. Fluorescence measurements using the inherent fluorophores of FA itself were sufficient for characterization of both FA and FA–surfactant complexes.

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