Article

On the Dodecylpyridinium Binding to Humic Acids and Fulvic Acids from Different Origins

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Abstract

The binding of dodecylpyridinium ($C_{12}Py^+$) ions with humic acids (HAs) as well as fulvic acids (FAs) from different origins has been studied in aqueous solution at 25 °C. The binding isotherms are determined using a potentiometric titration technique with surfactant-ion-selective membrane electrodes. All investigated HAs of different origins (both soil and aquatic) show the same independent sites binding behavior with $C_{12}Py^+$ ions, and the number of binding sites and binding constants are analyzed by Scatchard plot equation. In all FAs systems, the binding is highly cooperative and the binding constants and cooperative parameters are calculated by applying Hill's binding theory. The binding affinity of $C_{12}Py^+$ ions is stronger with soil HAs than with soil FAs. This suggests that the hydrophobicity of the backbone of HAs is higher than that of FAs, which tendency agrees with the higher carbon content and the lower oxygen content of HAs than FAs. The binding strength of $C_{12}Py^+$ with humic substances (HSs) varies among HS samples of different origins. In both HAs and FAs systems, $C_{12}Py^+$ binding is stronger with soil samples than that with aquatic samples showing that the hydrophobicity of HS is one of the key factors in $C_{12}Py^+$ binding to HS.

Introduction

Humic substances (HSs) are the break-down products of plants and biological origins found in almost all terrestrial and aquatic environments on the earth's surface. Depending on their solubility, HSs can operationally be divided into three fractions: fulvic acid (FA), humic acid (HA), and humin (Gaffney et al., 1996; Hesketh et al., 1996; Hayes, 1998). Humic substances possess a wide range of molecular weights and include both hydrophilic and hydrophobic moieties (Tombacz et al., 1999; Kam et al., 2001). So that they interact readily with hydrogen ions, metal cations and organic compounds, such as surfactants, pesticides and herbicides (Norden et al., 1996; Schmitt et al., 1996; Akim et al., 1998; Thieme et al., 1998; Jelinek et al., 1999). Among

these compounds, ionic surfactants play an important role in the environment because of their anthropogenic origin market everywhere from household detergents to explosives (Hargreaves, 2003) and they can accumulate in soils and waters. In case of the deposition of cationic surfactants in soils and waters, it is expected that these substances will readily bind to negatively charged humic substances (Bors, et al., 2001). The knowledge of the interactions of cationic surfactants with HSs is of particular importance, especially with respect to the fate and transport of organic pollutants in the environment.

There are several investigations on the interactions of HS with hydrophobic organic compounds as well as biocides (Paolis, et al., 1997; Spark, et al., 1998; Hesketh et al., 1996). The affinity of these organic compounds to HSs appears to vary among HSs sam-

ples from different origins. One approach to elucidate the source of this variability is to relate the observed binding capacities to the analyzed structural and chemical features of HSs used in the experiments. These studies have shown that the hydrophobicity of HSs is one of the main factors modifying the binding of organic compounds to HS. Concerning with the surfactant binding to HSs, there has been no systematic study which try to relate the binding affinity with the structural and chemical features of HSs. Only a few physicochemical studies have been reported on the particular case of the interaction of cationic surfactants with HSs (Otto, et al., 2003; Hiraide, et al., 1999).

Recently we reported the amphiphilic properties of HA and FA by alkylpyridinium binding study (Yee et at., 2006) and reveals the effectiveness of surfactant binding study to characterize the amphiphilicity of HSs. In this study we have investigated the binding of dodecylpyridinium ($C_{12}Py^+$) ions with HAs and FAs from different origins by potentiometric titration method based on surfactant-ion-selective membrane electrodes. Depending on their origin and the natural conditions prevailing their formation, HAs and FAs have different structural, physical and chemical properties. The eight samples in this study include four HAs and four FAs. Alternately, the samples may be classified by their origins from which they were isolated. On this basis, there are six soil samples (isolated from soil) and two aquatic samples (isolated from lake). Primary emphasis is placed on the comparison between the binding strength of $C_{12}Py^+$ to these HS samples. The understanding of the results are supported by the information obtained from the electropherograms of HSs from capillary electrolysis (CE).

Materials and Methods

Materials

Eight different HAs and FAs were studied. All samples used were Japanese origins. Dando and Inogashira HSs were purchased from Japan Humic Substances Society and other HSs were extracted by an international standard method recommended by IHSS (Swift, 1996; Yonebayashi, K. et. al., 2002). The elemental compositions and origins of the samples are listed in Table 1 (Watanabe et al., 1994; Yonebayashi, K. et. al., 2002). Dodecylpyridiniumbromide ($C_{12}Py^+ Br^-$) was synthesized by the conventional method and was purified by repeated recrystallizations from acetone. The critical micelle concentration (cmc) of $C_{12}Py^+$ obtained is 12.0 mmol dm^{-3}, which agrees with the literature value of 11.4 mmol dm^{-3} (Mukerjee and Mysels, 1971) in aqueous solution. By using tetraborate pH standard buffer solution ($Na_2B_4O_7$), all experimental solutions were kept at pH 9.18 to ensure the complete dissociation of COOH group and ionic strength of 0.03 mol dm^{-3}.

Potentiometry for surfactant binding study

The binding isotherms of $C_{12}Py^+$ to HAs and FAs were obtained by the potentiometric titration method using respective surfactant-ion-selective membrane electrodes operated at 25 °C. The surfactant-ion-selective membranes were composed of poly (vinyl chloride) (PVC) and polymeric plasticizer (Elvaroy 742, Du Pont). The potentiometric measurements were carried out by using a digital multimeter (Advantest TR6845) connected with the electrochemical cell: Ag/AgCl, KCl || sample solution | PVC membrane | reference solution || KCl, AgCl /Ag. The slope of the linear plots of the electromotive force (emf) vs. the logarithm of surfactant concentration (C_t) below the critical micelle concentration (cmc) showed theoretical Nernstian slope, i.e., 57.0–59.2 mV/decade. To assure the asymmetrical potential of the electrochemical cell, calibrations of respective surfactant-ion-selective membrane electrodes were carried out just before and after each binding measurement. The concentrations of HAs or FAs were kept constant at

Table 1. Elementary composition (% weight on an ash-free basis) of the studied samples.

Sample	Origin*	Abbreviation	Elemental composition			
			C	H	N	O
HA	Aso (active volcano soil)	AHA	60.9	2.8	2.5	32.4
	Inogashira (ando soil)	IHA	54.8	4.3	4.0	36.6
	Dando (brown forest soil)	DHA	53.0	5.3	4.5	36.9
	Lake Biwa (aquatic)	BHA	42.9	5.4	4.7	40.9
FA	Aso (active volcano soil)	AFA	43.4	3.7	1.7	51.8
	Inogashira (ando soil)	IFA	43.3	3.5	1.7	51.4
	Dando (brown forest soil)	DFA	47.6	3.5	0.8	48.1
	Lake Biwa (aquatic)	BFA	54.8	5.9	2.3	37.0

*all samples are Japanese origin

1.00 g dm^{-3} in all the binding measurements. The highest concentration of $C_{12}Py^+$ studied was far below the cmc of this surfactant.

Determination of proton-binding equilibria of FAs by potentiometric titration

In order to determine the carboxyl contents of FAs, potentiometric titration was carried out by using automatic titration system based on PC-compatible computer (KYOTO electronics, APB-410-20B), ion meter (ORION Model 720A) and a Ag/AgCl glass combination pH electrode (ORION, Model 91-01). The titrations were carried out under N_2 atmosphere to ensure a CO_2 free system and the temperature was kept constant at 25.0° C (±0.1° C).

A 500-mg dm^{-3} of FA solution was prepared directly in the titration cell by dissolving 0.0050 g of FA in 10 cm^3 of NaCl solution with the ionic strength of 0.03 mol dm^{-3}. The solutions were allowed to equilibrate under N_2 flowing for 30 min, and were then titrated with diluted carbonate-free NaOH standard solution. The ionic strength of the titrant was also kept at 0.03 mol dm^{-3} using a NaCl solution. Blank-titrations (calibration) using standard HCl solution as an analyte were also performed just before and after each measurement of sample solution to determine the standard potential of the electrochemical cell as well as to obtain the accurate concentration of standard NaOH solution. The titrations were made duplicate or triplicate.

Capillary Electrophoresis (CE)

The electrophoretic mobilities of HSs were measured at 25° C with CAPI-1000 CE system (Otsuka Electronic Co.) equipped with an UV detector and a software system for data acquisition on a PC. Samples of 1.00 g dm^{-3} HSs solutions were used for all CE measurements by dissolving the solid HSs samples in tetraborate pH standard buffer solution ($Na_2B_4O_7$) with pH 9.18 and ionic strength of 0.03 mol dm^{-3}. Tetraborate buffer with ionic strength of 0.03 mol dm^{-3} was used in order to keep the same experimental condition as in the binding measurements. The electrophoretic buffer was a solution of tetraborate pH standard buffered (pH 9.18), ionic strength of 3.0×10^4 mol dm^{-3}. Separation of HSs samples was performed by using a fused silica capillary (60cm×50μm; effective length 49cm) at a voltage of 20 kV. Injection was performed for 1 sec at the anode side of the capillary. Prior to sample injection, capillaries were washed with a portion of 0.1 mol dm^{-3} NaOH for 3 min, followed by a 3 min wash with the running buffer solution. The experiments were run for 1200 sec and measured at 200 nm UV absorbency.

Results and Discussion

Typical results of the potentiometric titration experiments are given in Fig. 1. The calibration curves clearly show an excellent performance of the surfactant-ion-selective membrane electrodes, namely the linear response with Nernstian slope and the good reproducibility before and after the binding measurement. The deviation from the calibration curve in the presence of HSs allows us to calculate the amount of bound surfactant, $C_b = C_t - C_f$. From the results obtained by the potentiometry, the binding isotherms can be constructed, where the binding degree, $n = C_b / C_{HS}$, defines as the amount of bound surfactant per concentration of humic substances, C_{HS}, expressed in g dm^{-3}, is plotted against the free surfactant concentration, C_f, in mmol dm^{-3}.
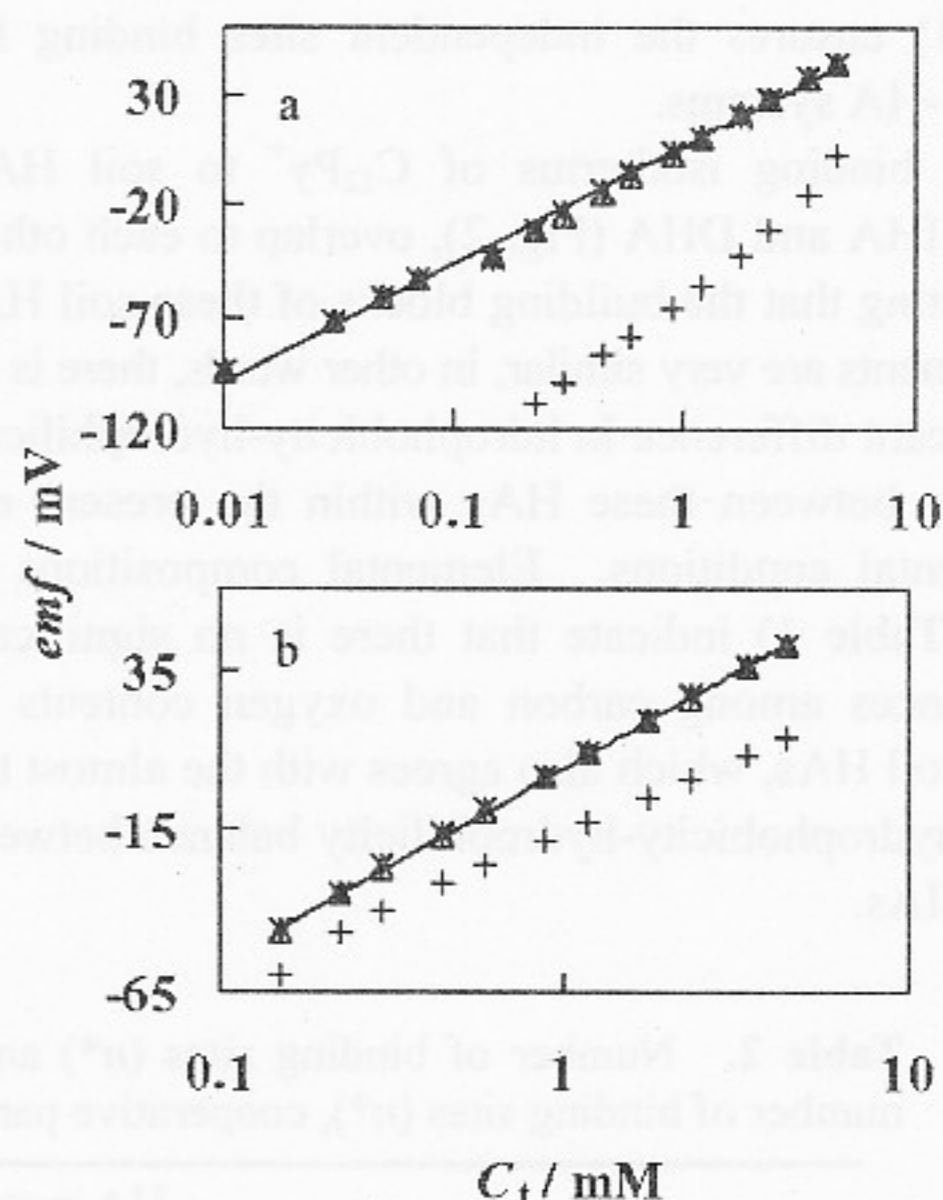

Figure 1. Potentiograms of (a) $C_{12}Py^+$-IHA system and (b) $C_{12}Py^+$-IFA system. (△) without FA or HA before binding measurements; (∗) without FA or HA after binding measurements; (+) with FA or HA; pH = 9.18, $I = 0.03$, $T = 25$°C.

Binding behavior in HA systems

Figure 2 shows the binding isotherms of $C_{12}Py^+$ to individual HAs (AHA, IHA, DHA, and BHA), where our previous results for $C_{12}Py^+$ to AHA have been included for comparison (Yee et at., in press). The cationic surfactant, $C_{12}Py^+$ ions, binds to HAs at very low equilibrium concentration, far below the cmc even in the presence of excess salt. All the investigated HAs (both soil and aquatic) give the same binding behavior with $C_{12}Py^+$ ions that is, the binding isotherms show gentle sigmoid shape and cooperative nature is not observed as in the case of AFA system. These binding isotherms are replotted as Scatchard plots (Scatchard, 1949) to see the binding mode through the all binding degree. The Scatchard plots (Fig. 3) give the straight lines with negative slopes, suggesting the independent sites binding behavior (Marshall, 1978) of surfactants to HAs. Applying the following equation,

$$n / C_f = n^* K - n K \qquad (1)$$

the number of binding sites, n^* and the binding constant, K are determined. The results are summarized in Table 2. The solid lines in Fig. 2 indicate the isotherms reproduced from the calculated values listed in Table 2. Good agreement of the experimental results with the calculated curve based on equation (1) ensures the independent sites binding for $C_{12}Py^+$-HA systems.

The binding isotherms of $C_{12}Py^+$ to soil HAs: AHA, IHA and DHA (Fig. 2), overlap to each other, suggesting that the building blocks of these soil HAs components are very similar, in other words, there is no significant difference in hdrophobicity-hydrophilicity balance between these HAs within the present experimental conditions. Elemental compositions of HAs (Table 1) indicate that there is no significant differences among carbon and oxygen contents of these soil HAs, which also agrees with the almost the same hydrophobicity-hydrophilicity balance between these HAs.
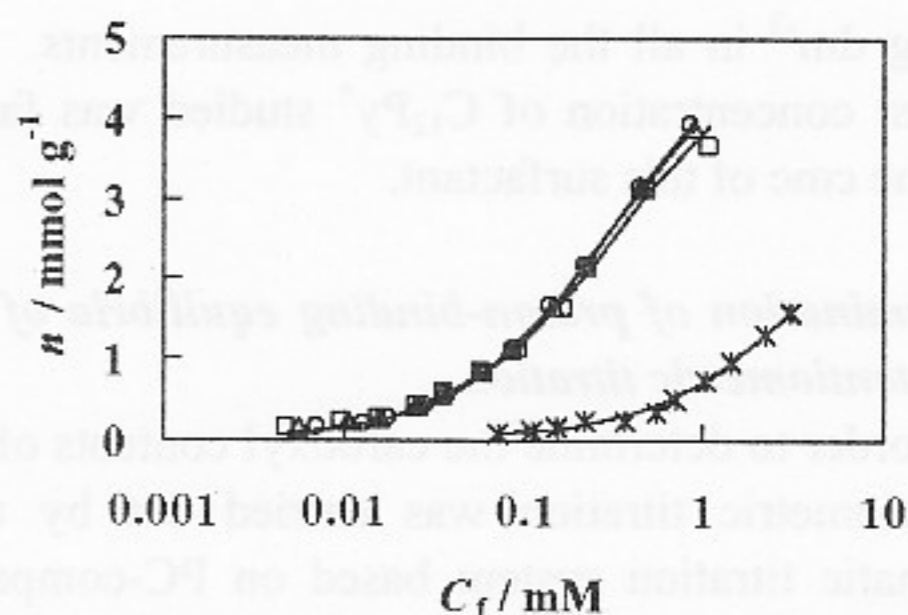

Figure 2. Binding isotherms of $C_{12}Py^+$ with HAs at 25°C. (○) AHA; (△) IHA; (□) DHA; (∗) BHA. Solid lines refer to the curves reproduced by using equation 1.
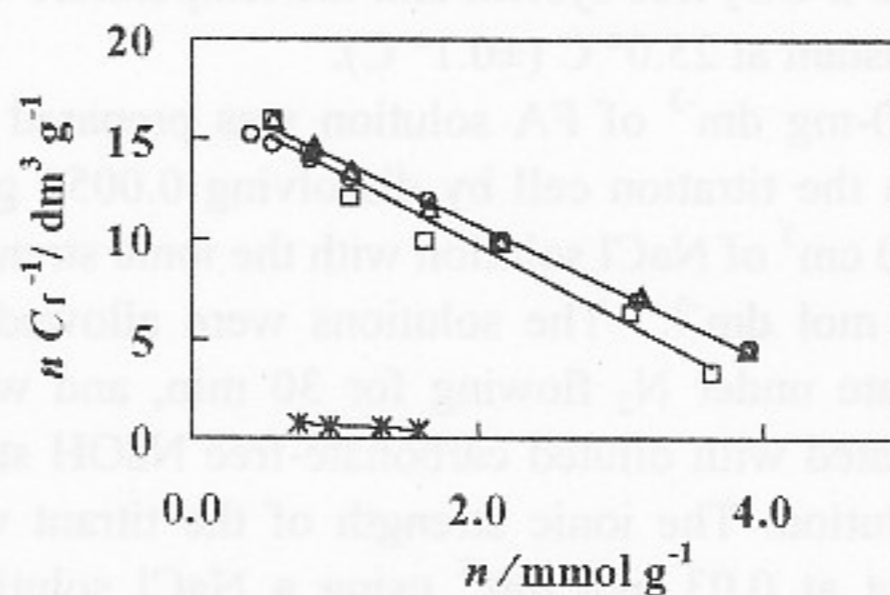

Figure 3. Scatchard plots for $C_{12}Py^+$-HA systems. (○) AHA; (△) IHA; (□) DHA; (∗) BHA.

The binding isotherm for aquatic BHA system, however, is considerably different from the other soil HA systems. The binding isotherm shifts to higher equilibrium concentration and the value of n^* and K are smaller than that of soil samples (Table 2). These results suggest that aquatic BHA is less charged and less hydrophobic than soil one since the greater in K for the soil HAs can be attributed to the interactions between the hydrophobic backbone of these HAs and the hydrocarbon chains of the surfactants. This type of hydrophobic interaction plays an important role in surfactant-HA interaction as we described in detail in the previous paper (Yee et al., 2006).

Table 2. Number of binding sites (n^*) and binding constant (K) for CnPy -HA systems and the number of binding sites (n^*), cooperative parameter (h), binding constant (K), for CnPy$^+$-FA systems.

Origin	HA system		FA system		
	n^* / mmol g^{-1}	K / mM^{-1}	n^* / mmol g^{-1}	h	K / mM^{-1}
Aso (soil)	5.4	3.01	9.6	2.9	0.70
Inogashira (soil)	5.3	3.15	7.7	1.9	0.57
Dando (soil)	4.9	3.20	5.6	1.7	1.23
Lake Biwa (aquatic)	3.9	0.23	5.1	1.8	0.42

Electrophoretic behavior of HAs.

To investigate the electrophoretic behavior of HAs from different origins, CE measurements were carried out. The migration behavior of molecules in CE depends on their charge to size ratio. If two HSs samples exhibit the same behavior in an electric field, then they are likely to have comparable charge to size ratio. Differences in the intensity and electrophoretic mobility are established by structural and chemical differences resulting from the different origins of HSs (Pompe, et al., 1996, Schmitt, et al., 1996).

Figure 4 indicates the electropherograms of HAs. The measurements are reproducible with respect to migration time and peak shape. It is noted that AHA, IHA and DHA exhibit comparable electrophoretic behavior; the intensity of first peak are almost the same for these three HAs and only a little difference is observed in the intensity of the second peak. Due to their similar migration behavior in the electric field, these HAs may have the same composition of these fractions with similar charge to size ratio. However, the intensity of first peak and second peak of aquatic BHA is much lower than the soil samples. One possible reason is that BHA contains smaller amount of these fractions than soil HAs. These peaks cannot be presently assigned to any individual substances because no standards are available for the individual fraction, however, the obtained information are in well agreement with the results of the binding study by potentiometric measurements.

Binding behavior in FA systems.

Figure 5 shows the binding isotherms of $C_{12}Py^+$ to individual FAs (AFA, IFA, DFA, and BFA). As in AFA system that we previously reported (Yee et al., in press) all $C_{12}Py^+$-FA systems studied exhibit a steep rise in the binding within a small change in equilibrium surfactant concentrations, which is characteristic for cooperative binding. Namely, the strength of surfactant binding to FA increases with the increase of the bound amount, n, because of the hydrophobic interaction between hydrocarbon chains of surfactant molecules. Such a cooperative nature is frequently observed in the interaction between surfactants and polyelectrolytes (Shirahama, 1998; Malovikova, et al., 1984; Hayakawa, et al., 1983; Liu et al., 1999). In this concern, the binding isotherm can be empirically analyzed by Hill's equation (Marshall, 1978):

$$\log\frac{n}{n^*-n} = \log\frac{\theta}{(1-\theta)} = h\log C_f + \log K_h \quad (2)$$

where n^* is the total number of binding sites expressed in mmol g^{-1} FA samples, θ is the fractional saturation, h is a quantitative measure of cooperativity, and K_h is the overall binding constant. The value of h gives a criterion by which the cooperativity can be estimated: $h = 1$ for noncooperative binding and $h > 1$ for cooperative binding (Hayakawa, et al., 1983). The value of n^* for all FA samples are given in Table 2, which are determined from the proton binding equilibria of FAs by potentiometric titration method at the ionic strength of 0.03 mol dm^{-3}. To determine the value of h and K_h, $(\theta / (1-\theta))$ is plotted in Fig. 6 against C_f. Then, the binding constant of a surfactant with an individual binding site, K, can be calculated by using the equation:

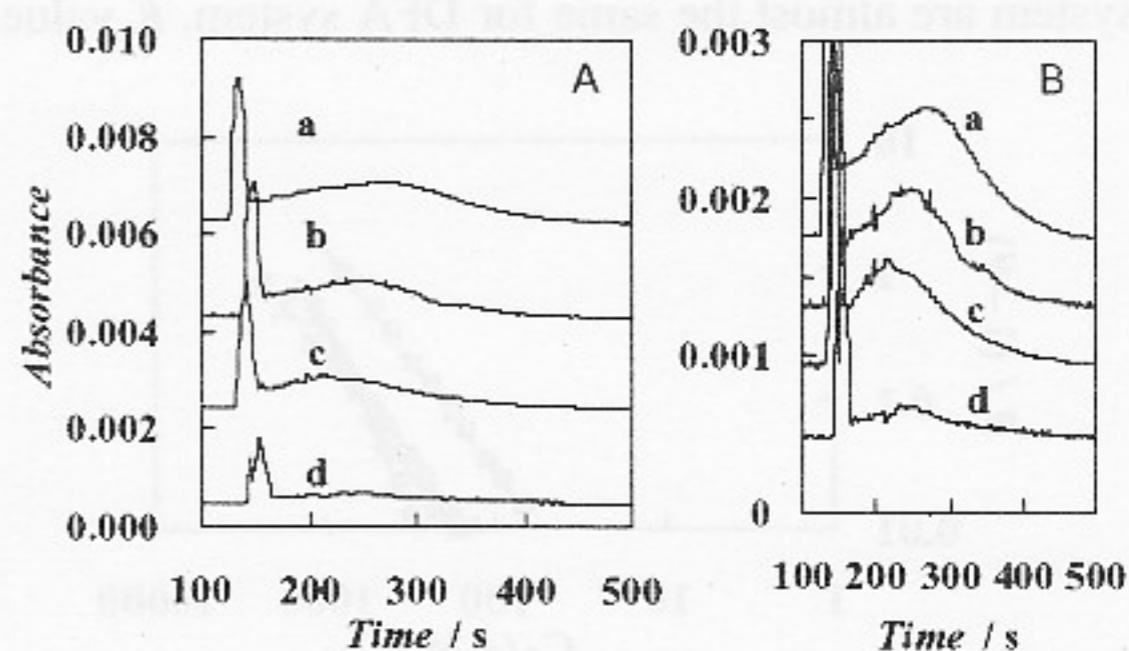$$K = (K_h)^{1/h} \quad (3)$$

Figure 4. Electropherograms of HAs analyzed with tetraborate buffer (pH 9.18) [20 kV, 25°C, detection at 200nm]. (A) First strong peak around 150 s and (B) Second weak peak around 250 s; (a) AHA; (b) IHA; (c) DHA; (d) BHA.
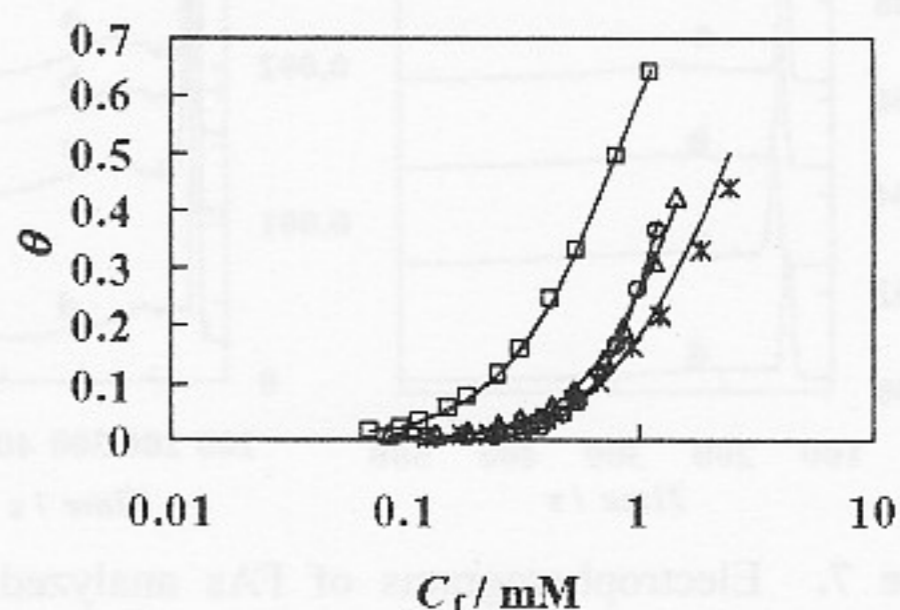
Figure 5. Binding isotherms for $C_{12}Py^+$-FA systems. (○) AFA; (△) IFA; (□) DFA; (✳) BFA. Solid lines refer to the curves reproduced by using equation 2.

The calculated h and K values for $C_{12}Py^+$ binding to AFA are summarized in Table 2. The solid lines in Fig. 5 indicate the isotherms reproduced from the calculated values listed in Table 2. Good agreement of the experimental results with the calculated curve ensures the cooperative binding for all FAs systems studied.

The binding strength is the strongest for DFA, smallest for BFA and almost the same for AFA and IFA systems. Among the three soil FAs: AFA, IFA and DFA, DFA has smallest n^*, cooperativity, h, and largest K value (Table 2). The greater in K and lower in h for $C_{12}Py^+$-DFA system may be attributed to hydrophobic interactions between the bound surfactant ions and DFA backbone, since such interaction would not contribute to the overall cooperative effect. According to the elemental composition, DFA has a little bit larger carbon content and lower oxygen content than that of AFA and IFA, and it possibly relates with the stronger binding of DFA system. Although the value of n^* and h for BFA system are almost the same for DFA system, K value

is much smaller. It may be expected that there is no effective hydrophobic interaction between the bound surfactant ions and BFA backbone.

Electrophoretic behavior of FAs.

Now we turn our attention to the electropherograms of FAs (Fig.7). No significant difference in the intensity of electrophoretic peaks is observed within the three soil origins (AFA, IFA, and DFA) even they show the different binding strength in the binding of $C_{12}Py^+$ ion. These FAs may have similar composition of these fractions with the same charge to size ratio. The first peak of aquatic FA, BFA, is almost the same with the soil samples. However, the intensity of the second peak of BFA is less pronounced than all other soil FAs. Aquatic BFA may have a smallest amount of this fraction than soil one. This different electrophoretic behavior originates from the different origin of the HSs. Presently, the strongest binding of $C_{12}Py^+$ to DFA among three soil FAs is difficult to explain with information obtained from CE analysis of FAs.

Comparison between the binding behavior of HAs and FAs systems

Differenct binding behavior is observed between HAs and FAs systems, that is, independent sites binding behavior in HAs system and cooperative binding in FAs systems due to the differences in functionality and hydrophobicity-hydrophilicity balance between these HS.

For a given type of soil origin, K value of HAs system is larger than that of FAs system (Fig. 8). K values for HAs are approximately, 4.5 times in Aso and Inogashira system and 2.5 times in Dando system, larger than that of FAs. Elemental analysis (Table. 1) indicates that high K values can be related to a large carbon content and to a rather low oxygen
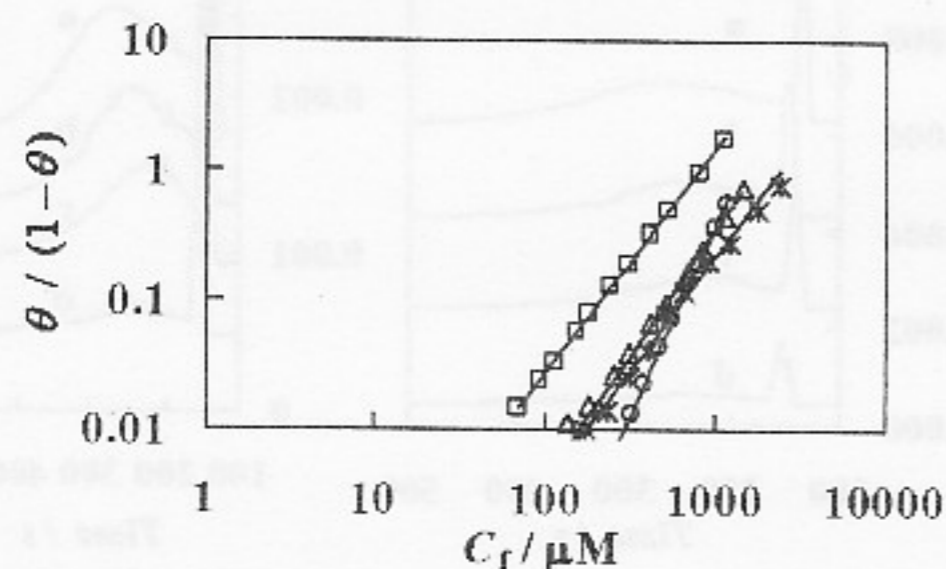

Figure 6. Hill's plots for $C_{12}Py^+$-FA systems. (○) AFA; (△) IFA; (□) DFA; (∗) BFA. Solid lines refer to the curves reproduced by Hill's equation.
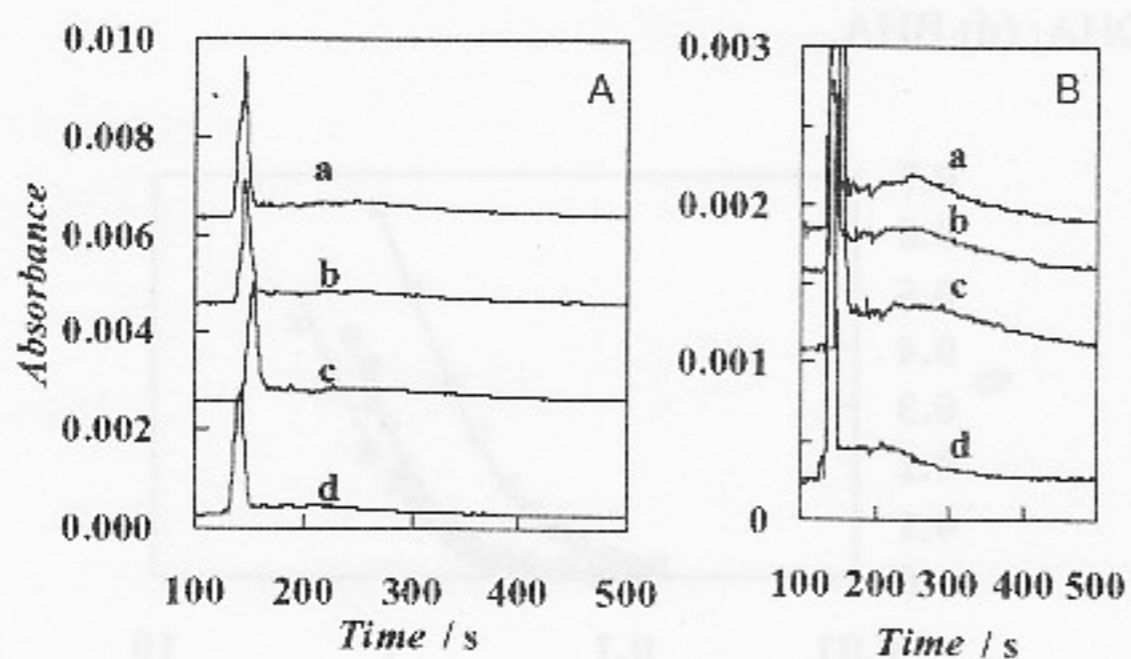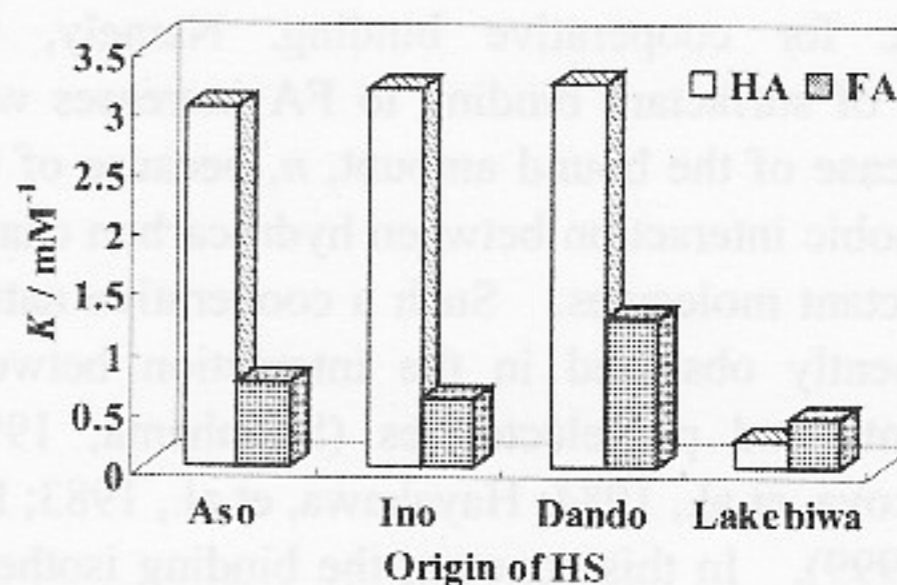

Figure 7. Electropherograms of FAs analyzed with tetraborate buffer (pH 9.18) [20 kV, 25°C, detection at 200nm]. (A) First strong peak around 150 s and (B) Second weak peak around 250 s; (a) AFA; (b) IFA; (c) DFA; (d) BFA.

Figure 8. Binding constant (K) for $C_{12}Py^+$ binding with HS of different origins.

content in HAs structures. In this regard, we can deduce that the hydrophobic interaction between the hydrocarbon tail of surfactants and hydrophobic part of HAs may possibly be one of the dominant forces apart from the electrostatic interaction in surfactant-HSs interactions. The difference in binding strength between DHA and DFA system is smaller compared with other Aso and Inogashira system. As explained in DFA system, the greater in K and lower in h for $C_{12}Py^+$-DFA system may be attributed to the hydrophobic interactions between the bound surfactant ions and DFA backbone, since such interaction would not contribute to the overall cooperative effect.

No significant difference in binding strength is observed between aquatic HAs and FAs system, even the binding behavior is different. Aquatic HAs are less hydrophobic than soil origin and consequently the hydrophobic interaction between hydrocarbon tail of surfactants and hydrophobic part of aquatic HA may be comparatively small. As a result, no distinct difference in binding strength is observed between BHA and BFA system.

Conclusions

The binding of $C_{12}Py^+$ ions to HSs vary depending on their origins. This variability can be attributed to the differences in hydrophobicity-hydrophilicity balance among HSs of different origins. The greater hydrophobic and smaller hydrophiphilic soil HAs show a stronger binding with cationic surfactant in comparison with smaller hydrophobic and greater hydrophiphilic soil FAs. No significant difference in binding strength is observed between aquatic HA and FA system. The binding is stronger with the soil samples than with aquatic one in both HAs and FAs system. These results show that hydrophobicity of HSs is one of the key factors in $C_{12}Py^+$ binding to HSs in addition to electrostatic interaction.

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