Comprehensive Study of Humic Substances-Ionic Surfactant Interaction in Aqueous Solution

By

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A Dissertation Presented to the Graduate School of Science and Engineering of Saga University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September 2006

Approval

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CERTIFICATE OF APPROVAL

Ph. D. Dissertation

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Acknowledgement

First and foremost, I would like to express my profound gratitude to my supervisors, Professor Dr. Noboru Takisawa and Professor Dr. Tohru Miyajima for their invaluable guidance, fruitful advice and encouragement throughout my research works. Special thanks are extended to their wives who help me to be smooth in daily life at the times of my earliest days in Japan.

I also owed my deepest and heartfelt thanks to Professor Dr. Kenichi Nakashima for his generous assistance in my research work. It seems impossible for me to express my appreciation with words to his wife (Mrs. Yuki Nakashima) for her kind help.

I wish to thank to the members of my dissertation committee for their comments and suggestions.

I would like to take this opportunity to express my gratitude to Emeritus Professor Dr. Keishiro Shirahama for his invaluable advices, suggestions and kind support.

I wish to convey my sincere acknowledgements to the Ministry of Education, Sport, Culture, Science and Technology of Japan (Monbukagakusho) for providing finical support for my doctoral degree.

My sincere thanks belong to the Ministry of Education, Myanmar for granting me to be in Saga University to accomplish my doctoral degree.

I acknowledge a debt of gratitude to Professor Dr. Maung Maung Htay, Department of chemistry, Yangon University, Myanmar, Emeritus Professor Dr. Nyunt Wynn and all of my teachers for their enthusiastic encouragement.

My deepest thanks are also given to my research colleagues from Takisawa lab, Miyajima lab, and all individuals who I mentioned yet, for their understanding and kind help.

Finally, thanks are also due to my beloved parents and family for their peerless love and support. I thank my husband for his favor and inspiring.

The author

Dedicated to my parents

Abstract

This work encompasses the several facets of humic substances-surfactant interaction in aqueous solution including the thermodynamic information, solution physico-chemistry, and conformational changes in their aggregation.

The subject matter is conveniently arranged into seven chapters. The first chapter covers the brief and effective introduction of humic substances (HSs) and surfactants together with their properties and applications.

The 2nd chapter deals with the amphiphilic properties of fulvic acid (FA) and humic acid (HA) evaluated by alkylpyridinium (C_nPy^+) binding study based on surfactant-ion-selective membrane electrode. The cooperative binding is found in C_nPy^+-Aso fulvic acid (AFA) system, where as the independent site binding is observed in C_nPy^+-Aso humic acid (AHA) system due to differences in charge density as well as hydrophobicity-hydrophilicity balance. In AFA system, the binding constants and cooperative parameters are calculated by applying Hill's binding theory. In AHA system, the number of binding sites and binding constants are analyzed by Scatchard plot equation. Apart from electrostatic interaction, two different hydrophobic interactions are involved in HS- surfactants interaction: hydrophobic interaction among surfactants themselves so called cooperative binding (C_nPy^+-AFA system) and hydrophobic interaction between the hydrocarbon tail of surfactant and the backbone of HS (C_nPy^+-AHA system). The binding strength is increased with increasing carbon number of surfactant in both AFA and AHA systems owing to these hydrophobic interactions.

In chapters 3 and 4, the thermodynamic information of $C_{12}Py^+$ binding with AFA and AHA are presented respectively, including the effect of pH, ionic strength, and the concentration of HSs on their binding. Thermodynamic parameters facilitate to give much deeper insight in binding mechanism. In $C_{12}Py^+$-AFA system, the binding strength is increased with increasing temperature. The cooperative binding of $C_{12}Py^+$ with AFA is the endothermic process driven by the positive entropy resulting possibly from the dehydration of hydrophobically hydrated water molecules around the hydrocarbon chains of the bound $C_{12}Py^+$ ions. Meanwhile, the temperature dependence of binding strength is not found in $C_{12}Py^+$-AHA system and the enthalpy of binding is

slightly negative. The entropy of binding ($\Delta S°$) in AFA and AHA systems is 95 and 61 J mol^{-1} K^{-1} respectively.

In both AFA and AHA systems, the binding is obviously pH dependent and is most pronounced at pH 9.18. In AFA system, the effect of pH on the binding is investigated at two pH regions, i.e., at pH>7 and pH<7 while the ionic strength of the system is kept constant at 0.03 mol dm^{-3}. Different binding phenomena are observed: the cooperative binding at pH>7 and non cooperative binding at pH<7.

Moreover, the binding strength is decreased with increasing ionic strength due to ion screening effect in both AFA and AHA systems. The sensitivity of binding strength to electrolyte concentration is higher in AHA system than that in AFA system suggesting that the more counterions are condensed on the oppositely charged AFA chains at certain pH and ionic strength. Thus, relatively smaller extent of change in binding is observed with the additional changing of ionic strength. This observation is in consistent with the greater entropy of binding in AFA system.

In chapter 4, the hydrodynamic diameters ($2R_h$) of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates, investigated by using dynamic light scattering (DLS), are also included. In the absence of cationic surfactant, the hydrodynamic diameter of AHA is unattainable within the experimental condition because of their inherent polydispersity. In the presence of surfactant, however, the hydrodynamic diameter of $C_{12}Py^+$-AFA or $C_{12}Py^+$-AHA aggregates becomes measurable with high reproducibility due to the coagulation force of cationic surfactant. In both systems, the hydrodynamic diameter increases with increasing $C_{12}Py^+$ concentration due to the growth of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates while maintaining a constant pH, ionic strength, and AFA/AHA concentration at 9.18 and 0.03 mol dm^{-3}, 0.05 g dm^{-3}, respectively. The hydrodynamic diameters of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregate increase with increasing ionic strength, which is more pronounce in AHA system. This results point up a mark for higher sensitivity of binding strength to electrolyte concentration in $C_{12}Py^+$-AHA system than that in $C_{12}Py^+$- AFA system.

Chapter 5 focuses the study of the interaction between anionic surfactant, sodium dodecyl sulfate (SDS), with AHA by potentiometric titration and dynamic light scattering (DLS) methods at pH 9.18 (ionic strength 0.03 mol dm^{-3}) and pH 3.98 (ionic strength 0.10 mol dm^{-3}). There is no binding between SDS with AHA at pH 9.18 and

ionic strength of 0.03 mol dm^{-3} since the strong electrostatic repulsion between these molecules outweighs any specific interaction. At pH 3.98 and high ionic strength some interaction is observed by DLS measurement since electrostatic repulsion is suppressed by counterions at this solution condition.

In order to study the various aspects of HSs-ionic surfactants interaction, the effect of cationic surfactant headgroup on the binding with HSs is also reported in this chapter. The binding of dodecyltrimethylammonium ($DTMA^+$) ions with AFA or AHA is weaker than that of $C_{12}Py^+$ ions, due to steric hindrance of headgroup of $DTMA^+$ ions. On one way, the binding of $C_{12}Py^+$ ions with AFA or AHA is stronger than that of $DTMA^+$ due to stronger attractive force induced by resonance effect of benzene ring carried by $C_{12}Py^+$ ions. From DLS measurements, it is found that the hydrodynamic diameter of $DTMA^+$-AFA/$DTMA^+$-AHA aggregates is smaller than that of $C_{12}Py^+$-AFA/$C_{12}Py^+$-AHA aggregates and $DTMA^+$-AHA aggregates is smaller than $DTMA^+$-AFA aggregates.

The affinity of $C_{12}Py^+$ to HSs appears to vary among HSs samples of different origins since HS are continuously subject to alterations in the biosphere. In chapter 6 the binding of dodecylpyridinium ($C_{12}Py^+$) ions with FA and HA of different origins are examined by potentiometric titration method and the variability in binding strength is related with the structural and chemical features of analyzed HSs. On the binding with $C_{12}Py^+$ ions, all investigated FA of different origins (both soil and aquatic) exhibit cooperative binding behavior and all investigated HA exhibit independent sites binding behavior. However, the binding strengths are different depending on their origins. The binding affinity of $C_{12}Py^+$ ions is stronger with soil HA than with soil FA. In both FA and HA systems, $C_{12}Py^+$ binding strength is stronger with soil samples than that with aquatic samples. These results show that hydrophobicity of HSs is one of the key factors in HS- cationic surfactant binding since soil HS is more hydrophobic than aquatic one as well as HA is more hydrophobic than FA.

Overall, the substantial informations are summarized in chapter 7.

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1. Introduction

This chapter lays a foundation for humic substances (HSs) - surfactant studies by providing an overview of HSs, ionic surfactants, and some fundamental concept of potentiometric titration based on surfactant-ion-selective membrane electrode.

1.1. Humic Substances: Structure, Compositions, and Properties

Humic substances (HSs) are breakdown products of plants and biological origins found in almost all terrestrial and aquatic environments that can not be exactly classified as any other chemical class of compounds (e.g., polysaccharides, proteins, lignin, etc.) [1,2]. The pathways proposed for the formation of HSs during the decay of plant and animal remains in soil, is shown in Fig. 1 [3]. The size, molecular weight, elemental compositions, structure, and the number and position of functional groups of HSs vary depending on their origin, method of extraction, and natural condition prevailing their formation[4-6]. HS are operationally be classified as three fractions according to their solubility in water: fulvic acid (FA), humic acid (HA) and humin. FA are those organic materials that are soluble in water at all pH values. HA are those materials that are soluble only above pH 2. Humin is the fraction of natural organic materials that is insoluble in water at all pH [7-11].

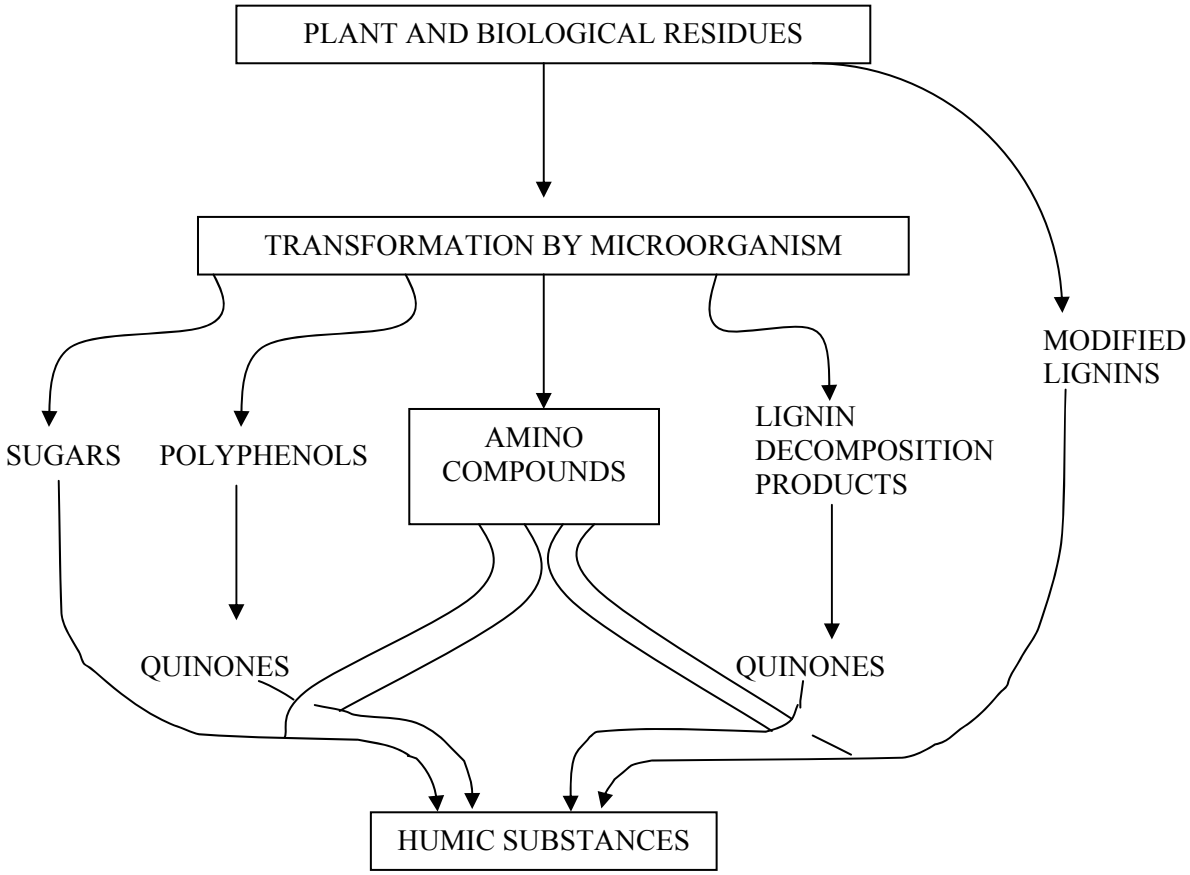


Figure 1. Purposed Mechanism for the formation of humic substances

(from Stevenson)

1.1.1. Structures of HS

Although precise structure of HA and FA is unattainable, the knowledge of the basic structure is required for a full understanding of the properties and function of these constituents in the environment [12-14]. The main advantages of hypothetical models are: (1) as a means of representing the average properties of HA and FA, (2) to help in the formulation of new hypotheses regarding their structures and the development of innovative experimental schemes for the investigation, and (3) for illustrating mechanisms of the binding of metal ions and organic compounds [15]. The hypothetical structures of HA and FA are given in Figs. 2 and 3 [15, 16].

HSs have a wide range of molecular weights and sizes, ranging from a few hundred to as much as several hundred thousands atomic mass units. In general, FA are lower molecular weight than HA, and soil-derived materials are larger than aquatic materials [17]. The structures of FA are somewhat more aliphatic and less aromatic than HA, and FA are richer in carboxylic acid, phenolic, and ketonic groups [18, 19]. This is responsible for their higher solubility in water at all pH. HA, being more highly aromatic, become insoluble when the carboxylated groups are protonated at low pH values. This structure allows HS to function as amphiphilic compounds, with the ability to bind both hydrophobic and hydrophilic materials [20, 21].

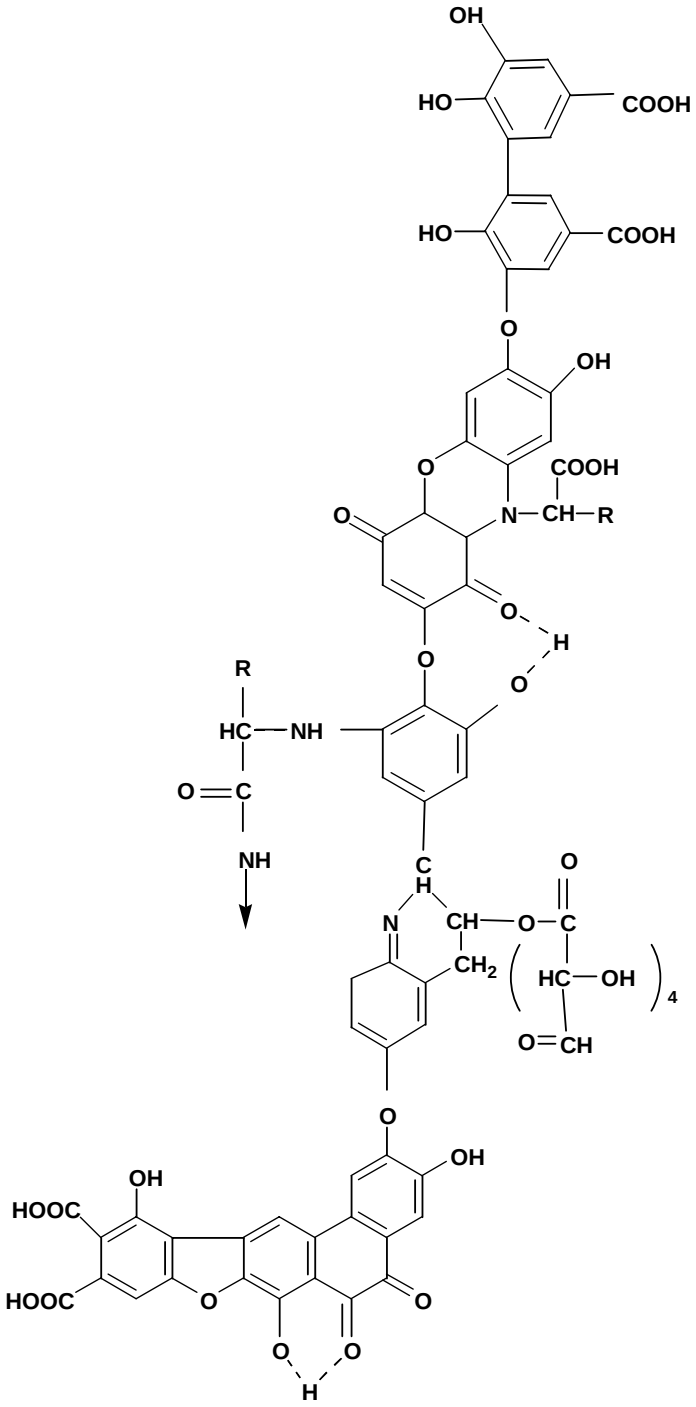


Figure 2. Model structure of HA (Steveson)

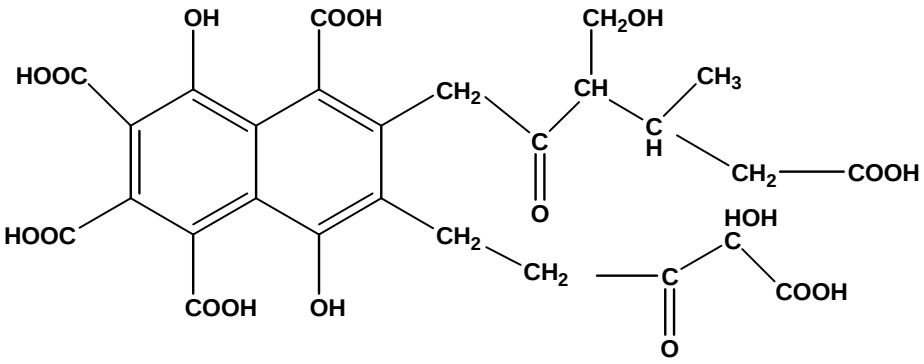


Figure 3. Model structure of FA according to Buffle et al. (1977)

1.1.2. The Compositions of HSs

The compositions of HSs refer to the elemental composition, functional groups, building blocks, and actual HSs molecules. The major elements in their composition are C, H, O, N, and S. These elements are always present regardless of their origin and country or continent. The usual range for the elemental compositions of HSs are described in Table 1 [22, 23].

Generally, HA contains more carbon and less oxygen than FA. A variety of functional groups, including COOH, phenolic OH, enolic OH, quinone, hydroxyquinone, lactone, ether, and alcoholic OH, have been reported in HSs.

Table 1. Usual Range for the Elemental Composition of HS

Element	Humic Acids / %	Fulvic Acid/%
Carbon	53.8–58.7	40.7–50.6
Oxygen	32.8–38.3	39.7–79.8
Hydrogen	3.2–6.2	3.8–7.0
Nitrogen	0.8–4.3	0.9–3.3
Sulfur	0.1–1.5	0.1–3.6

1.1.3 Methods of Characterization

Nearly every method available to the analytical chemist has been used in attempt to characterize HS, to unravel the complex properties and behavior of HSs. Some of the more widely used methods are listed in Table 2 [24, 25].

Table 2. Methods for Analysis and Characterization of Humic Materials

Molecular weight Determination
Viscosity
Vapor pressure osmometry
Ultracentrifugation
Gel filtration
Laser Light Scattering
Functional Group Analysis
Fourier transform infrared spectroscopy
^{1}H NMR
^{13}C nuclear magnetic resonance
Electron Spin Resonance
Pyrolysis-gas chromatography
Pyrolysis-mass spectrometry
Pyrolysis-Fourier transform infrared
spectroscopy
pH titration
Binding Studies
Cation exchange
Fluorescence
Photoacoustic spectroscopy
Dialysis
Potentiometric titration

These include both chemical, physical, degradative and nondegrdative methods. As yet, no single analytical method can provide data for absolute characterization of the structure and properties of humic materials. Therefore, a combination of several techniques, with the comparison and confirmation of results from each method are used to clear up the complex issues of humic composition and properties.

1.1.4. The Versatile Properties of HS and its Application

HSs represent one of the greatest carbon reservoirs on earth. Approximately 80% of the total carbon in terrestrial media and 60% of the dissolved in aquatic media are made up of humic substances. They seem to be purpose built for many life-sustaining functions [26]. According to the accepted principles of colloid science, HS systems are considered to exhibit colloidal properties when the dimensions of the dissolved or dispersed components (in hydrophobic or hydrophilic colloids, respectively) are in the range of 1 to 1000nm [27-29]. Hydropobic moieties such as long alkyl side-chains from fatty acid residues provide amphiphilic character in humic molecules. Therefore, analog properties to those of surface-active agents can also be expected [30].

The specific properties of HSs such as a high cation exchange capacity, the ability to chelate metals, the ability to adsorb organic, a high water holding capacity, an ease of precipitation at low pH or in the presence of coagulants, and an ease of combustion due to its organic nature are useful for agriculture, environmental, industry and biomedicine [31].

The agricultural applications include a slow release of the micronutrients for plant and microbial growth, a high water-holding capacity, a buffering capacity that results in plant growth stimulation. Currently, humic materials are used as additives in fertilizers [32]. Different salts of HSs, such as calcium humate and ammonium humate are used to increase soil fertility and found to have a significant grow-stimulation effect [33].

The environmental applications include metal removal by chelation, removal of organics by adsorption, neutralization of acidic water streams, removal of anions, reduction of metal species and explosives and chemical agent destruction [34, 35].

So far, industrial and biomedical applications of HSs and humus-derived products are rare. These are used in building as additives to control the setting rate of concrete and

in the preparation of lather as a leather dye and as an ingredient of a solution to furnish leather [36].

Several studies of the medicinal properties of humic materials have been reported and are used in veterinary and human medicine. Thiel et al found that preincubation of cell cultures with ammonium humate avoided infection by the herpesvirus noted the function of HS as protectors of the organism [37]. Pflug et al reported that HA are able to interact with the bacterium Micococcus luteus. In this case humic materials protected the organism against cell-wall disruption by the enzyme lysozyme [38].

1.1.5. Interaction between HSs with Inorganic, Organic, and Amphiphilic Materials

As described above, HSs are the most widely spread natural complexing agent occurring in nature and have an ability to interact with many other classes of compounds. For this reason the study of interaction between HSs with inorganic constituents such as heavy metal ions, hydrated metal oxides, and clay minerals and with organic materials such as hydrophobic organic pollutants have been the subject of long-standing and continued research.

The complexation of metal ions with HSs is of great interest in understanding of metal ion transport, toxicity and bioavilability of metal ions [39]. Numerous studies about metal interactions with HSs, applying many different analytical methods, including potentiometry, anodic stripping voltametry, fluorescence techniques, nuclear magnetic resonance spectroscopy, luminescence spectroscopy, capillary electrophoresis and ion-selective electrodes have been reported [40-43]. Although there are a variety of functional groups in the HA and FA structures, the carboxylate groups are primarily responsible for binding of metals and radionuclides under most natural conditions.

The presence of HSs can also promote the solubilization of nonpolar hydrophobic compounds (eg. dichlorodiphenyltrichloroethane (DDT)). This act to decrease the sorption of these materials to the soils or sediments or to decrease the volatility rate of the more volatile organic (e.g. polychlorinated biphenyl) [44]. As in the case of metal ion interaction, there are many studies of interaction between HSs and hydrophobic organic compounds using various methods: physical phase separation method,

equilibrium dialysis method, Solid Phase Microextraction (SPME) method, fluorescence quenching (FQ), rapid solid phase extraction (SPE), flocculation, and solubility enhancement etc [45-47].

Despite a large number of studies carried out on the metal complexation of HSs as well as on the interaction with hydrophobic organic compounds, studies on the binding with other amphiphilic compounds such as surfactants, which are widely utilized in various fields, has still received limited attention.

1.2. Surfactants

Surfactants are molecules with long hydrophobic chain and hydrophilic head group that alter solution surface tension. Most familiar of all surfactants is soap. A well-known feature of surfactant solutions is micelles formation and their ability to dissolve a variety of oil soluble materials, e.g. hydrocarbons, perfumes, dyes and so on. The interfacial activity of surfactants, which can be explained in terms of their molecular structure, gives rise to a wide range of surface chemistry functions: wetting, emulsifying, solubilising, rheology modifying, lubricity and surface condition. The aggregation of surfactants in aqueous solution is governed by the subtle balance of hydrophobic, hydrophilic and ionic interactions [48, 49].

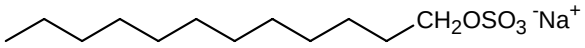
1.2.1. Classification of Surfactants and its Applications

Anionic surfactants, which include soap, are most widely used for cleaning processes because many are excellent detergents. One another important application of anionic surfactant is routine in most biochemical field [50].

Cationic surfactants comprise a long chain hydrocarbon as the lipophile with a quaternary amine nitrogen as hydrophile, and halide ions as counterions. An important property of cationics is that they are attracted to surfaces carrying a negative charge, upon which they adsorb strongly. Proteins and synthetic polymers can thus be treated with cationics to provide desirable surface characteristics. For example, hair conditioners and fabric softeners are cationic surfactants.

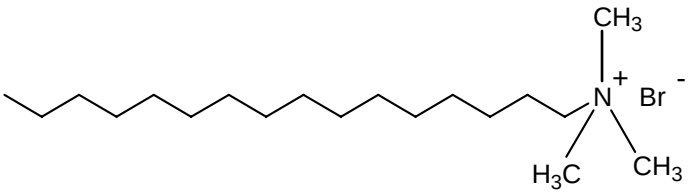
Amphoteric surfactants comprise a long hydrocarbon chain (lipophile) attached to a hydrophile containing both positive and negative charges, which give it the properties of a zwitterion. The simplest amphoterics can therefore behave as a cation or anion depending on pH. Mild and with low irritancy, amphoterics are widely used as in shampoos.

Non-ionic surfactants are second to anionics in cleaning applications and are frequently used in conjunction with them. Figure 4 shows the structures of some common surfactants [51, 52].



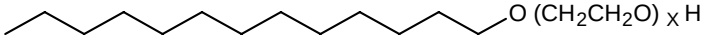
Anionic

(1) Sodium lauryl sulphate



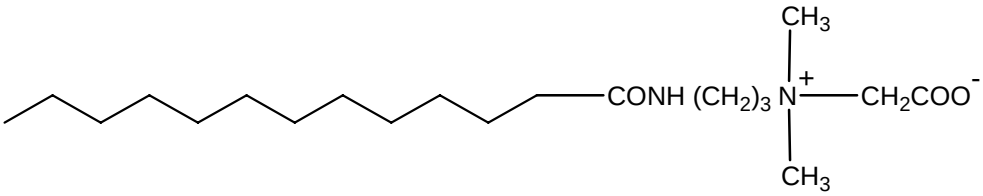
Cationic

(2) Cetyl trimethyl ammonium bromide, Cetrimide



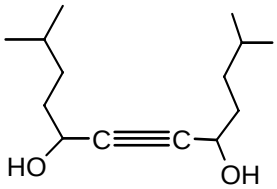
Non-ionic

(3) Lauryl alcohol ethoxylate



Amphoteric

(4) Laurylamidopropyl betaine



Non-ionic Gemini

(5) Surfynol acetylenic diol

Figure 4. Structure of some common surfactants

1.3. Binding of Surfactants with HSs

As mention above surfactants markets everywhere from household detergents to explosives and have invaded almost every sector of industry. Because of widespread and persistence uses, surfactants can be introduced into the environment through waste water or direct contamination and can accumulate in soils and waters. Natural plant-derived surfactants have been detected in river water at concentrations sufficiently high to produce persistent foam [53]. In case of the deposition of cationic surfactants in the soils and waters, it is expected that these substances will readily bind to negatively charged humic substances [54]. Thus the knowledge of the interactions of cationic surfactants with HS is of particular importance, especially with respect to fate and transport of organic pollutants in the environment.

On the other hand, ionic surfactants might be used in order to make better understanding the nature and effect of HSs in the environment. For example alkylammonium ions increase the order of disorder materials. In this regard, Tombaz et al studied the X-ray diffraction patterns of alkylammonium humate complexes and discussed the possible structure [55]. Thieme et al. investigated the interaction of colloid soil particles, humic substances and cationic detergent by X-ray microscopy and explored that how the coagulation force of cationic detergents will change the structure formed by the soil colloids when the HS are presents [56]. Otto et al. reported the NMR diffusion analysis of surfactant-humic substance interactions, and it was found that cetyltrimethylammonium bromide interacts more strongly with HA than with FA [57]. Adou et al demonstrated that cationic surfactant-HA interactions could lead to phase separation of the dissolved humics. The author stated that HA is removed from the aqueous phase by forming neutral hydrophobic complexes with cationic surfactants, however, no further evidence for the binding mechanism was presented [58]. Thus, it seems to be worthwhile to study the binding behavior of surfactants with HS in some detail from the viewpoint of academic research as well as applications. As yet, such a study has not been explored.

1.3.1. Binding Isotherms

There are many methods to study interactions between surfactants and oppositely charged polyelectrolytes in solution. One of the fundamental and necessary methods is the determination and analysis of the binding isotherm [59]. The binding isotherm expresses the amount of "bound" surfactant as a function of the free surfactant concentration. In order to determine the binding isotherm, the equilibrium free surfactant concentration needs to be determined in solution containing both polyelectrolyte and surfactant. In the early stages of polymer- surfactant research, binding isotherms were derived from changes in viscosity or surface tension. Equilibrium dialysis is a standard method. More recently, the surfactant-ion-selective electrodes are available to measure the free surfactant concentration directly in polyelectrolyte solution [60].

1.3.2. Preparation of Surfactant-Ion-Selective Membrane Electrodes

The following concentration cell: Ag/AgCl, KCl || reference solution, C_1| PVC membrane | sample solution, C_2 || KCl, AgCl/Ag was constructed as shown in Fig. 5. Where PVC is a surfactant-selective membrane containing 80% bis (2-ethylhexyl) phthalate (DOP) and 20% poly (vinyl chloride) (PVC, average degree of polymerization is about 1300). To a slurry mixture of DOP and PVC, tetrahydrofuran (THF) was added to obtain a clear viscous solution after warming for a while. The PVC solution was cast on a flat glass plate, and the solvent was gradually evaporated in a dry atmosphere over a day. A piece of the gel membrane (0.2–0.3 mm thick) is cut out and glued on one end of a PVC tube (1-cm diameter and 11cm long), with a PVC–THF solution being a good adhesive. The gel membrane was annealed at 40°C under reduced pressure for several hours before use.

The *emf* of this symmetrical cell is expressed by the Nernst equation (neglecting activity coefficient differences),

$$E = E^{\circ} + (RT/zF) \log (C_2/C_1) \quad (1)$$

where, E and E° are the potential and standard potential of the electrochemical cell.
R is the gas constant, T temperature in Kelvin, z the number of electrons transferred in the balanced net reaction, and F faraday constant. C_2 and C_1 are the concentration of

sample and reference solution respectively. The electrode performance is judged by the following criteria:

Is the plot of E vs. log C_2 a straight line with RT/zF equal to 59.1 at 25°C?

Is E at C_2 equal C_1, zero?

Do the data correctly reflect solution properties such as the critical micelle concentration (*cmc*)?

Does the electrode have a sufficient selectivity against other ions in the system? [61-64]

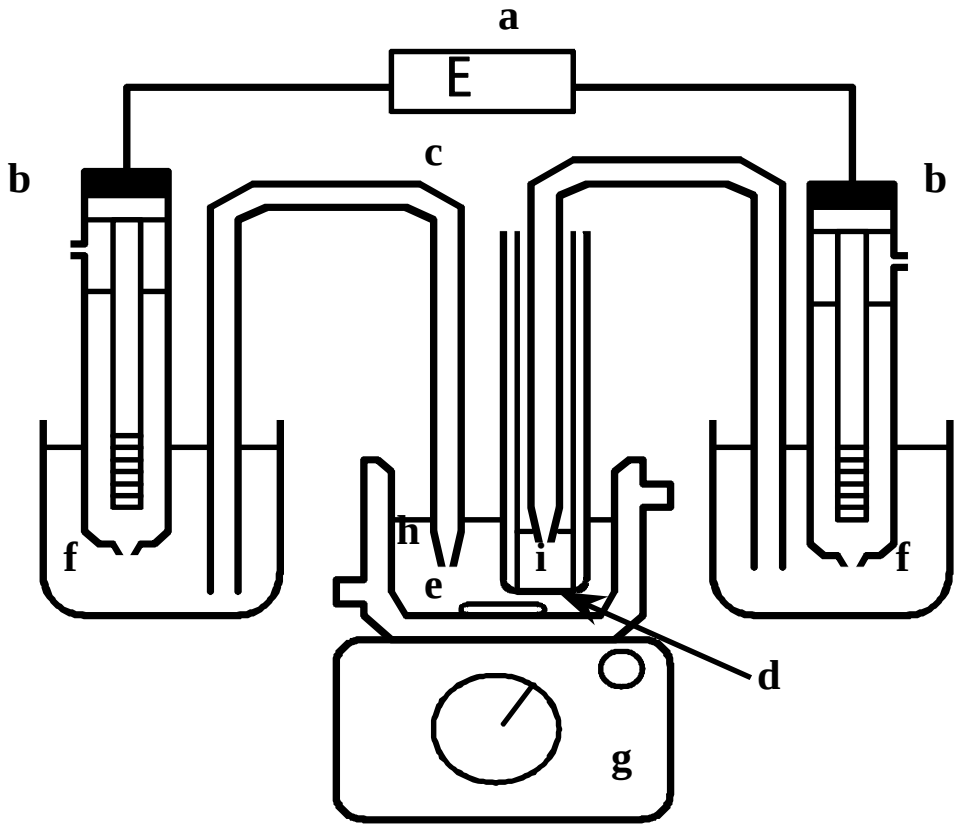


Figure 5. The Schematic diagram of surfactant-ion-selective membrane electrode

a = digital multimeter
b = Ag/AgCl electrode
c = KCl saltbridge
d = surfactant selective polyvinyl chloride membrane
e = sample solution
f = KCl solution
g = magnetic stirrer
h = Glass cell
i = inner reference solution

1.3.3. Basic Concept for Binding Isotherm Determined by Surfactant-Selective Membrane Electrodes

Many investigators have employed surfactant-ion-selective electrodes to study a wide variety of surfactant solutions [65-69]. In this method, the surfactant electrode electromotive force (*emf*) relative to standard electrode is determined for a calibration curve in the absence of polyelectrolytes, followed by *emf* measurements in the presence of constant polyelectrolyte concentration (Fig. 6, triangles and plus symbols, respectively). In the absence of polyelectrolytes, surfactant-ion-selective membrane electrode shows a nernstian response below cmc. In the presence of polyelectrolytes, *emf* valve deviates sharply from the calibration curve. The amount of bound surfactant and the degree of surfactant binding, *n*, defined as mole of bound surfactant per mole polyelectrolyte repeating unit or ionic group, can be determined. Care must be taken to ensure stability of the calibration curve over the course of the measurement, best achieved by "sandwiching" calibration between unknown determinations.

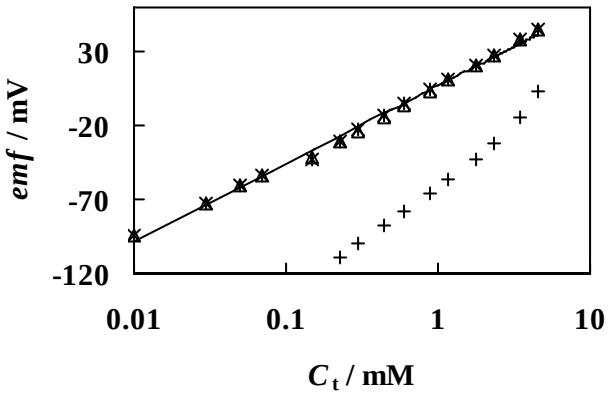


Figure 6. Example of potentiometric titration curve determined with cationic surfactant electrode. System: Inogashira Humic acid (IHA) (1 g/ L)-dodecylpyridinium bromide, (Δ); (∗) without IHA; (+) with IHA; pH = 9.18, I = 0.03, T = 25°C.

Because of the rapid response of the electrodes, full binding isotherms can be determined in relatively short times, and with minimal materials use. Binding isotherms have played a key role in the development of polyelectrolytes-surfactant research, yielding information about the nature of the binding process and allowing conclusions

about the structure of the aggregates [70-74]. Because of these advantages, this method seems to be promising tool to study the surfactant-HS interaction.

The objective of this study is to investigate the nature of phenomena of ionic surfactants-HS binding and to characterize their mutual interaction. This will permit us to assess in greater detail the relative importance of surfactant-HSs interaction in the academic and practical fields. In general, the amphiphilic property of humic substances is primarily responsible for the binding with heavy metals, persistent organic xenobiotics, and mineral surfaces and plays a crucial role in the detoxification of hazardous compounds, the fate and transport of organic substances, and substantial agriculture and environmental quality. In this regard, we evaluated the amphiphilic properties of HSs by alkylpyridinium binding study in chapter 2 [75].

The thermodynamic informations are essential for the better understanding of surfactant-HS binding mechanism. Thus, the thermodynamic information of dodecylpyridinium ion ($C_{12}Py^+$) binding with HA and FA is investigated and described in chapters 4 and 5, respectively, including the effect of pH and ionic strength on this system. These chapters also depict the substantial differences and similarities between HA and FA. With regard to the physical characterization of the surfactant-HS system, one of the most important parameter that strongly affects the diffusion coefficient is size distribution. For this reason, the subject matters concerning with the hydrodynamic diameters of $C_{12}Py^+$-FA and $C_{12}Py^+$-HA aggregate are also presented in this chapter [76, 77].

It has been hypothesized that the hydrophobic interaction is one of the driving forces in the interaction between ionic surfactants and HSs. In this context, the interaction between anionic surfactant, sodium dodecyl sulfate (SDS), with a more hydrophobic and less charged HA is investigated in chapter 6. Furthermore, the change in headgroup size should have a certain influence on the surfactant binding. The study of the headgroup effect is also included in this section [78].

The affinity of cationic surfactants to HSs appears to vary among HS samples from different origins. The variability of binding capacities can be correlated with the structural and chemical features of analyzed HSs. Thus, to unreavel the complex properties and behavior of HSs, it is substantial to investigate HSs from different origins.

We reported the study of binding between surfactant and HSs of different origins in chapter 3 [79].

Ultimately, the experiments described in this work have been systematically designated to examine the surfactants-HS interaction in greater detail. This study provides an insight the surfactant-humic substances intermolecular binding including the thermodynamic information, the effect of pH, ionic strength and concentration and origins of HSs affected on this system. This stage seems set for substantial progress of the new scientific studies.

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Evaluation of Amphiphilic Properties of Fulvic Acid and Humic Acid by Alkylpyridinium Binding Study

Amphiphilic properties of Aso fulvic acid (AFA) and Aso humic acid (AHA) are evaluated through the study on the binding of *N*-alkylpyridinium bromide ($C_nPy^+Br^-$, n = 12, 14, 16), using a potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18 and ionic strength of 0.03 M at 25°C. Different binding behaviors are observed between AFA and AHA due to the differences in the density of carboxylate groups as well as hydrophobicity-hydrophilicity balance. Independent sites binding behavior is observed in the C_nPy^+-AHA system, however, cooperative binding is observed in C_nPy^+-AFA system. The binding of C_nPy^+ to AHA is stronger than that to AFA, reflecting the importance of hydrophobic interaction between surfactant molecules and the backbone of AHA molecules. The chain length dependence of the free energy of binding per CH_2 group amounts to about 3.1 kJ/mol in C_nPy^+-AFA system, which is comparable to C_nPy^+ binding to dextran sulfate and that for C_nPy^+-AHA system is 2.3 kJ/mol.

2.1. Introduction

Humic substances (HS), found in soils and waters, are heterogeneous organic constituents with structurally complex backbones and various functionalities, and can be characterized as a polyelectrolyte. Depending on their solubility, HS can operationally be divided into three fractions: fulvic acid (FA), humic acid (HA), and humin [1–3]. HS also exhibit surface active and charged colloidal behaviors in aqueous system [4]. HS are known to interact not only with inorganic constituents, such as metal ions, hydrated metal oxides, clay minerals, but also with organic compounds, such as hydrophobic organic pollutants [5–8]. Through their amphiphilic properties, HS play a significant role in (1) the formation of soil aggregates, (2) the control of soil acidity, (3) the cycling nutrients, (4) the detoxification of hazardous compounds, (5) the fate and binding of solutes, and (6) the sustainable agriculture and environmental quality. It can be anticipated that their amphiphilic properties and their quantity directly and/or indirectly affect our environment and human health [9,10]. The objective of the present study is to investigate the amphiphilic properties, i.e., both polyelectrolytic and hydrophobic properties of HS.

A considerable amount of works has been carried out on the interaction between naturally occurring and synthetic polyelectrolytes and charged surfactants because of their essential role in biological and industrial systems. Many key developments have resulted from the applications of physical and/or chemical methodologies such as, surface tension measurement, viscometry measurement, conductance measurement, potentiometry, as well as spectroscopic tools such as NMR and fluorescence measurements [11–19]. Binding isotherms have provided important information such as the nature of the binding process, the degree of binding, and the structure of the aggregates [20]. Satake and Yang [21] initiated the investigation on polyelectrolyte–surfactant ion binding using surfactant-ion-selective electrodes; they were successful in the expression of surfactant binding behavior to various types of polymers. Many researchers found that ionic surfactants are cooperatively bound to oppositely charged polyelectrolytes and the binding starts at the free surfactant concentrations the order of magnitude below the critical micelle concentration (cmc), and is influenced by various

factors such as the added salt concentration, surfactant chain length, pH, temperature, etc. [22–30]. It has been well recognized that the binding is not only because of electrostatic interactions but also through other specific interactions.

Based on these fundamental information [20–30] studies for surfactants and well-characterized polymers, it may be worth while to study the surfactant binding to heterogeneous HS by use of surfactant-ion-selective electrodes in order to evaluate the amphiphilic properties of HS. However, the number of publications on the interaction of HS with surfactants is rather small and the understanding of the binding at molecular level is incomplete even at the present moment. In this study we have examined the binding behavior of cationic surfactant to humic substances by potentiometric titration method based on surfactant-ion-selective membrane electrodes. The effect of surfactant chain length on the binding isotherm has also been investigated.

2.2. Experimental Section

2.2.1. Materials

Aso fulvic acid (AFA) and humic acid (AHA) were collected from the Aso area of Kyushu Island of Japan and extracted by an international standard method, recommended by IHSS [31]. *N*-Alkylpyridiniumbromide (C_nPyBr, n = 12, 14, 16), i.e., dodecylpyridinium bromide ($C_{12}PyBr$), tetradecylpyridinium bromide ($C_{14}PyBr$), and hexadecylpyridinium bromide ($C_{16}PyBr$), were purchased from Wako Pure Chemical Industries, Ltd., and were purified by repeated recrystallizations from acetone. Tetraborate pH standard buffer solution (pH 9.18, ionic strength 0.03 M) was used to fix the pH and ionic strength of the sample solutions. Deionized water (Millipore Milli-Q system) was used in the preparation of all experimental solutions.

2.2.2. Potentiometry for Surfactant Binding Study

The binding isotherms of C_nPy^+ to AFA and AHA were obtained by the potentiometric titration method using respective surfactant-ion-selective membrane

electrode 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 || reference solution| PVC membrane | sample solution || KCl, AgCl/Ag. The slope of the linear plots of the electromotive force (emf) vs. the logarithms of surfactant concentration (C_t) below the critical micelle concentration (cmc) showed 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 electrode were carried out just before and after each binding measurement. The concentration of AHA was kept at 1.00 g/dm^3 in the binding measurements of $C_{12}Py^+$ and $C_{14}Py^+$, whereas for the binding experiment of $C_{16}Py^+$ the concentration was lowered to 0.10 g/dm^3. In the case of AFA, the concentration was 1.00 g/dm^3 for the binding study of $C_{12}Py^+$, however, it was 0.10 g/dm^3 for the binding study of $C_{14}Py^+$ and $C_{16}Py^+$. Because of the limitation of the electrode response to the strong binding experiment of $C_{16}Py^+$ to AHA and $C_{14}Py^+$ and $C_{16}Py^+$ to AFA, the concentration of AHA and AFA was to be reduced. The highest concentration of C_nPy^+ studied was far below the corresponding cmc of these surfactants.

2.2.3. Determination of Proton-Binding Equilibria of HS by Potentiometric Titration

In order to determine the carboxyl contents of AFA, potentiometric titrations 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).

500 mg dm^{-3} of AFA solution was prepared directly in the titration cell by dissolving 0.0050 g of AFA in 10 cm^3 of NaCl solution with the ionic strength of 0.03M. The solutions were allowed to equilibrate under N_2 flowing for 30 min, and were then titrated with diluted carbonate-free NaOH solution. The ionic strength of the titrant was

also kept at 0.03M using NaCl solution. Blank-titrations (calibration) using standard HCl solution as an analyte were also performed just before and after each measurement of sample solutions to determine the standard potential of the electrochemical cell and to obtain the accurate concentration of NaOH solution. The titrations were made duplicate or triplicate. On the other hand, HAs are less soluble than FAs and so back titration method was used in the case of AHA. But, it was difficult to determine the precise and accurate carboxyl contents of AHA because of some hysteresis phenomena.

2.3. Results and Discussion

2.3.1. Binding Isotherms of $CnPy^+$ to AFA and AHA

Figure 1 shows the representative potentiogram of $C_{12}Py^+$ binding to AFA and AHA at 25 °C. Other surfactants, $C_{14}Py^+$ and $C_{16}Py^+$ exhibit the same behavior in the binding with the same samples. The respective surfactant selective electrode shows Nernstian response below cmc in the absence of AFA or AHA. In the presence of AFA or AHA, emf values deviate far from the Nernstian response at a defined surfactant concentration. Here, the different deviation manner is observed: deviation increases with surfactant concentration in $C_{12}Py^+$-AFA system as shown in Fig.1a, however deviation starts at very low surfactant concentration levels and it becomes smaller at higher C_t in $C_{12}Py^+$-AHA system as shown in Fig. 1b. This deviation allows us to calculate the free surfactant concentration, C_f and the degree of binding, n, by using the following equations,

$$C_f = 10^{E-Eo/S} \quad (1)$$

$$n = (C_t - C_f) / C_{HS} \quad (2)$$

where S is the slope of the Nernstian response, E_o is the asymmetric potential of the electrochemical cell, and C_{HS} is the concentration of humic substances expressed in g/dm^3. By utilization of these data, the binding isotherms can be constructed. Representative binding isotherms, the plots of n vs. C_f, are shown in Fig. 2 for $C_{12}Py^+$ binding to AFA and AHA. Also, the binding isotherms are re-plotted as Scatchard plots

[32] to see the binding mode through the all-binding degree (Fig. 3). Some part of the binding isotherm are constructed over the lower limit concentration of the Nernstian response i.e., round about 0.01mM, because the surfactant-ion-selective membrane tends to reasonably respond to the concentration down to one decade below this lowest limit of calibration in the presence of HS. The extension of the calibration may affect the accuracy of the binding isotherm at the lower $C_{f,}$ however, the good agreement of the experimental and calculated curves support the validity of these isotherms.

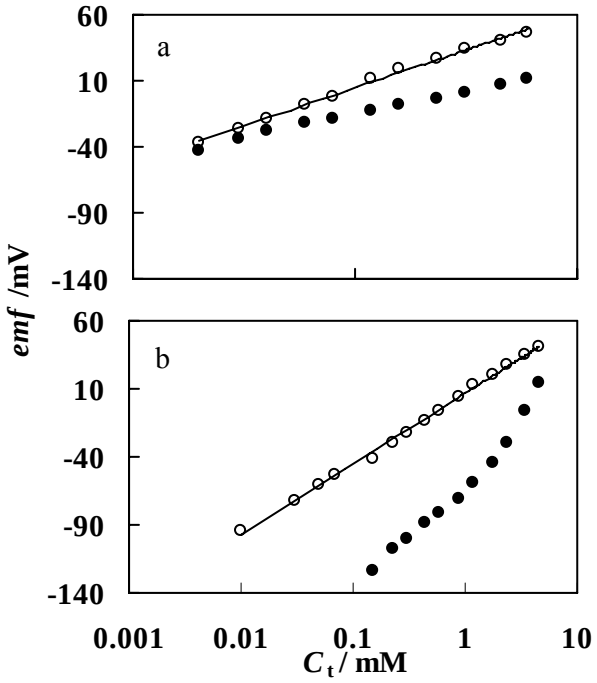

Figure 1. Potentiograms of (a) $C_{12}Py^+$-AFA system and (b) $C_{12}Py^+$-AHA system.

(o) without FA or HA; (•) with FA or HA; pH = 9.18, I = 0.03, T = 25°C.

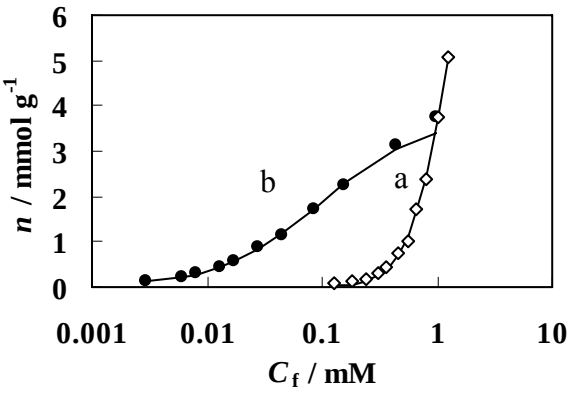

Figure 2. Binding isotherms of (a) $C_{12}Py^+$-AFA and (b) $C_{12}Py^+$-AHA at 25°C. pH = 9.18, I = 0.03. Solid lines refer to the curves reproduced by using equation 3 and 5 respectively.

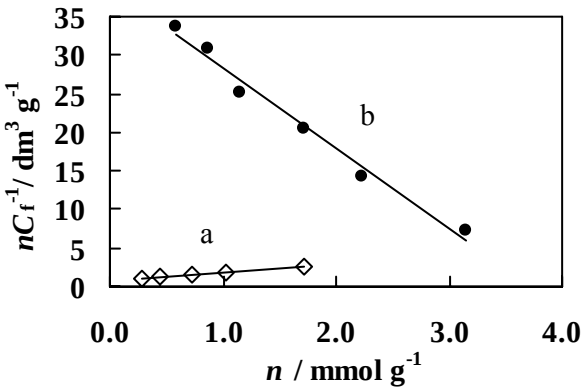

Figure 3. Scatchard plots for (a) $C_{12}Py^+$-AFA and (b) $C_{12}Py^+$ -AHA systems.

2.3.2. Analysis of $C_{12}Py^+$ Binding Equilibria to AFA and AHA

As shown in Fig. 2a, $C_{12}Py^+$-AFA system exhibits a steep rise in the binding within a small change in equilibrium surfactant concentration, which is characteristic for cooperative binding. The positive slope of Scatchard plot (Fig. 3a) also suggests the cooperative binding behavior. Such a cooperative nature was frequently observed in the interaction between surfactants and polyelectolytes [12, 23–26]. In this concern, the binding isotherm can be empirically analyzed by Hill's equation [33]:

$$\log \frac{n}{n^* - n} = l\log \frac{\theta}{(1-\theta)} = h \log C_f + \log K_h \quad (3)$$

where n^* is the total number of binding sites expressed in meq/g AFA sample, 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 independent sites binding and $h > 1$ for cooperative binding [34]. The value of n^* in AFA is 9.56 meq/g, which is determined from the proton binding equilibria of AFA by potentiometric titration method at the ionic strength of 0.03M. To determine the value of h and K_h, (θ / (1-θ)) is plotted in Fig. 4a against with C_f. Then, the binding constant of a surfactant with an individual binding site, K can be calculated by using the equation:

$$K = (K_h)^{1/h} \quad (4)$$

The calculated h and K values for $CnPy^+$ binding to AFA are summarized in Table 1. The solid lines in Figs. 2a and 4a indicate the isotherms reproduced from the calculated values listed in Table 1. The good agreement of the experimental results with the calculated curve supports the cooperative binding model for the AFA system.

On the other hand, no steep rise in the binding is observed in $C_{12}Py^+$-AHA system (Fig. 2b). The binding isotherm of $C_{12}Py^+$-AHA system shows a gentle sigmoidal curve and the Scatchard plots give the straight lines with a negative slope (Fig. 3b), which suggests that the binding can be treated as independent sites binding [34]. In this case, the binding can be analyzed by using the equation:

$$n / C_f = n^* K - K n \quad (5)$$

$$\theta = n / n^* \quad (6)$$

The value of n^* and K are determined from the plot of n / C_f vs. n. The calculated n^* and K values for $C_{12}Py^+$ binding to AHA are described in Table 1 together with the values calculated for $C_{14}Py^+$ and $C_{16}Py^+$ bindings. For the sake of evidence, the binding isotherm is also expressed by Hill's equation (Fig. 4b).

Here, we use the n^* value determined from the equation 5 because it is difficult get the precise and accurate n^* value from the proton binding titration. The slope of the Hill's plot is almost unity, confirming the independent sites binding of C_nPy^+- AHA system. Since the ionic strength of the solutions are kept constant by using pH standard buffer, the negative- or anti-cooperative C_nPy^+ binding due to the change in charge

density of AHA is not observed. *K* of equation 5 may be expected to be independent of the binding degree. The observed isotherms in Figs. 2b and 4b reproduced from the calculated values are in very reasonable agreement with the experimental results.

Table 1. The total number of binding sites (*n**), cooperative parameter (*h*), binding constant (*K*), for C_nPy^+-AFA system and number of binding sites (*n**) and binding constant (*K*) for C_nPy^+-AHA system.

CnPyBr	AFA system			AHA system	
	*n**	*h*	*K* (mM^{-1})	*n**	*K* (mM^{-1})
C_{12}PyBr		2.69	0.8	3.7	10.4
C_{14}PyBr	9.65	2.42	8.6	3.8	80.3
C_{16}PyBr		2.71	130.9	4.8	496.8

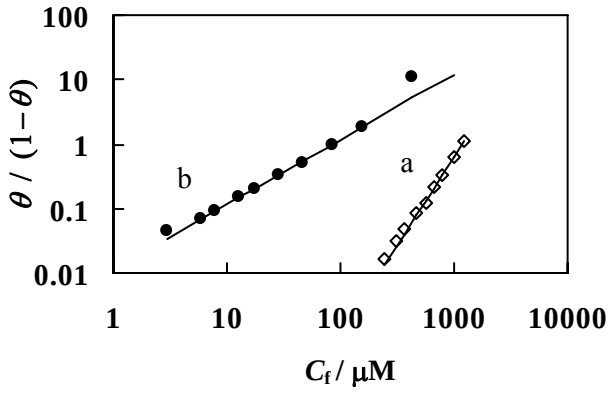

Figure 4. Hill plots for (a) $C_{12}Py^+$-AFA, and (b) $C_{12}Py^+$ -AHA systems. Solid lines refer to the curves reproduced by the Hill's equation.

2.3.3. Evaluation of Amphiphilic Properties of Humic Substances from the Surfactant Binding Behavior

The binding behavior of C_nPy^+ to AFA is clearly different from that to AHA as described just above. Cationic surfactants, C_nPy^+, bind cooperatively with AFA, however the cooperativity is not observed with AHA system. These differences in the binding behaviors reflect the differences in functionality and hydrophobicity-hydrophilicity balance between AFA and AHA molecules.

The presence and absence of cooperative nature in C_nPy^+-AFA and C_nPy^+-AHA system respectively, strongly suggests the different distribution modes of the ionic binding site in AFA and AHA. It has been well-recognized that ionic surfactants are cooperatively bound to polyelectrolytes with opposite charges. Shirahama et al. have well established the highly cooperative characteristic of surfactant–polyion interactions [12–14]. They have revealed that the cooperative nature in the surfactant binding to oppositely charged polyelectrolyte is caused by the hydrophobic interactions between bound surfactants themselves. Moreover, as indicated in Table 1, the number of binding sites, n^*, of AFA is greater than that of AHA, even FAs appear to be smaller than HAs from the structural point of view [2]. In these regards, we can deduce that the ionic sites in AFA are probably located close enough to each other to allow the hydrophobic interaction between bound surfactants. The binding sites in AHA seem to be far apart compared with AFA, resulting in the lower density of ionic sites and preventing the cooperative binding.

It is apparent as well that the binding of $C_{12}Py^+$ to AHA is much stronger than that of AFA based on the K value and binding isotherms (Fig. 2 and Table 1). It gives the straightforward information on the difference in the hydrophobicity of these molecules. Although the electrostatic interaction between cationic head group of surfactant and anionic sites of AFA or AHA molecules is one of the main driving forces in the binding of C_nPy^+, the intrinsic strength of this electrostatic interaction may be of the same order in both systems because AFA and AHA have similar types of functional groups, i.e., carboxylate groups [1, 2]. If so, why C_nPy^+ interacts stronger with AHA than with AFA? There may be additional hydrophobic interaction between hydrocarbon tail of surfactant and the hydrophobic backbone of AHA molecule. Although, both FA and

HA are the fractions of HS, the structure of HA is somewhat more aromatic and less aliphatic than FA; and HA molecules are poorer in carboxylic acid and phenolic groups compared with FA molecules. As a result, HA molecules are less soluble and more hydrophobic than FA molecules [1, 8], and the hydrocarbon tail of the surfactant molecules may interact with the backbone of HA molecules through the hydrophobic interaction. On the contrary, the backbone of FA molecules is rather hydrophilic and there is no effective hydrophobic interaction with surfactant's tail.

Otto et al. [8] studied NMR diffusion analysis of surfactant-humic substance interactions, and it was found that cetyltrimethylammonium bromide interacts more strongly with HA than with FA. They mentioned the importance of hydrophobic effect in humic acid-surfactant interactions, but did not clarify the specific type of hydrophobic interaction. As we have discussed above, two different hydrophobic interactions are considered to be involved in surfactant-humic substances systems: one is hydrophobic interaction between the hydrocarbon tail of surfactant and the backbone of humic substances and another is the hydrophobic interaction among the bound surfactants themselves. The former contributes to the greater binding strength of AHA than AFA and the latter causes the cooperative binding in AFA system. The model used to explain these hydrophobic interactions is shown in Fig. 5.

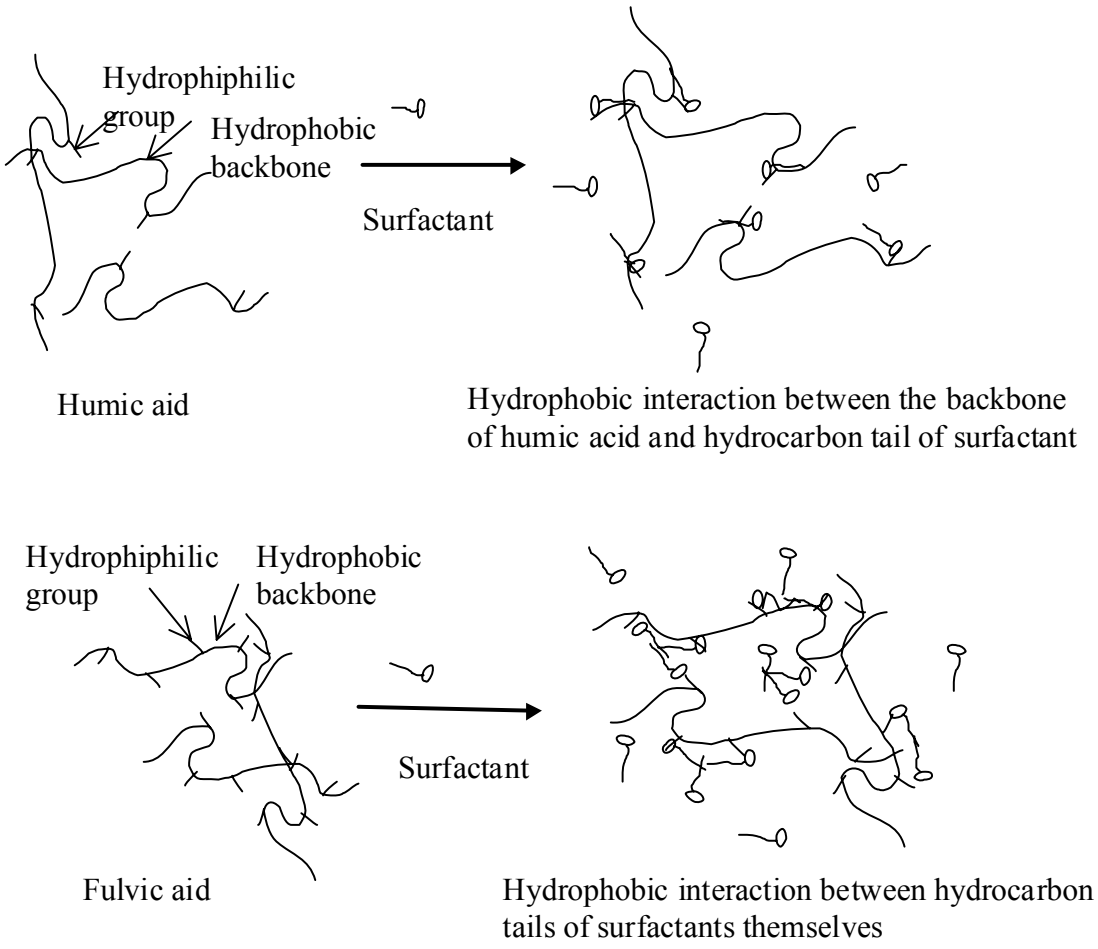

Figure 5. Schematic representation of the hydrophobic interactions involved in surfactant-humic substances systems.

2.3.4. Evaluation of the Hydrophobicity in Humic Substance-Surfactant Aggregate from the Surfactant Chain Length Dependence of C_nPy^+ Binding To AFA and AHA

The binding isotherms corresponding to respective surfactant systems are represented in Figs. 6 and 7 in order to see the effect of surfactant chain length on the binding. In both AFA and AHA systems, the binding shifts to lower equilibrium concentration with increasing carbon number of surfactant. The *h* and *K* values calculated for C_nPy^+ (n = 12, 14, 16) binding to AFA using equations 3 and 4 and *n** and *K* value for AHA system calculated by using equation 5 are summarized in Table 1. The solid lines in the figures indicate the isotherms reproduced from the calculated values listed in Table 1. The good agreement of the experimental results with the calculated curves ensures the cooperative binding in AFA system and independent sites binding in AHA system.

The binding increases with increasing carbon number of surfactant in both AFA and AHA system (Figs. 6, 7 and Table 1). Regarding the hydrocarbon chain length dependence, we can evaluate the hydrophobicity in the system. In order to examine the extent of the increase in binding constants with increasing surfactant chain length, *RT* ln *K* is plotted against surfactant chain length in Fig. 8, where *R* is the gas constant, and *T* is the absolute temperature, i.e., 298K for the present case. The negative value of the slope of these plots gives the free energy of transfer per surfactant CH_2 group from water phase to humic substance-surfactant aggregate. Both plots show a linear relationship with the slope of 3.1 kJ/mol in C_nPy^+-AFA system and 2.3 kJ/mol in C_nPy^+-AHA system.

The increase in *RT* ln *K* for each CH_2 group in C_nPy^+-AFA system, 3.1 kJ/mol, is comparable to the chain length dependence of *RT* ln *uK* of 3.2 kJ/mol per CH_2 group found for *N*-alkylpyridinium binding to dextran sulfate and 3.0 kJ/mol per CH_2 group for alkyltrimethylammonium binding to DNA [23,24]. Where, *u* is the cooperative parameter characterized by Satake and Yang equation [21]. This indicates that the hydrophobic moiety of C_nPy^+-AFA aggregate resembles as those of surfactant-polyion aggregates.

In order to compare the hydrophobicity in C_nPy^+-AFA aggregate with that in surfactant micelle itself, log *K* value is plotted in Fig. 9a against the logarithmic values

of cmc. The slope of this plot, -1.7, is greater than unity, suggesting that the hydrophobicity of surfactants in C_nPy^+-AFA system is larger than that of miceller system. Actually the free energy of transfer of CH_2 group in C_nPy^+-AFA system, 3.1 kJ/mol, is almost the same order with that for the micelle formation of nonionic surfactant, 2.9 kJ/mol [35]. This may reflect the difference behavior between the binding of surfactant aggregate on AFA and the micelle formation of surfactants itself. Each surfactant in aggregate binds to the ionic site of AFA, thus it may behave like a nonionic surfactant.

In the binding of C_nPy^+ to AHA, the free energy change for a mole of methylene group to be transferred from water phase to polyelectrolyte phase is calculated to be –2.3 kJ/mol. This binding can be treated as the transfer of surfactant from water phase to AHA phase, and the obtained value can be compared with the free energy change for a methylene group to be transferred from water phase to a pure hydrocarbon phase e.g, dodecane, which is about –3.5 kJ/mol. The ratio of these values is 0.66, suggesting that the hydrocarbon tail of bound surfactant on AHA may not be perfectly surrounded by the hydrophobic moiety of AHA molecules because of the stiffness of the backbone. By comparing with the micelle formation (Fig. 9b), it can be deduced that the hydrophobicity of AHA-surfactant aggregate is slightly larger than that of surfactant micelle.

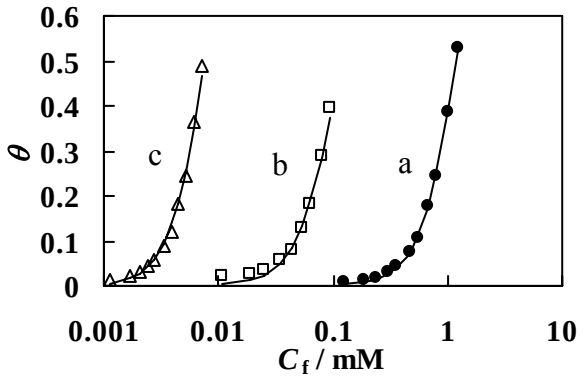

Figure 6. Binding isotherms for AFA system. (a) $C_{12}Py^+$, (b) $C_{14}Py^+$, (c) $C_{16}Py^+$. Solid lines refer to the curves reproduced by the Hill's equation.

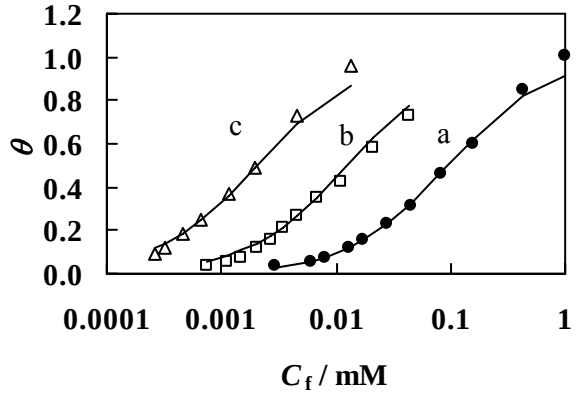

Figure 7. Binding isotherms for AHA system. (a) $C_{12}Py^+$, (b) $C_{14}Py^+$, (c) $C_{16}Py^+$. Solid lines refer to the curves reproduced by equations 5 and 6.

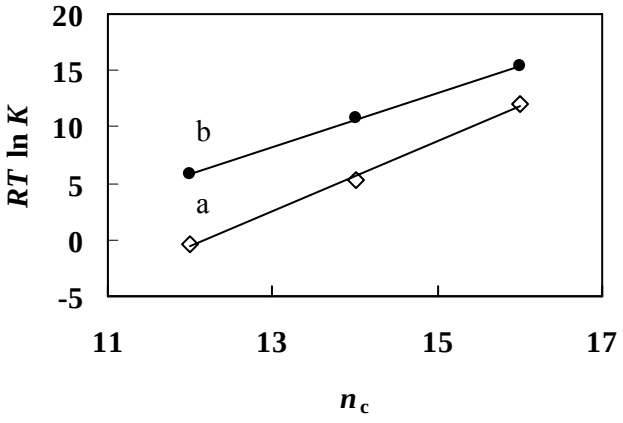

Figure 8. Free energy change as a function of surfactant chain length. (a) C_nPy^+-AFA system; (b) C_nPy^+-AHA system.

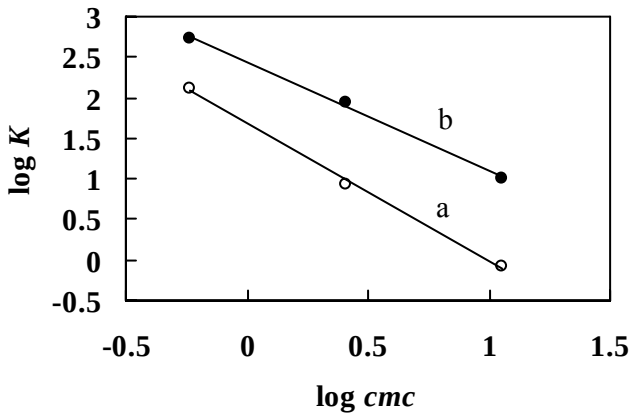

Figure 9. Binding constants *K* as a function of log *cmc*. (a) C_nPy^+-AFA system; (b) C_nPy^+-AHA system.

2.4. Conclusion

The amphiphilic properties of humic substances, i.e., fulvic acid and humic acid, can be evaluated through the binding study with cationic surfactant by using surfactant-ion-selective membrane electrode. In addition to the electrostatic interaction, two different hydrophobic interactions are involved in surfactant-humic substance interactions: one is hydrophobic interaction between the hydrocarbon tail of surfactant and the backbone of humic substances (C_nPy^+-AHA system) and another is the hydrophobic interaction among surfactants themselves (C_nPy^+-AFA system). The different binding behavior of C_nPy^+ to AFA and AHA is observed due to the differences in the number of binding sites and the hydrophobicity between humic substances. The more hydrophobic the humic substance, the greater the binding of C_nPy^+ to humic substances will be, through the hydrophobic interaction between C_nPy^+ and the backbone of humic substances. The hydrophobicity of humic substances-surfactant aggregates can be evaluated through the surfactant chain length dependence. Investigating specifically the binding behavior of C_nPy^+ to humic substances has resulted in noteworthy observations.

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Thermodynamic Studies of Dodecylpyridinium Ion Binding with Fulvic Acid

The binding of dodecylpyridinium ions ($C_{12}Py^+$) with Aso fulvic acid (AFA) has been studied from the thermodynamic point of view by using potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18 and at the ionic strength of 0.03 mol dm^{-3}. The cooperative binding of $C_{12}Py^+$ with AFA is the endothermic process driven by the positive entropy resulting possibly from the dehydration of hydrophobically hydrated water molecules around the hydrocarbon chains of the bound $C_{12}Py^+$ ions. The binding is obviously pH dependent and is most pronounced at pH 9.18. Different binding modes are observed at two pH regions, i.e., cooperative binding at pH>7 and non cooperative binding at pH<7. The effect of ionic strength is also important in binding phenomenon. As the ionic strength decreases, the $C_{12}Py^+$ binding with AFA is enhanced, probably due to the lowering of screening effect of counterions. The sensitivity of binding to ionic strength is larger at high pH than that at low pH in AFA system.

3.1. Introduction

Humic substances (HSs) are inherently composite materials in both chemical and structural points of view [1,2]. They are ubiquitous in the environment, occurring in any soils, waters, and sediments of the ecosphere [3]. HSs have a substantial capacity to interact with inorganic constituents, organic compounds, and amphiphilic compounds [4–6]. The versatile properties of humic substances such as high cation exchange capacity, the ability to chelate metal ions, the ability to adsorb organic substances, high water holding capacity, and an ease of combustion due to its organic nature, are very useful for agricultural and environmental purposes [7]. The investigation on HSs properties and their complexation behavior is therefore of considerable interest. Along with the recent rapid developments in the study of interaction between humic substances and inorganic compounds as well as clay minerals [8–10], the needs is increasing for the deeper understanding of the mechanism. Because of widespread and persistence use, surfactants can be introduced into the environment through waste water or direct contamination and can interact with natural amphiphilic compounds such as humic substances. Only a few physicochemical studies have been reported on the interaction between one of the naturally occurring polyelectrolytes, i.e., HSs with surfactants [11–13].

Recently, we reported the surfactant binding study with AFA and AHA, where the different binding behavior was observed between AFA and AHA system due to the differences in hydrophobicity-hydrophilicity balance of these HSs [14]. In accordance with our experiences and previous polyelectrolytes-surfactant studies, it has been observed that the more detailed the thermodynamic information about the polyelectrolytes-surfactant interaction, the better should become our understanding in mechanism [15–18]. For example, Wang and Tam [19] recently depicted the new binding model of dodecyltrimethylammonium bromide to neutralized poly (acrylic acid) and methylacrylic acid /ethyl acrylate from the thermodynamic parameters obtained by using isothermal titration calorimetry.

It is also well known that the solution conditions such as pH of the system and ionic strength of the medium control the size, shape, molecular weight characteristics, and

various functions of HSs and have a profound effect on their interactions with other components [20–22]. Recently, Adou et al. reported that PPL (polypropylene) removal efficiency of HSs is strongly pH dependent and higher removal can be achieved at pH greater than 7 [24]. Liu et al. studied the surface features of humic acid (HA) by using AFM and concluded that the ionic strength and pH of a system greatly affect the behavior of HA through modifying the molecular conformation of HA [24]. Avena et al. expressed that HA and FA molecules behave as flexible entities that can swell or shrink in response to changes in pH and ionic strength [25]. Balnois et al. however, supported that HSs generally exist as small, semirigid spherocolloids at environmentally relevant pH and ionic strength [26].

In the present paper, we have examined the thermodynamic parameters of dodecylpyridinium ($C_{12}Py^{+}$) binding to fulvic acid (FA) by potentiometric titration method based on surfactant-ion-selective membrane electrode. The effect of pH, ionic strength, and the concentration of FA on the binding are also investigated to reveal their profound effect on the binding.

3.2. Experimental Section

3.2.1. Materials

Aso fulvic acid (AFA) was collected from the Aso area of Kyushu Island of Japan and extracted by an international standard method, recommended by IHSS [27]. 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 $C_{12}PyBr$ obtained is 12.0 mmol dm^{-3} in aqueous solution. For thermodynamic studies all experimental solutions were kept at pH 9.18 and ionic strength of 0.03 mol dm^{-3} by using tetraborate pH standard buffer solution. During the potentiometric measurements, the temperature was controlled at the desired value (293, 298, 303, and 308K) by circulating thermostated water through the jacketed glass cell.

3.2.2. Ionic strength and pH condition

To study the effect of pH and ionic strength, sample solutions were prepared in the pH range of 4-10 and ionic strength of 0.03-0.10 mol dm^{-3}. NaBr was used as the supporting electrolyte for experiments performed at pH 3.97 (I = 0.03 and 0.10 mol dm^{-3}) and the pH was adjusted by analytical grade hydrochloric acid. Various pH standard buffer solutions (Wako Pure Chemical Industries, Ltd.,): phosphate pH standard solution (0.00866 mol KH_2PO_4 + 0.03031 mol Na_2HPO_4 per kilogram H_2O), tetraborate pH standard solution (0.01mol $Na_2B_4O_7$ per kilogram H_2O), and carbonate pH standard solution (0.025 mol $HNaCO_3$ + 0.025 mol Na_2CO_3 per kilogram H_2O) were used in order to keep the sample solutions at pH 7.41, 9.18, and 10.01 respectively. The ionic strength of the buffer solutions was computed using the following general equation on the assumption of completely dissociation of each electrolyte:

$$I = \frac{1}{2}\sum_{i=1}^{n} z_i^2 C_i \qquad (1)$$

where, I is the ionic strength, C_i is the concentration of the ion i, z_i is charge of the ion i, and n is the number of ions in buffer solutions. The ionic strength of these buffer solutions was then controlled at desired values by adding of corresponding quantity of NaBr and deionized water (Millipore Milli-Q system). The pH values of the final solutions were verified by a digital pH meter (ORION, model 91-01). From experiment to experiment, only one parameter was changed at a time; the others remained unchanged.

3.2.3. Potentiometry for Surfactant Binding Study

The binding isotherms of $C_{12}Py^+$ to AFA were obtained by the potentiometric titration method using the surfactant-ion-selective membrane electrode operated at desired temperature. The surfactant-ion-selective membranes were composed of poly (vinyl chloride) (PVC) and polymeric plasticizer (Elvaroy 742, Du Pont). The potentiometric measurements were made by using a digital multimeter (Advantest TR6845) connected with the electrochemical cell: Ag/AgCl, KCl || reference solution| PVC membrane | sample solution || KCl, AgCl/Ag. The linear plots of the electromotive force (emf) vs. the logarithms of surfactant concentration (C_t) below the critical micelle concentration

(cmc) gave Nernstian slope, i.e., 57.0–59.2 mV/decade. The calibrations has been carried out just before and after each binding measurement to assure an asymmetrical potential of the electrochemical cell. In the presence of AFA, emf values deviated far from the Nernstian response at a defined surfactant concentration. From this deviation, the free surfactant concentration, C_f and the degree of binding, $n = (C_t\text{-}C_f)/C_{HS}$ was calculated. Where C_{HS} is the concentration of AFA and was kept constant at 1.00 g dm^{-3} in all binding measurements. The added concentration of $C_{12}Py^+$ was far below the corresponding cmc of these surfactants.

3.2.4. Determination of Total Number of Binding Sites of AFA by Potentiometric Titration

In order to determine the total number of binding sites and the degree of ionization of AFA, potentiometric titrations were 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 AFA solution was prepared directly in the titration cell by dissolving 0.0050 g of AFA in 10 cm^3 of NaCl solution with the required ionic strength (0.03, 0.05 or 0.10 mol dm^{-3}). The solutions were allowed to equilibrate under N_2 flowing for 30 min, and were then titrated with a diluted carbonate-free NaOH solution. The ionic strength of the titrant was also kept the same as analyte (0.03, 0.05 or 0.10 mol dm^{-3}) using a NaCl solution. Blank-titrations (calibration) using standard HCl solution as an analyte were also performed just before and after the measurement of sample solutions to determine the standard potential of the electrochemical cell and to obtain the accurate concentration of NaOH solution. The titrations were made twice or thrice to check the reproducibility.

Figure 1 shows the representative pH titration curve of AFA at ionic strength of 0.03 mol dm^{-3}. The derivative of the titration curve is estimated using a simple differential equation:

$$\frac{\mathrm{dpH}}{\mathrm{d}V} \cong \frac{\mathrm{pH_{i+1}} - \mathrm{pH_i}}{V_{i+1} - V_i} \tag{2}$$

where V is the volume of NaOH added and the maximum value of the derivative curve is taken as the end-point for the titration of carboxyl groups. Then, the carboxyl content of AFA can be calculated by using equation:

$$[\mathrm{COOH}] = \frac{C_b * V_{b.eq}}{m} \tag{3}$$

where, C_b and $V_{b.eq}$ are the concentration and end point volume of NaOH and m is the weight of AFA used in the titration. Also, the degree of dissociation (α) is defined as:

$$\alpha = \{C_b V_b + [\mathrm{H^+}]\ (V_o + V_b)\} / C_b\ V_{b,eq} \tag{4}$$

where, V_b is the volume of NaOH added and V_b is the initial volume of AFA solution.

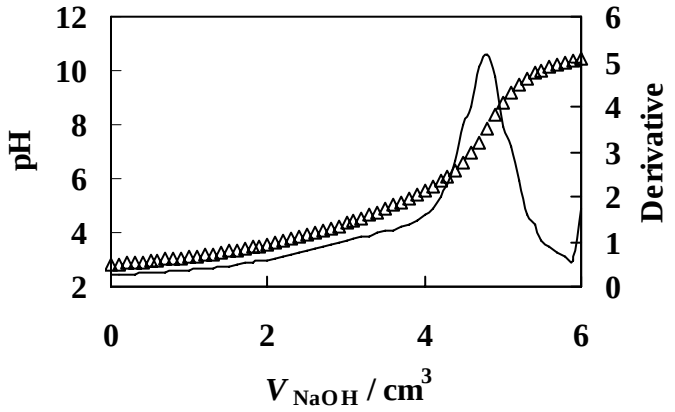

Figure 1. Titration curve of AFA at 25°C and I = 0.03 mol dm^{-3}. (Δ) pH, (—) derivative.

3.3. Results and Discussion

3.3.1. Effect of Temperature on $C_{12}Py^+$-AFA System

Figure 2 shows the binding isotherms of $C_{12}Py^+$-AFA system at various temperatures. $C_{12}Py^+$ ions bind to AFA at very low equilibrium concentration, far below the cmc even in the presence of excess salt. All the binding isotherms exhibit a steep rise in binding within a small change in the equilibrium surfactant concentration, which is characteristic for cooperative binding [14,28-31]. In this context, the binding isotherm can be empirically analyzed by Hill's equation [32]:

$$\log \frac{n}{n^* - n} = \log \frac{\theta}{(1-\theta)} = h \log C_f + \log K_h \qquad (5)$$

where n^* is the total number of binding sites expressed in meq 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 cooperativity can be estimated from the value of h: $h = 1$ for noncooperative binding and $h > 1$ for cooperative binding [31]. The values of n^* for AFA are given in Table 1, which were determined from the proton binding equilibria of AFA by using potentiometric titration method at the ionic strength of 0.03 mol dm^{-3}. To determine the value of h and K_h, (θ / (1-θ)) is plotted against C_f, both in logarithm scale in Fig. 3. Then, the binding constant of a surfactant with an individual binding site, K can be calculated by using the following equation:

$$K = (K_h)^{1/h} \qquad (6)$$

The calculated h and K values for $C_{12}Py^+$ binding with AFA at various temperatures are summarized in Table 1. The solid lines in Fig. 2 indicate the isotherms reproduced from the calculated values, which are in good agreement with the experimental results.

As shown in Fig. 2 the binding isotherms shift to lower equilibrium concentration with increasing temperature i.e. the binding strength increases with increasing temperature indicating an endothermic binding process. The value of K at a certain temperature is used for the calculation of Gibbs free energy change ($\Delta G°$):

$$\Delta G° = - RT \ln K \qquad (7)$$

where R is the gas constant, and T is the absolute temperature expressed in Kelvin. The value of $\Delta G°$ becomes more negative with increasing the temperature. The enthalpy ($\Delta H°$) of $C_{12}Py^+$ binding with AFA can be obtained from the temperature dependence of the binding constants (K) (Fig. 4) using the Van't Hoff relation:

$$\Delta H° = -R \frac{d \ln K}{d(1/T)} \quad (8)$$

As shown in Fig. 4, a good linearity in ln K vs. $1/T$ is observed between 293 and 303 K, but a less difference in ln K between 303 and 308 K. This less difference may be possibly due to the small systematic error within the experiment and the value of $\Delta H°$ has been calculated from the slope of the straight line. However, in surfactant-polyelectrolyte systems, the reversal of slope around 310K is often occurred due to hydrophobic interaction [33]. Once the Gibbs free energy and enthalpy of binding are obtained, the entropy of interaction ($\Delta S°$) can be determined by using the following equation.

$$\Delta S° = 1/T\,(\Delta H° - \Delta G°) \quad (9)$$

The thermodynamic parameters thus obtained for $C_{12}Py^+$-AFA system are summarized in Table 1.

On the basis of the observed thermodynamic parameters, it is observed that the binding of $C_{12}Py^+$ ions to AFA molecules is an endothermic process driven by positive entropy. Such an entropy driven binding was frequently observed in the case of the surfactant-ionic polymer interactions. For examples, Alizadeh [15] et al. reported that the enthalpy of binding between sodium dodecyl sulfate (DS^-) ion with lysozyme showed an endothermic process occurred at without and low ethanol concentration and the binding was entropy controlled. Bai et al. [16] also observed that the enthalpy of interaction of hydrophobically modified polyacrylamide (HMPAM) and poly (acrylamide)-co-(acrylic acid) (HMPAM-AA) with cationic gemini surfactant is an endothermic process. Such entropy driven binding suggests the importance of the hydrophobic interaction in binding process. The positive entropy change in AFA-$C_{12}Py^+$ ions interaction is possibly attributed to the dehydration of the hydrophobically hydrated water molecules around the hydrocarbon chain of the bound $C_{12}Py^+$ ions. The positive enthalpy change with binding, $\Delta H°$ = 12 kJ/mol, suggests the binding of surfactant ion to AFA is not the simple binding but partly the ion exchange reaction

between $C_{12}Py^+$ ion and the small counterion. In such an ion exchange reaction, the enthalpy gain by the electrostatic interaction may be cancelled out and the hydrophobic interaction may play a major role in the thermodynamics of binding.

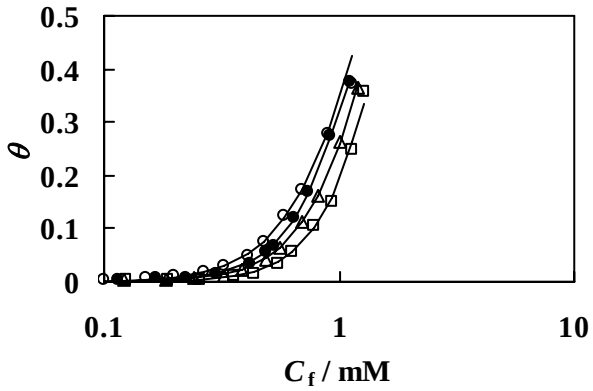

Figure 2. Binding isotherms of $C_{12}Py^+$-AFA system at pH = 9.18, I = 0.03 mol dm^{-3}. (□) 293K, (Δ) 298K, (•) 303K, (o) 308K. Solid lines refer to the curves reproduced by using equation 5.

Table 1. The carboxyl content of AFA (n*), cooperative parameter (h), binding constant (K), and the thermodynamics parameters for $C_{12}Py^+$-AFA system at pH 9.18 and ionic strength 0.03 mol dm^{-3}.

T (K)	n* (meq g^{-1})	h	K (M^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta S°$(J mol^{-1} K^{-1})	$\Delta H°$ (kJ mol^{-1})
293		3.0	619	-15.7		
298	9.65	2.8	684	-16.2	95	27
303		2.8	763	-16.7		
308		2.5	778	-17.1		

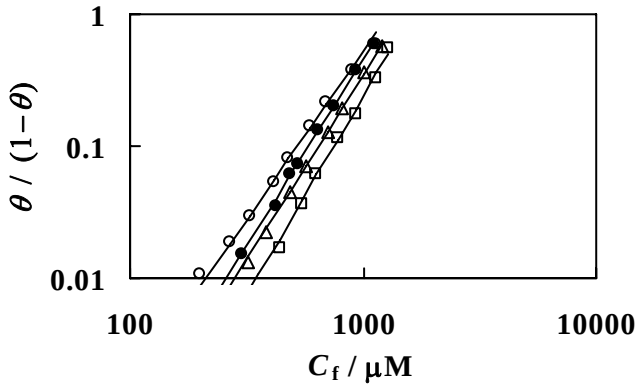

Figure 3. Hill plots for $C_{12}Py^+$-AFA system (□) 293K, (Δ) 298K, (•) 303K, (o) 308K. Solid lines refer to the curves reproduced by Hill's equation.

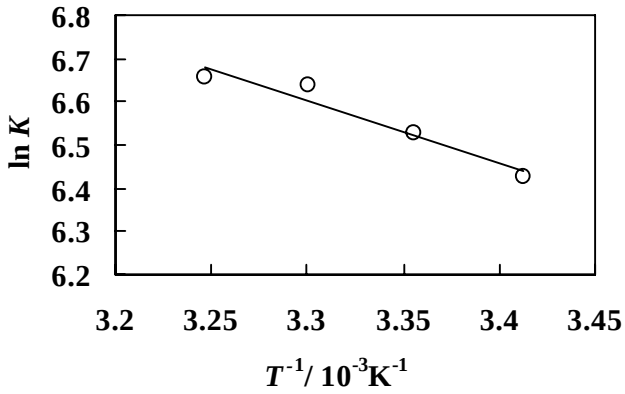

Figure 4. Temperature dependence of binding constant (K) for $C_{12}Py^+$-AFA at pH = 9.18, I = 0.03 mol dm^{-3}.

3.3.2. Effect of pH on $C_{12}Py^+$- AFA System

The effect of pH on the binding is investigated at two pH regions, i.e., at pH >7 and pH < 7. Figures 5a and 5b show the binding isotherms of $C_{12}Py^+$-AFA system expressed as the function of pH (3.97, 7.41, 9.18,and 10.01) at ionic strength of 0.03 mol dm^{-3} and 0.10 mol dm^{-3} respectively. The binding isotherms are analyzed by Hill's equation as well. The calculated *h* and *K* values are summarized in Table 2.

At high pH (pH >7) and at ionic strength of 0.03 mol dm^{-3}, the binding isotherm shifts to lower equilibrium concentration with increasing pH from 7.41 to 9.18, suggesting that the binding is enhanced due to the ionization of AFA functional groups with increasing pH. The development of negative charges at the surface of AFA molecules with increasing pH causes the stronger binding of cationic $C_{12}Py^+$ ions. However, no difference in the binding is observed between pH 9.18 and pH 10.01. At about pH 9, the carboxylate functional groups of AFA are fully ionized and thus no significant change in binding occurs with increasing pH. It has been verified by the measurement of the degree of dissociation (α) of AFA as a function of pH by potentiometric titration. As shown in Fig. 6, α increases with increasing pH and reaches to unity when pH > 8.

The isotherm for $C_{12}Py^+$- AFA system at low pH region (pH < 7) is different from that at high pH region (pH >7). At pH 3.97 the steep rise in binding isotherm within a small change in the equilibrium surfactant concentration is not observed (Fig. 5). At this pH, the cooperative parameter, *h* is unity (Table 2), i.e., noncooperative binding is occurred. Weconcluded that at low pH, with lower AFA ionization, hydrophilicity is reduced. The low charge density at the surface of AFA molecules prevents the cooperative binding and also causes the weaker binding of $C_{12}Py^+$ ions. This is in agreement with the observation by Lead et al. [22]. These authors studied the diffusion coefficients of HSs by fluorescence correlation spectroscopy and role of solution conditions and mentioned that opposite diffusion phenomenon was observed between pH>7 and pH<7 for Suwannee River Fulvic Acid.

At ionic strength of 0.10 mol dm^{-3} (Fig. 5b), no change in the binding of $C_{12}Py^+$ with AFA is observed with increasing pH from 7.41 to 10.01. This is because the full ionization of AFA molecules occurs at pH 6.5 at ionic strength 0.10 mol dm^{-3} as shown in Fig. 6. On the other hand, the similar behavior is observed as in the case of lower

ionic strength (0.03 mol dm^{-3}) when pH is decreased from 7.41 to 3.97. The magnitude of binding decreases with increasing ionic strength at a certain pH (Table 2) because of the ion-screening effect. We will discuss the ionic strength effect in detail in the following section.

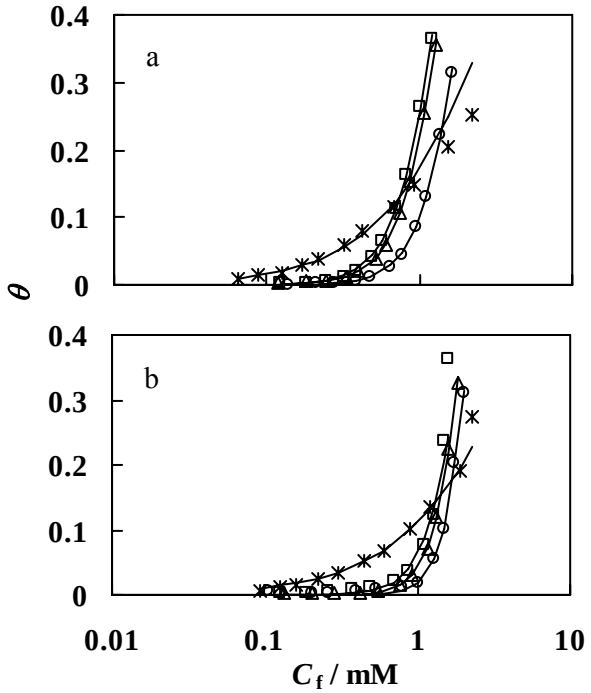

Figure 5. Binding isotherms for $C_{12}Py^+$-AFA system as a function of pH at (a) I = 0.03 mol dm^{-3} and (b) I = 0.10 mol dm^{-3} (∗) pH 3.97, (o) pH 7.41, (□) pH 9.18, (Δ) pH 10.01.

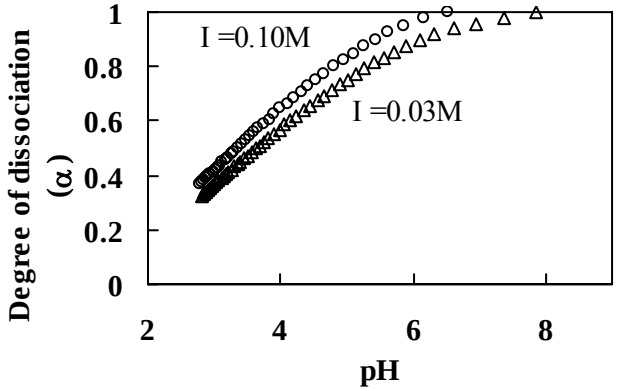

Figure 6. The degree of dissociation of AFA expressed as a function of pH. (o) 0.10 mol dm^{-3} NaCl, (Δ) 0.03 mol dm^{-3} NaCl.

Table 2. The total number of binding sites of AFA (*n**), cooperative parameter (*h*), and binding constant (*K*), for $C_{12}Py^{+}$-AFA system at different pH and at 25°C.

Solution conditions		*n** (meq g^{-1})	*h*	*K*(M^{-1})
I (mol dm^{-3})	pH			
0.03	3.97	9.65	1	313
	7.41		3	469
	9.18		3	684
	10.01		3	627
0.10	3.97	8.61	1	233
	7.41		5	422
	9.18		4	458
	10.01		4	448

3.3.3. Effect of Ionic Strength on $C_{12}Py^+$-AFA System

Figure 7a shows the binding isotherms of $C_{12}Py^+$-AFA system expressed as the function of ionic strength (0.03, 0.05,and 0.10 mol dm^{-3}) at pH 9.18. The *h* and *K* values calculated for $C_{12}Py^+$-AFA system at various ionic strengths are summarized in Table 3. The binding isotherms shift to lower equilibrium concentration with decreasing ionic strength, i.e., the binding strength increases with decreasing ionic strength. As the salt concentration decreases, the concentration of counterions also decreases, which lowers the magnitude of ion-screening effect on the AFA molecules. This increases the binding of $C_{12}Py^+$ ions with AFA.

For a deeper understanding of the concomitance effect of pH and ionic strength, the ionic strength effect has been also examined at low pH (pH 3.97). Similar characteristic is observed (Fig. 7b), i.e., the binding strength decreases with increasing ionic strength. However, all the binding is noncoopreative at this pH. For the comparison of the effect of ionic strength at both pHs, the logarithm of *K* is plotted in Fig.7 against the root of ionic strength. The linearity of the plots suggests the effect of ionic strength followed by the electrostatic interaction. The observed intrinsic binding strength, K_0, is 3.0 at pH 9.18 and 2.6 at pH 3.97. The negative slope of this plot at pH 9.18 (-1.2) is greater than that at pH 3.97 (- 1.0). It suggests that the sensitivity of the binding to ionic strength of the system is greater at higher pH. This observation is in agreement with the expected one from the viewpoint of electrostatic interaction. At higher pH, AFA molecules are more deprotonated and the effect of ionic strength is greater than at lower pH.

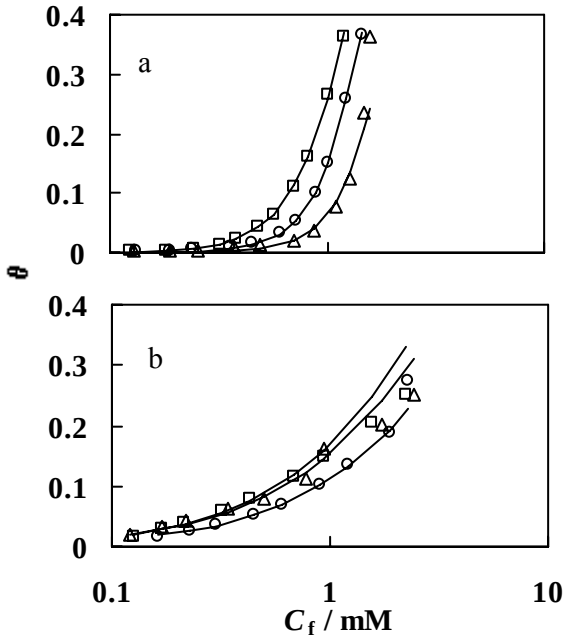

Figure 7. Binding isotherms of $C_{12}Py^+$-AFA system as a function of ionic strength at (a) pH 9.18 and (b) pH 3.97. (□) I = 0.03 mol dm^{-3}, (o) I = 0.05 mol dm^{-3}, (Δ) I = 0.10 mol dm^{-3}.

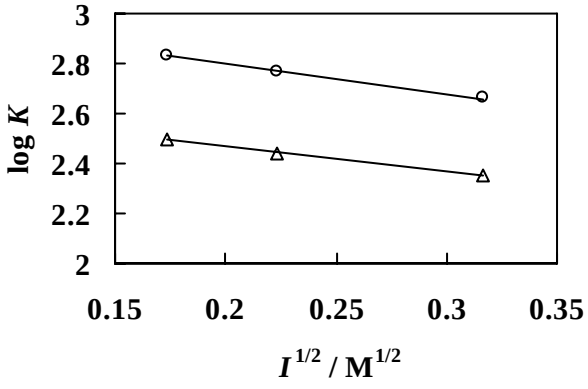

Figure 8. Log K as a function of the root of ionic strength at (o) pH 9.18 and (Δ) pH 3.97.

Table 3. The total number of binding sites of AFA (n*), cooperative parameter (h), and binding constant (K), for $C_{12}Py^+$-AFA system at different ionic strength and at 25°C.

Solution conditions		n*(meq g^{-1})	h	K(M^{-1})
pH	I (mol dm^{-3})			
9.18	0.03	9.65	3	684
	0.05	8.90	3	589
	0.10	8.61	4	458
3.97	0.03	9.65	1	313
	0.05	8.90	1	277
	0.10	8.61	1	233

3.3.4. Effect of FA Concentration on $C_{12}Py^+$-AFA System

The effect of concentration of AFA on $C_{12}Py^+$-AFA system is also examined by potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18, ionic strength of 0.03 and at 25°C. Figure 9 shows the binding isotherms of $C_{12}Py^+$-AFA system at various AFA concentrations. As shown in the figure, no significant change in the binding is observed with changing the AFA concentration in the range of 0.2 - 1.5 g dm^{-3}. Under these experimental conditions, any self-aggregation of AFA molecules does not affect the binding of $C_{12}Py^+$ ions and our results suggest that the hydrophilicity of AFA is strong enough to fully disaggregate in water at low pH.

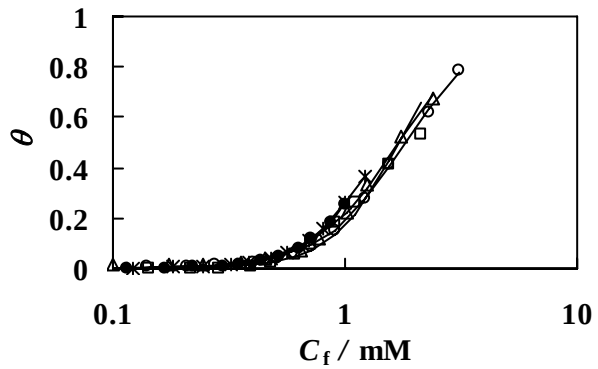

Figure 9. Binding isotherms of $C_{12}Py^+$-AFA system at different AFA concentrations and at pH = 9.18, I = 0.03, T = 25°C. (o) 0.20 g dm^{-3}, (Δ) 0.35 g dm^{-3}, (◊) 0.50 g dm^{-3}, (∗) 1.0 g dm^{-3}, (•) 1.5g dm^{-3}. Solid lines refer to the curves reproduced by using equation 5.

3.4. Conclusion

On the basis of the observed thermodynamics parameters, the binding of $C_{12}Py^+$ ions to AFA molecules is an endothermic process driven by the positive entropy resulting possibly from the disruption of water structure and/or the conformational change in AFA by the bound $C_{12}Py^+$ ions. The pH and ionic strength greatly affect the $C_{12}Py^+$ binding with AFA. An increase in the solution pH from 7.41 to 9.18 leads to the development of negative charges on the AFA molecules and consequently increases the $C_{12}Py^+$ binding. Different binding modes are observed at pH>7 and pH<7: cooperative binding at pH>7 and noncooperative binding at pH<7. An increase in ionic strength results in the increase in ion screening, and which depresses the binding. In the $C_{12}Py^+$-AFA system the sensitivity of binding to electrolyte concentration is larger at high pH than that of low pH. It is realized that the effect of pH and the ionic strength on the binding behavior can only be evaluated when it is interpreted together. In a subsequent paper we will report the thermodynamic parameters for the binding of $C_{12}Py^+$ ions with Aso humic acid (AHA) and the factors influencing this binding.

3.5. References

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(Chapter 4)

Thermodynamic Studies of Dodecylpyridinium Binding to Humic Acid and Effects of Solution Parameters on their Binding

Thermodynamic information of dodecylpyridinium ions ($C_{12}Py^+$) binding to Aso humic acid (AHA) has been investigated by using potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18. No significant change in binding has been observed with changing the temperature in $C_{12}Py^+$- AHA system. The enthalpy of $C_{12}Py^+$ ions binding with AHA is slightly negative. The solution parameters such as pH and ionic strength affect the binding. The binding is most pronounced at pH 9.18 since the functional groups of AHA are fully ionized and the degree of dissociation (α) is unity at around pH 9.18. The binding strength decreases with increasing ionic strength due to the ion-screening effect. The sensitivity of binding to electrolyte concentration is higher in AHA system than that in Aso fulvic acid (AFA) system. The hydrodynamic diameters of $C_{12}Py^+$- AHA and $C_{12}Py^+$- AFA aggregates are measurable as a probe of their molecular interaction by dynamic light scattering (DLS) microscopy. In both AHA and AFA system, the hydrodynamic diameters increase with increasing surfactant concentration. DLS measurements also give the similar results of lower sensitivity of binding strength to electrolyte concentration in AFA system. AHA concentration does not interfer the binding strength within the concentration range of 0.2 - 1.5 g dm^{-3}.

4.1. Introduction

Interaction between ionic surfactants and humic substances (HS) has attracted increased attention because of their roles in academic researches, environmental fields, and so forth [1,2]. For example, dodecyltrimethylammonium bromide has been used in the study of removal efficiency of HS by polypropylene (PPL) [3]. Samuel et. al has studied the association of linear alkylbenzenesulfonates, which are widely exploited in detergent industry, with dissolved HS and assessed its effect on aquatic system [4]. Despite the extensive use of ionic surfactants together with HS, there is a little systematic data on their molecular interaction. As yet, the understanding in their binding is still ambiguous.

In this regard, we have already reported the binding behavior and thermodynamic parameters of *N*-alkylpyridinium bromide (C_nPyBr)-HS binding and depicted the influencing factors on their binding [5,6]. In the present study, we report the results of an investigation in which the thermodynamic parameters of $C_{12}Py^+$ ions binding with AHA and the influencing factors such as pH, ionic strength, and AHA concentration on the binding has been studied. Then the results are discussed by comparing the previous analogous study of $C_{12}Py^+$- Aso fulvic acid (AFA) system. The substantial differences are observed between two investigations of $C_{12}Py^+$ ions binding with FA and humic acid (HA). It is well known that HA and FA have different properties concerning with their sizes, charges, and hydrophobicities although they are the fraction of humic substances (HS). The structure of HA is more aromatic and less aliphatic than FA, and HA molecules are poorer in carboxylic acid and phenolic groups in compared with FA molecules. As a result, HA molecules are less soluble and more hydrophobic than FA molecules [7-11]. The intermolecular interaction between $C_{12}Py^+$ with AFA/AHA is also probed by measuring the hydrodynamic diameters of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates.

4.2. Experimental Section

4.2.1. Materials

Aso fulvic acid (AFA) and humic acid (AHA) were collected from the Aso area of Kyushu Island of Japan and extracted by an international standard method, recommended by IHSS [12]. 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 $C_{12}PyBr$ is 11.4 mmol dm^{-3} in aqueous solution. Tetraborate pH standard buffer solution was used to keep all experimental solutions at pH 9.18 and ionic strength of 0.03 mol dm^{-3} in thermodynamic studies.

4.2.2. pH and Ionic Strength Condition

To study the effect of pH and ionic strength, sample solutions were prepared in the pH range of 7–10 and ionic strength of 0.03-0.10 mol dm^{-3}. Various pH standard buffer solutions (Wako Pure Chemical Industries, Ltd.,): phosphate pH standard solution (pH 7.41), tetraborate pH standard solution (pH 9.18), and carbonate pH standard solution (pH 10.01) were used in order to keep the sample solutions at desired pH. The pH values of the solutions were verified by digital pH meter (ORION, model 91-01). The ionic strength of the buffer solution was computed as described in previous paper [7]. The ionic strength of the sample solutions was controlled by the addition of the corresponding quantity of sodium bromide (NaBr) and deionized water (Millipore Milli-Q system).

4.2.3. Potentiometry for Surfactant Binding Study

The binding isotherms of $C_{12}Py^{+}$ to AHA were obtained by the potentiometric titration method using the surfactant-ion-selective membrane electrodes operated at desired temperature at 293, 298, 303, and 308K. The temperature was controlled at the desired value by circulating thermostated water through the jacketed glass cell. The electrochemical cell: Ag/AgCl, KCl || reference solution| PVC membrane | sample solution || KCl, AgCl/Ag was constructed. The preparation of the electrode was

described elsewhere [13]. The surfactant-ion-selective membranes were composed of partially ionized poly (vinyl chloride) and polymeric plasticizer (Elvaroy 742, Du Pont).

In the absence of AHA, the slope of the linear plots of the electromotive force (emf) vs. the logarithms of surfactant concentration (C_t) below the critical micelle concentration (cmc) showed an Nernstian slope, i.e., 57.0–59.2 mV/decade. In the presence of AHA, however, a deviation from the linearity was observed, suggesting that a part of surfactant bound with AHA. Under assumptions that the membrane is only sensitive to free surfactants, but not to the bound ones, the free surfactant concentration, C_f and the degree of binding, n, can be calculated from the deviation. The concentration of AHA was kept constant at 1.00 g dm^{-3} in all the binding measurements. The added concentration of $C_{12}Py^+$ was far below the corresponding cmc.

4.2.4. Dynamic Light Scattering Measurements

Dynamic light scattering measurements (DLS) were carried out with an Otsuka ELS-800 instrument at a fixed 90° scattering angle. Correlation functions were analyzed by a cumulant method and used to determine the diffusion coefficient (D) of the sample solutions. If one assumes that the scattering species can roughly be taken as spheres, then the hydrodynamic radius (R_h) was calculated from D by using the Stokes–Einstein equation [14,15]

$$R_h = k_B T / (6\pi\eta D) \quad (1)$$

where k_B is the Boltzmann constant, T the absolute temperature, and η the solvent viscosity.

A stock solution of AFA and AHA were prepared in tetraborate pH standard buffer solution (pH 9.18, ionic strength 0.03 M) at a concentration of 0.5 g dm^{-3}. A known amount of $C_{12}Py^+$ was dissolved in similar pH standard solution to obtain the concentration of 75 mmol dm^{-3}. Then, known volume of $C_{12}Py^+$ solution was added to the 0.5 g $dm^{-3}$of AFA or AHA solution to give the final concentration of 1–4 mmol dm^{-3} which are far below the corresponding *cmc* of these surfactants. The final concentration of AFA or AHA in the sample solution was 0.05g dm^{-3}.

4.3. Results and Discussion

4.3.1. Effect of Temperature on $C_{12}Py^+$-AHA System

Figure 1 shows the binding isotherms of $C_{12}Py^+$-AHA system at various temperatures. $C_{12}Py^+$ bind to AHA at very low equilibrium concentration, far below the *cmc*. The gradually increasing binding isotherm indicates that the binding is non-cooperative [16-19]. The Scatchard plots[20] (not shown) give the straight line with negative slope suggesting the independent sites binding behavior [21] of surfactants with AHA. Applying the equation:

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

the number of binding sites, n^* and the binding constant, K are determined. The results are summarized in Table. 1. The solid lines in Fig. 1 indicate the isotherms reproduced from the calculated values listed in Table 1. The good fit of the calculated binding isotherms to the experimental data gives the confidence of the binding mechanism.

As shown in Fig.1 no significant changed in binding is observed with changing the temperature. Usually humic acids (HA) are thermally stable and do not undergo significant destruction of the skeleton and retain the content of functional groups during isothermal heating at temperatures up to 250°C [22]. The value of K at a certain temperature is used for the calculation of Gibbs free energy change (ΔG). The enthalpy (ΔH) of $C_{12}Py^+$ binding with AHA can be obtained from the temperature dependency of the binding constants (K) using the Van't Hoff relation [23,24] and the observed thermodynamic parameters are summarized in Table 1. The enthalpy of $C_{12}Py^+$ binding to AHA is approximately zero suggesting that the binding is not only because of electrostatic interaction but also through the hydrophobic interaction. This observation confirms the already reported estimations of important role of hydrophobic interaction between the hydrocarbon tail of surfactant and the backbone of AHA in the binding [5]. We previously reported that in $C_{12}Py^+$-AFA system thebinding strength increased with increasing temperature. The cooperative binding of $C_{12}Py^+$ with AFA is endothermic process driven by the positive entropy [6].

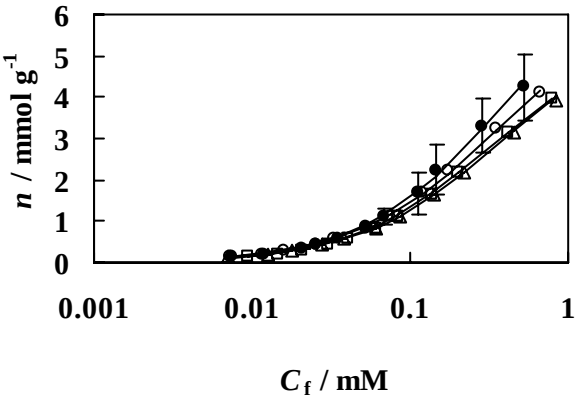

Figure 1. Binding isotherms of $C_{12}Py^+$-AHA system at pH = 9.18, I = 0.03 mol dm^{-3}. (□) 293K, (Δ) 298K, (•) 303K, (o) 308K. Solid lines refer to the curves reproduced by using Eq. (1).

Table 1. Total number of binding sites (n*), binding constant (K), and the thermodynamics parameters for $C_{12}Py^+$-AHA system at pH 9.18 and ionic strength 0.03 mol dm^{-3}.

T (K)	n*	K (M^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔH (kJ mol^{-1})
293	5.6	3142	-19.6		
298	5.7	2878	-19.7	61	-1.5
303	6.4	2975	-20.1		
308	6.2	3005	-20.5		

4.3.2. Effect of pH on $C_{12}Py^+$- AHA System

The effect of pH on the binding of $C_{12}Py^+$ to AHA is investigated at two different ionic strengths. Figures 2a and 2b show the binding isotherms of $C_{12}Py^+$-AHA system as a function of pH (7.41, 9.18, and 10.0) at the ionic strength of 0.03 mol dm^{-3} and 0.10 mol dm^{-3} respectively. The n^* and K values for $C_{12}Py^+$ binding to AHA at various pH calculated by using Eq. 1 are summarized in Table 2. The binding isotherm shifts to lower equilibrium concentration with increasing pH from 7.41 to 9.18. It may be attributed to the increase in ionization degree of AHA functional groups with increasing pH. The development of negative charges at the surface of AHA molecules causes the stronger binding of cationic $C_{12}Py^+$ ions. However, no difference in the binding was observed between pH 9.18 and pH 10.01 at both ionic strengths. At pH 9.18, the functional groups of AHA may be fully ionized and thus no significant change in binding occurs with increasing pH. It has been verified by the investigation in the degree of dissociation (α) of AHA as a function of pH at the ionic strength of 0.03 mol dm^{-3} and 0.10 mol dm^{-3}. It has been found that α increases with increasing pH and reaches to unity when pH > 7 at the ionic strength of 0.10 mol dm^{-3} and pH > 8 at the ionic strength of 0.03 mol dm^{-3}.

The magnitude of binding strength decreases with increasing ionic strength at a certain pH because of the ion-screening effect. To elucidate the combined effect of pH and ionic strength, the value of $K/K_{pH7.4}$ is calculated (Table 2). This value is lower at low ionic strength, ca.1.29, and is larger at higher ionic strength, ca. 1.5, suggesting that the effect of pH on the binding is more pronounced at lower ionic strength. The effect of pH on the binding when pH is below 7 has not been investigated because of inherent difficulties of solubilization of AHA at low pH.

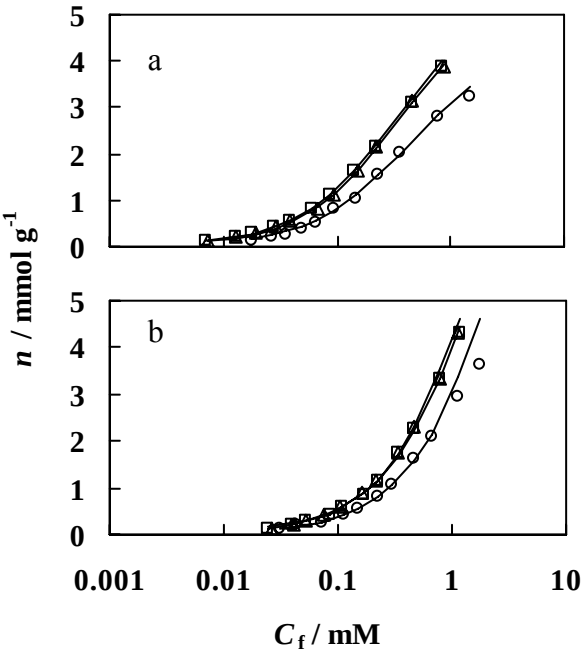

Figure 2. Binding isotherms for $C_{12}Py^{+}$-AHA system as a function of pH at (a) I = 0.03 mol dm^{-3} and (b) I = 0.10 mol dm^{-3}. (o) pH 7.41, (□) pH 9.18, (Δ) pH 10.01.

Table 2. The total number of binding sites of AHA (n*), and binding constant (K), for $C_{12}Py^+$-AHA system at various pH and ionic.

Solution conditions		n*(mmol g^{-1})	K (M^{-1})	K / $K_{pH7.41}$
I (mol dm^{-3})	pH			
0.03	7.41	4.5	2223	1.00
	9.18	5.7	2878	1.29
	10.01	5.6	2754	1.24
0.10	7.41	14.1	273	1.00
	9.18	13.8	406	1.42
	10.01	14.3	415	1.52
0.03	9.18	5.7	2878	
0.05		10.6	1020	
0.10		13.8	406	

4.3.3. Effect of Ionic Strength on$C_{12}Py^+$-AHA System and Comparison of the Extent of Ionic Strength Effect between AFA and AHA System

Figure 3 shows the binding isotherms of AHA expressed as a function of ionic strength (0.03, 0.05,and 0.10 mol dm^{-3}) at pH 9.18. The n* and K values calculated for $C_{12}Py^+$-AHA system at various ionic strengths are summarized in Table 2. The binding isotherms shift to higher equilibrium concentration level with increasing ionic strength, i.e., the binding strength decreases with increasing ionic strength. As the salt concentration decreases, the concentration of counterions also decreases, which lowers the magnitude of ion-screening effect on the HS molecules and increases the binding of $C_{12}Py^+$ ion. It has been observed that the value of n* increases with increasing ionic strength. This tendency is possibly due to the decrease of cmc with increasing ionic

strength and it makes the difficulty in distinguish of the saturation of binding from the micelle formation.

In order to compare the sensitivity of binding to electrolyte concentration between AFA [6] and AHA systems, ln K is plotted in Fig. 4 against with ionic strength. Interestingly, the slope of this plot for AHA-$C_{12}Py^+$ system is higher than that for AFA-$C_{12}Py^+$ system, that is, AFA system is distinctly less sensitive to electrolyte concentration than AHA system. This may be attributed to the magnitude of counterion condensation, which is expected to be higher in AFA system than in AHA system because of the greater charge density of FA molecules [25]. In AFA system, the more counterions, that is Na^+ ions, are condensed on the oppositely charged AFA binding sites even at low ionic strength that may reduce the effective charge density of AFA. Thus, relatively smaller change in binding can be observed with the additional changing of ionic strength. On the other hand, in AHA system, the less or no counterions may condense on AHA chains at low ionic strength. Thus, the binding strength between oppositely charged AHA and $C_{12}Py^+$ is remarkably strong (K = 2878 M^{-1} in AHA system and K = 684 M^{-1} in AFA system). In this case, the extent of binding is greatly influenced by additional change of ionic strength. This result is in agreement with the observation by Tombácz et al. They reported that FA is significantly less sensitive to electrolytes than HA in the study of the effect of sodium chloride on interaction of fulvic acid and fulvate with montmorillonite [26]. In addition, a small decrease in binding strength with increasing ionic strength is observed in AHA system due to increase of hydrophobic interaction with increasing ionic strength and may lead to formation of larger agregation. This assumption will be convinced by investigation in their hydrodynamic diameter.

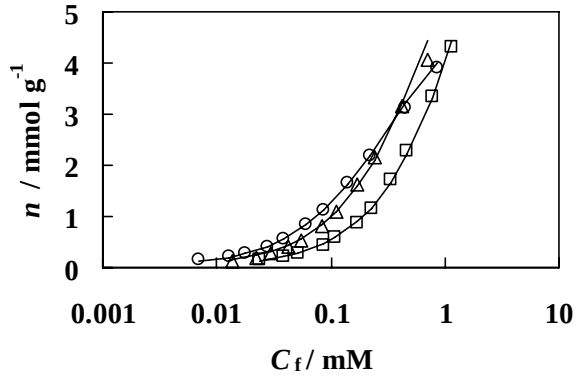

Figure 3. Binding isotherms of $C_{12}Py^+$-AHA system as a function of ionic strength: (o) I = 0.03 mol dm^{-3} (Δ) I = 0.05 mol dm^{-3}, (□) I = 0.10 mol dm^{-3}.

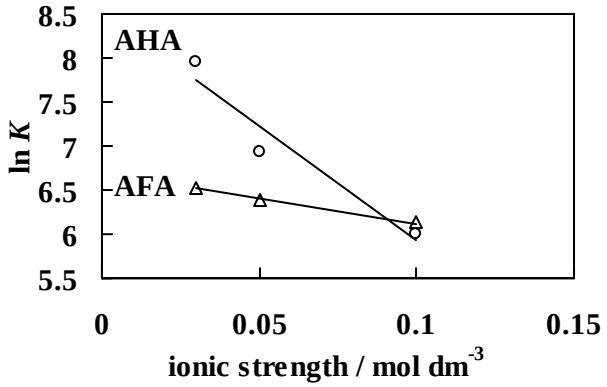

Figure 4. ln K as a function of ionic strength for (o) AHA system and (Δ) AFA system.

4.3.4. Hydrodynamic Diameter of $C_{12}Py^+$- AFA and $C_{12}Py^+$-AHA Aggregates

In order to get the better understanding in binding characteristic of $C_{12}Py^+$ ions in AFA and AHA system, DLS measurements have been carried out. The intermolecular interaction between $C_{12}Py^+$ and AFA/AHA are probed by measuring the hydrodynamic diameters ($2R_h$) of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates in which $C_{12}Py^+$ concentration are systematically changed while maintaining a constant concentration of AFA/AHA (0.05g dm^{-3}). Without cationic surfactant, the hydrodynamic diameter of

AHA is unattainable within the experimental condition because of their inherent polydispersity. As shown in Fig. 5a, the result is incredible in the absence of surfactant and often, can not be measured absolutely. However, in the presence of surfactant the hydrodynamic diameter of $C_{12}Py^+$-AFA or $C_{12}Py^+$-AHA aggregates is measurable (Fig. 5b) with high reproducibility due to the coagulation force of cationic surfactant.

Figure 6 represents the variation of hydrodynamic diameters of $C_{12}Py^+$- AFA and $C_{12}Py^+$-AHA aggregates as a function of binding degree at pH 9.18 and ionic strength of 0.03 mol dm^{-3}. The hydrodynamic diameter increases with increasing $C_{12}Py^+$ concentration in both systems. Thieme et al. also observed the increase in size of dodecyltrimethylammonium bromide (DTB)-HS aggregates with increasing DTB concentration in the investigation by X-ray microscopy [27]. The addition of $C_{12}Py^+$ ions causes charge neutralization and enhances the hydrophobic interaction between surfactant-HS aggregates. As a result, larger aggregates may be induced. When we increase the concentration of $C_{12}Py^+$, the larger aggregates are formed, which gives the larger hydrodynamic diameter.

If we look at the change in hydrodynamic diameter more closely, one can see the difference between $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates. The hydrodynamic diameters of these aggregates are approximately the same at low $C_{12}Py^+$ concentrations (1,1.5 mmol dm^{-3}), however, the size of $C_{12}Py^+$-AFA aggregates are apparently larger than that of $C_{12}Py^+$-AHA aggregates at higher surfactant concentration. This can be explained by the considerable factor: the different binding behavior between AFA and AHA due to the differences in hydrophobicity-hydrophilicity balance. As we reported in early paper [5], $C_{12}Py^+$ ions are cooperatively bound with AFA where the binding constant, *K*, is relatively smaller (684 M^{-1} at pH 9.18, I=0.03 mol dm^{-3}). In AHA system, independent sites binding behavior is observed and *K* is comparably larger (2878 M^{-1} at pH 9.18, I=0.03 mol dm^{-3}). There may be additional hydrophobic interaction between surfactant tail and the hydrophobic backbone of AHA molecule in addition to electrostatic interaction [5]. The stronger interaction between AHA backbone and surfactant tail in turn causes the smaller aggregates, because the hydrocarbon tail of the surfactant can not contribute to the aggregation of surfactant-HS aggregates.

Figure 7 shows the change in hydrodynamic diameters of $C_{12}Py^+$- AFA and $C_{12}Py^+$-AHA aggregates as a function of surfactant concentration at pH 9.18 and at different ionic strength. The size of the aggregate increase with increasing ionic strength, which is more pronounce in AHA system (Fig. 10). This means that the effect of ionic strength on the $C_{12}Py^+$ binding in AHA system is apparently higher in AFA system. This observation is in agreement with the results of the binding measurements where the sensitivity of binding to electrolyte concentration is much greater in $C_{12}Py^+$-AHA system than that of $C_{12}Py^+$- AFA system.

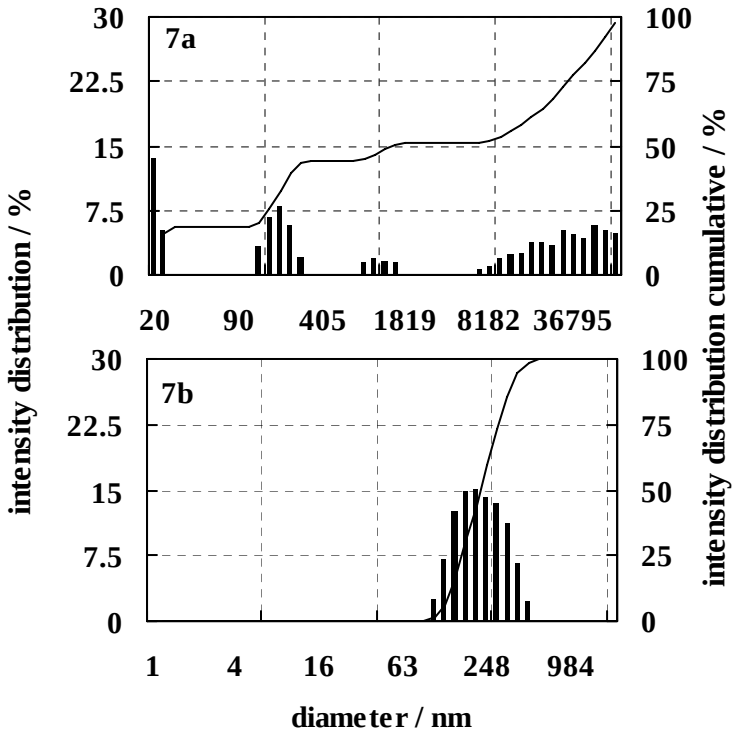

Fig. 5. Representative histogram of the particle size distribution for $C_{12}Py^+$-AFA system (1.5 mmol dm^{-3} $C_{12}Py^+$ and AFA 0.05 g dm^{-3}) at pH 9.18 and ionic strength 0.03M. (a) without $C_{12}Py^+$, (b) with $C_{12}Py^+$.

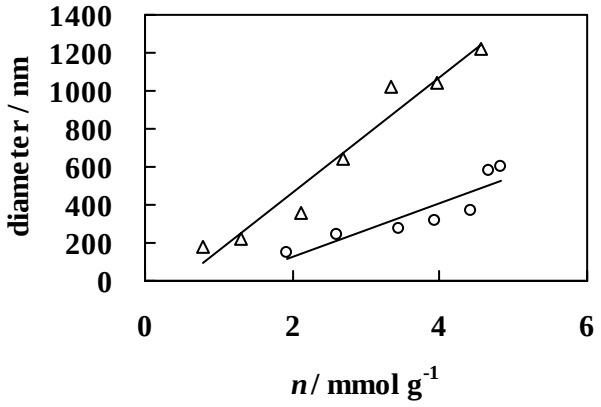

Fig. 6. Dependence of hydrodynamic diameter of the $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates as a function of binding degree**:** (Δ) AFA system, (o) AHA system.

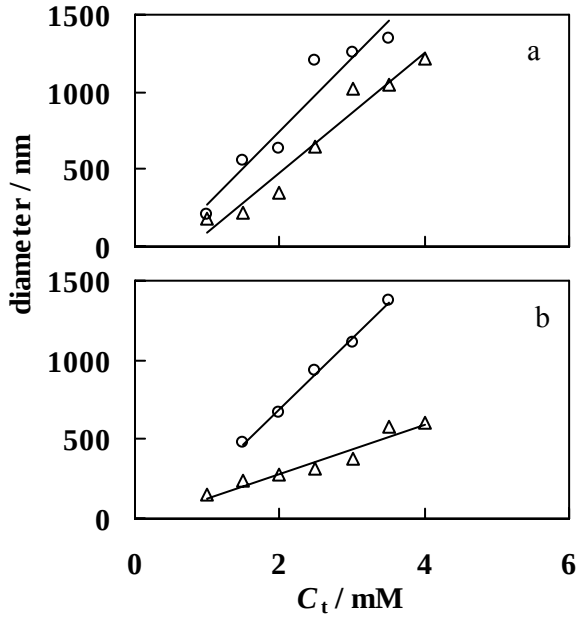

Figure 7. Dependence of hydrodynamic diameter of the $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregrates on total surfactant concentration at pH 9.18 and at ionic strength (Δ) 0.03M, (o) 0.10M. (a) AFA system, (b) AHA system.

4.3.5 Effect of AHA Concentration on $C_{12}Py^+$-AHA System

The effect of concentration of AHA on $C_{12}Py^+$-AHA system is also examined by potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18, ionic strength of 0.03 and at 25°C. As in AFA system, no significant change in binding is observed with changing the AHA concentration in the range of 0.2 - 1.5 g dm^{-3}. Under this experimental condition, any self-aggregation of AHA does not affect the binding strength of $C_{12}Py^+$ ions.

4.4. Conclusion

In $C_{12}Py^+$-AHA system, independent sites binding is observed and the enthalpy of binding is only slightly negative. On the contrary, cooperative binding is found in $C_{12}Py^+$-AFA system and the binding is endothermic process driven by positive entropy. The binding is obviously pH and ionic strength dependent in both systems. However, no HS concentration dependence of binding is observed in both systems. DLS measurements also provide the evidences of some similarity and difference between AHA and AFA system: the hydrodynamic diameter of aggregates increase with increasing $C_{12}Py^+$ concentration in both AHA and AFA system, however, the hydrodynamic diameter of $C_{12}Py^+$-AFA aggregates are larger than that of $C_{12}Py^+$-AHA aggregates.

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(Chapter 5)

Study of Ionic surfactants Binding to Humic Acid and Fulvic Acid by Potentiometric Titration and Dynamic Light Scattering

The binding of anionic surfactant, sodium dodecyl sulfate (SDS) with Aso humic acid (AHA) has been studied by potentiometric titration and dynamic light scattering (DLS) methods at two pH regions and ionic strengths, that is pH 9.18 (ionic strength 0.03 mol dm^{-3}) and pH 3.98 (ionic strength 0.10 mol dm^{-3}). At pH 9.18 and low ionic strength no binding is observed between SDS and AHA in the investigation by both methods, whereas some interaction is observed at pH 3.98 and at high ionic strength by DLS measurement since electrostatic repulsion is suppressed by counterions at this solution condition. The binding between cationic surfactant, dodecyltrimethylammonium ion ($DTMA^+$) with Aso fulvic acid (AFA) and AHA has also been investigated by potentiometric titration and (DLS) methods and compared with the binding of dodecylpyridinium ion ($C_{12}Py^+$). The binding of $DTMA^+$ ions with AFA or AHA is weaker than that of $C_{12}Py^+$ ions, presumably due to steric hindrance of headgroup of $DTMA^+$ ions and higher attractive for binding of $C_{12}Py^+$ ions induced by resonance effect of benzene ring. The hydrodynamic diameter of $DTMA^+$-AFA/$DTMA^+$-AHA aggregates is smaller that of $C_{12}Py^+$-AFA/ $C_{12}Py^+$-AHA aggregates.

5.1. Introduction

Interaction between ionic surfactants and humic substances (HS) is interesting because of their intriguing properties[1-7]. HS are the most abundant organic materials in nature and play a crucial role in the environment. Nonetheless, their structure and physicochemical properties are still mysterious[8-10]. Several researchers have been attempting to clear up the complex issues of humic composition, and properties[11-15]. In some cases, ionic surfactants might be used in order to make better understanding the nature and effect of HS in the environment.

For example alkylammonium ions increases the order of disorder materials. In this regard, Tombácz et al. used alkylammonium ions in the study of X-ray diffraction patterns of humic acid (HA) [16]. Adou et al used dodecyltrimethylammonium bromide in the study of removal efficiency of HS by polypropylene (PPL). The authors stated that it was impossible to remove the bound hydrophobic organic compounds (HOCs) to dissolved organic matters without using dodecyltrimethylammonium bromide [17]. However they didn't clarify the binding nature of ionic surfactants and HS.

Thus, it seems to be valuable to study the binding of ionic surfactants with HS in some details from the view point of academic research as well as applications. In this context, we have already reported the binding behavior and thermodynamic parameters of *N*-alkylpyridinium bromide (C_nPyBr)-HS interaction from different origins and depicted the influencing factors on their binding [18-20]. It has been observed that the subtle balance of ionic, hydrophobic, and hydrophilic interactions governs their binding as a function of pH, ionic strength, temperature, and hydrocarbon chain length etc..

Otto et al. found that even negatively charged surfactants such as SDS interact with humic material at submicellar surfactant concentrations by NMR (Nuclear Magnetic Resonance) diffusion analysis [21]. Traina et al. also reported the association of alkylbenzenesulfonates with dissolved humic substances and its effect on bioavailability [22].

In the present study, the binding of sodium dodecyl sulfate (SDS), a typical anionic surfactant, with humic acid (HA) is examined by potentiometric titration with surfactant-ion-selective membrane electrode and also by dynamic light scattering (DLS) method. As yet, such an investigation has not been reported. We carry out some

experiments to see how the pH and ionic strength affect on their binding. Also, the binding between cationic surfactant, dodecyltrimethylammonium ($DTMA^+$) with HA and FA is investigated and compared with the dodecylpyridinium ion ($C_{12}Py^+$) binding from the previous result [19,20] in order to study the head group effect. This paper is an extension of our previous works on surfactants-HS interaction, which we are still pursuing.

5.2. Experimental Section

5.2.1. Materials

Sodium dodecyl sulfate (SDS) and dodecyltrimethyl ammonium bromide ($DTMA^+Br^-$) were purchased from Wako Pure Chemical Industries, Ltd.,. SDS was used as received and $DTMA^+$ was purified by repeated recrystallization from acetone. Aso humic acid (AHA) and Ao Fulvic acid (AFA) were collected from the Aso area of Kyushu Island of Japan and extracted by an international standard method, recommended by IHSS [23].

5.2.2. Ionic Strength and pH Condition

The binding of SDS with AHA was investigated at two pH regions i.e., at pH 9.18 and at pH 3.98. NaBr was used as the supporting electrolyte for experiments performed at pH 3.98 (ionic strength 0.10 mol dm^{-3}) and the pH was adjusted by analytical grade hydrochloric acid. Tetraborate pH standard buffer solution was used to fix the pH and ionic strength of the sample solutions at 9.18 and 0.03 mol dm^{-3}, respectively in SDS binding and $DTMA^+$ binding study. Deionized water (Millipore Milli-Q system) was used in the preparation of all experimental solutions.

5.2.3. Potentiometry for Surfactant Binding Study

The binding of SDS with AHA was investigated 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 membrane potential was measured by using a digital multimeter (Advantest TR6845) connected with the electrochemical cell: Ag/AgCl, KCl || sample solution| PVC membrane | reference solution || KCl, AgCl/Ag.

5.2.4. Dynamic Light Scattering Measurements

A series of DLS measurements were carried out for mixed AHA-SDS solutions at pH 9.18 (ionic strength 0f 0.03 mol dm^{-3}) and pH 3.98 (ionic strength 0.1 mol dm^{-3}) in which AHA concentration was kept constant at 0.05g/L and SDS concentration was varied in the range of 1–4 mmol/L. Dynamic light scattering measurements (DLS) were carried out with an Otsuka ELS-800 instrument at a fixed 90° scattering angle. Correlation functions were analyzed by a histogram method and used to determine the diffusion coefficient (D) of the samples. Hydrodynamic radius (R_h) was calculated from D by using the Stokes–Einstein equation: [24,25]

$$R_h = k_B T/ (6\pi\eta D)$$

where k_B is the Boltzmann constant, T the absolute temperature, and η the solvent viscosity.

5.3. Results and Discussion

5.3.1. SDS-AHA Binding by Potentiometric Titration

The emf (electromotive force) responses in aqueous surfactant solutions, in the presence and absence of AHA at different pH is shown in Fig.1, where emf is plotted against the logarithms scale of the total SDS concentration (C_t). As shown in figure, the electrode shows excellent performance with Nernstian response, i.e., the slopes are about 58.5 mV/dec. The break found around 3.6mM SDS also confirm the sensitivity of the electrode to SDS ions, since the critical micelle concentration (cmc) of SDS in the medium is round about 4mM [26].

Figure 1a displays the experimental results performed at pH 9.18 and ionic strength of 0.03 mol dm^{-3}. There is no difference between the two titration curves; one is in the absence (open cycles and stars) and the other is in the presence (triangles) of AHA, meaning that SDS does not bind with AHA within the experimental conditions. One possibility is that any specific interaction can not overwhelm the strong electrostatic repulsion between negatively charged SDS and AHA molecules.

In order to reduce the electrostatic repulsion between SDS and AHA, we carried out the potentiometric titration at low pH and high ionic strength. Figure 1b shows the potentiogram of SDS binding to AHA at pH 3.98 and ionic strength of 0.10 mol dm^{-3}. A deviation from the Nernstian response, which is a sign of binding, is not observed. The cmc value shifts to the lower concentration ca. 1.8mM at higher ionic strength, since micellization is favored by the addition of salt that screens the electrostatic repulsion between the surfactant head group. At both pH; pH 9.18 and pH 3.98, the value of cmc does not affected by the presence of AHA. No experiment has been performed for SDS binding with Aso fulvic acid (AFA) because AFA is rather hydrophilic than AHA and no binding can be expected.

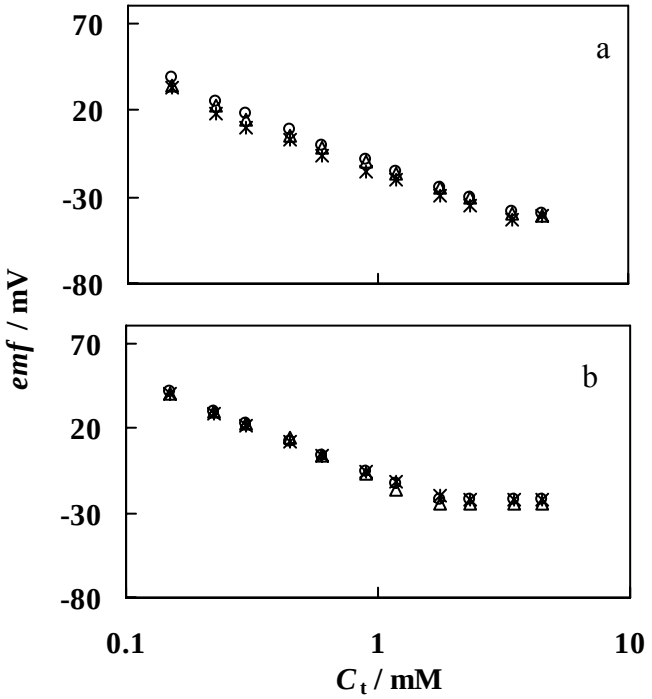

Figure 1. Potentiograms of SDS-AHA system (a) at pH 9.18 (I = 0.03 mol dm^{-3}) and (b) at pH 3.98 (I = 0.10 mol dm^{-3}).

5.3.2. SDS-AHA Bbinding by DLS Measurements

In order to chase the binding between SDS and AHA, DLS measurements have been carried out at two pH region, i.e., pH 9.18 (ionic strength 0.03 mol dm^{-3}) and pH 3.98 (ionic strength 0.10 mol dm^{-3}) in which SDS concentration are systematically changed in the range of 1–4 mmol/L and AHA concentration is kept constant at 0.05g/L. The hydrodynamic diameter of AHA alone is unattainable within the experimental condition because of their inherent polydispersity. However, we have reported that the hydrodynamic diameter of dodecylpyridinium ions ($C_{12}Py^{+}$)-AHA aggregates is measurable as a probe of their intermolecular binding in the previous study [19]. As in the case of AHA alone, the precise determination of hydrodynamic diameter of SDS-

AHA is still unattainable at pH 9.18 suggesting that there is no binding between SDS and AHA.

At pH 3.98, it has been observed that the hydrodynamic diameter is measurable with high reproducibility for the sample solution containing 0.05g/L of AHA with 3.5 mmol/L of SDS and is about 180nm (Fig. 2). It suggests that at low pH and high ionic strength the electrostatic repulsion between SDS and AHA is suppressed and there is a specific interaction between them. In order to validate their interaction, DLS measurements for AHA or SDS alone have been performed at the same experimental conditions. Apparently, the hydrodynamic diameter is unattainable without AHA even SDS concentration used is above cmc. In the presence of AHA, AHA molecules may wrap around surfactant micelle, with decreasing the area of hydrocarbon core of the micelle which is exposed to water. An indirect clue to the formation of such aggregates is provided by experimental results which show that the hydrodynamic diameter is measurable only above cmc. As described above, Otto et al. and Traina et al. observed the interaction between negatively charged surfactants such as SDS and alkylbenzenesulfonates with HS. On the other hand, Koopal et al. reported that no significant binding was observed between SDS and purified Aldrich humic acid (PAHA) at pH 7 and ionic strength of 0.025 mol dm^{-3}. These discrepancies can be explained by the ambiguous nature of HS, the solution parameter, the sample preparation, and the analytical technique used in the experiment. We conclude that there is a specific weak interaction between SDS and AHA at low pH and high ionic strength in which their electrostatic repulsion is suppressed by the counterions and only be able to detect by some relevant method as a function of solution parameter.

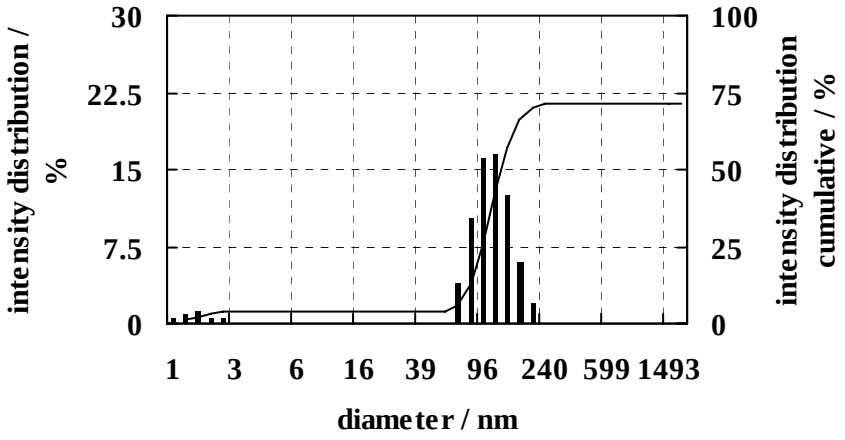

Figure 2. Representative histogram of the particle size distribution for $C_{12}Py^+$-AHA system (3.5 mmol dm^{-3} $C_{12}Py^+$ and AHA 0.05 g dm^{-3}) at pH 3.98 and ionic strength 0.10M.

5.3.3. Effect of Cationic Surfactant Head Group (Potentiometric Titration)

Alternatively, the binding of dodecyltrimethylammonium ions ($DTMA^+$) with AHA and AFA has been studied by using potentiometric titration method with surfactant-ion-selective membrane electrodes in aqueous solution of pH 9.18 and at the ionic strength of 0.03 mol dm^{-3}. Figure 3 shows the potentiogram of $DTMA^+$ binding to AFA and AHA at 25 °C. In contrast with SDS, deviation from the calibration line is observed. From the deviation a binding isotherm is constructed by plotting $n = C_b / C_{HS}$ vs. C_f in logarithm scale, where C_b, the amount of bound surfactant, is a difference between the total (C_t) and equilibrium (C_f) concentrations, and C_{HS} the concentration of humic substances expressed in g dm^{-3}.

Figure 4 and 5 show the binding isotherm of $DTMA^+$ binding to AFA and AHA respectively, where our previous results for $C_{12}Py^+$ to AFA and AHA have been included in order to study the effect of cationic surfactant headgroup [19,20]. The binding of $DTMA^+$ to AFA and AHA is the same behavior with that of $C_{12}Py^+$ to AFA and AHA. In AFA system, the binding is highly cooperative and the binding constants

and cooperative parameters are calculated by applying Hill's binding theory. In AHA system, independent sites binding behavior is observed with $DTMA^+$ ions and the number of binding sites and binding constants are analyzed by Scatchard plot equation. The calculated results are shown in Table 1and 2. In both systems, $DTMA^+$ binding is weaker than $C_{12}Py^+$ ions binding and this can be explained by two considerable factors. One factor is due to steric hindrance produced by a larger headgroup size of the trimethylammonium group in the binding with anionic HS. Another factor is that $C_{12}Py^+$ ions also has resonance effect due to benzene ring and may be more actively attractive for binding. Free energy decrease is about 2.2 kJ mol^{-1} for a change of the headgroup from $DTMA^+$ to $C_{12}Py^+$, which can be collated to the free energy loss of 2 kJ mol^{-1} for a change of dodecyldimethylammonium chloride (DDAC) to dodecylamethylammonium chloride (DMAC) in the interaction with poly(L-glutamic acid) [27].

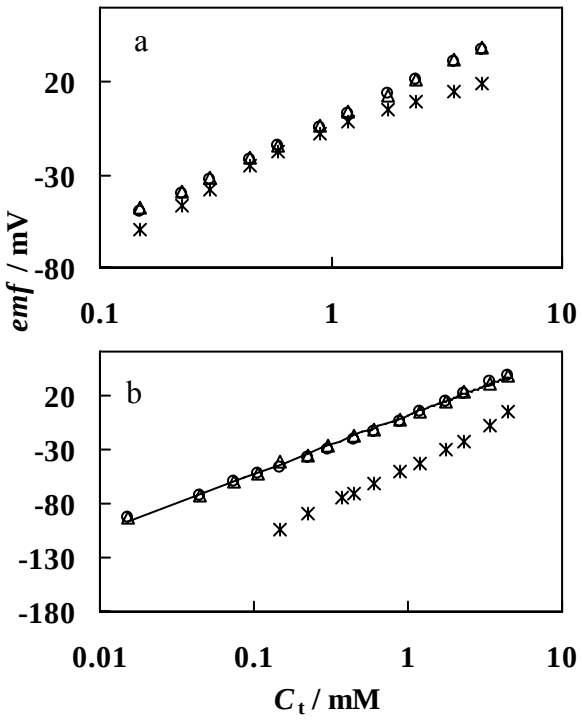

Figure 3. Potentiograms of (a) $DTMA^+$-AFA (b) $DTMA^+$-AHA systems at pH 9.18 (I = 0.03 mol dm^{-3}.

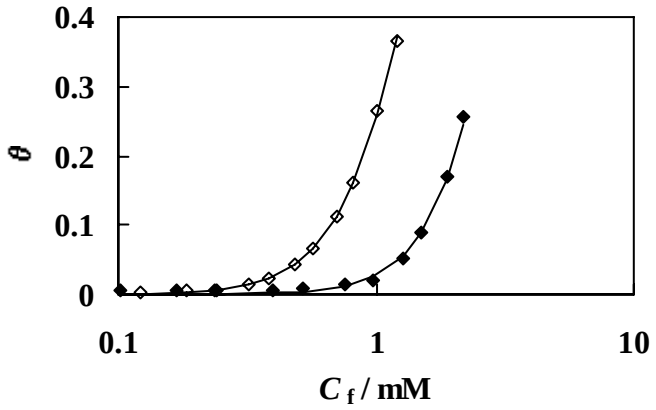

Figure 4. Binding isotherms of $DTMA^+$-AFA and $C_{12}Py^+$-AFA system at pH 9.18 (I = 0.03 mol dm^{-3}).

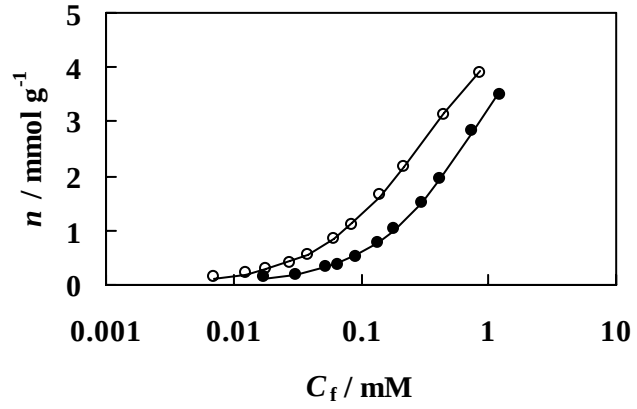

Figure 5. Binding isotherms of $DTMA^+$-AHA and $C_{12}Py^+$-AHA system at pH 9.18 (I = 0.03 mol dm^{-3}).

Table 1. The total number of binding sites of AFA (n*), cooperative parameter (h), and binding constant (K), for $DTMA^+$-HS and $C_{12}Py^+$-HS and systems at pH 9.18 (I = 0.03 mol dm^{-3}) and at 25°C

Systems	n* (meq g^{-1})	h	K (M^{-1})	$\Delta G°$(kJ mol^{-1})
$DTMA^+$-AFA	9.65	3	317	-14.27
$C_{12}Py^+$-AFA	9.65	3	684	-16.17
$DTMA^+$-AHA	6.4		1015	-17.15
$C_{12}Py^+$-AHA	5.7		2878	-19.73

5.3.4. Effect of Cationic Surfactant Head Group (DLS Measurements)

The hydrodynamic diameters ($2R_h$) of $DTMA^+$-AFA and $DTMA^+$-AHA aggregates have been examined in order to probe their intermolecular interaction. Figure 6 represents a comparison of the change in hydrodynamic diameters due to cationic surfactants with a different ionic head group. In which the hydrodynamic diameters of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA have been added from the previous result [19]. In all systems, the hydrodynamic diameters increase with increasing surfactant concentration. It strongly suggests the formation of cationic surfactant-HS nanoaggregates due to charge neutralization and the size of aggregates become growth as a function of surfactant concentration. As seen in figure, it has been found that the hydrodynamic diameters of $DTMA^+$- aggregates are smaller than that of $C_{12}Py^+$ aggregates in both AFA and AHA systems. If we compare $DTMA^+$-AFA and $DTMA^+$-AHA aggregates, $DTMA^+$-AHA aggregates are smaller that that of AFA aggregates. Thus, the size of the aggregates might be affected by both HS and cationic surfactants and may be the function of binding behavior, solution conditions, and morphological change in AFA and AHA molecules induced by surfactant binding.

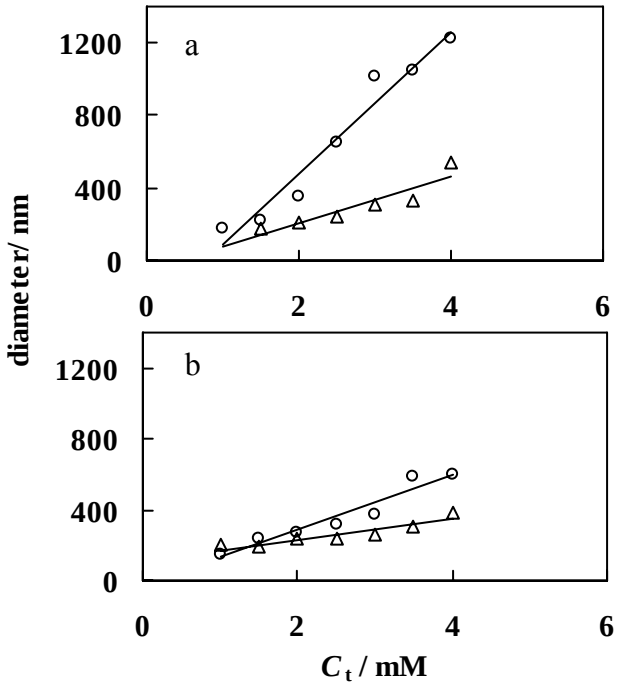

Figure 6. Dependence of hydrodynamic diameter of the cationic surfactant-HS aggregates on total surfactant concentration at pH 9.18 and ionic strength 0.03 mol dm^{-3} (a) AFA system, (b) AHA system. (o) $C_{12}Py^+$, (Δ) $DTMA^+$.

5.4. Conclusion

At high pH and low ionic strength no interaction is observed between SDS and AHA by the investigation of potentiometric titration method and DLS measurement. However, some specific interaction is observed between SDS and AHA at low pH and high ionic strength by DLS measurement, in good agreement with expectation given the different nature of measurements. The polar headgroup of cationic surfactant is one of the influencing factors on the binding with HS and it can be verified by both potentiometric titration and DLS measurements.

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(Chapter 6)

On the Dodecylpyridinium Binding Study of Humic Substances from Different Origins

The binding of dodecylpyridinium ($C_{12}Py^+$) ions with humic acids (HAs) as well as fulvic acids (FAs) from different origins have 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 soill and aquatic) show the same independent sites binding behavior in binding with $C_{12}Py^+$ ions, and the number of binding sites and binding constants are analyzed by Scatchard plot equation. In all FAs system, the binding is highly cooperative. 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 soil Fas, which tendency agrees with the higher carbon content and the lower oxygen content 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.

6.1. Introduction

Humic substances (HSs) are the break-down products of plants and biological origins found in almost all soil 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 [1-3]. HSs possess a wide range of molecular weights and include both hydrophilic and hydrophobic moieties [4,5]. So that they interact readily with hydrogen ions, metal cations and organic compounds, such as surfactants, pesticides and herbicides [6-10]. Among these compounds, ionic surfactants play an important role in the environment because of their anthropogenic origin market everywhere from household detergents to explosives [11], and they can accumulate in soils and waters. In case of the deposition of cationic surfactants in the soils and waters, it is expected that these substances will readily bind to negatively charged humic substances [12]. The knowledge of the interactions of cationic surfactants with HSs is of particular importance, especially with respect to 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 [13,14]. The affinity of the organic compounds to HSs appears to vary among HSs samples 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 the 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 [15,16].

Recently we reported the amphiphilic properties of HA and FA by alkylpyridinium binding study [17] 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).

6.2. Experimental Section

6.2.1. Materials

Eight different HAs and FAs were studied. All samples used were Japanese origins and were extracted by an international standard method recommended by IHSS [18]. The elemental compositions and origins of the samples are listed in Table 1. 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 11.4mmol dm^{-3} (Mukerjee and Mysels, 1971) in aqueous solution. All experimental solutions were kept at pH 9.18 and ionic strength of 0.03 mol dm^{-3} by using tetraborate pH standard buffer solution ($Na_2B_4O_7$).

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
	Lakebiwa (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
	Lakebiwa (aquatic)	BFA	54.8	5.9	2.3	37.0

*all samples are Japanese origin

6.2.2. 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 logarithms 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 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.

6.2.3. 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 standardsolution. 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.

6.2.4. Capillary Electrophoresis (CE)

The electrophoretic mobilities of HSs were measured at 25° C with CAPI-1000 CE system 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 sample 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 running buffer solution. The experiments were run for 1200 sec and measured at 200nm UV absorbency.

6.3. 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, $C_b = C_t - C_f$, where C_f is free surfactant concentration. 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 C_f, in mmol dm^{-3}.

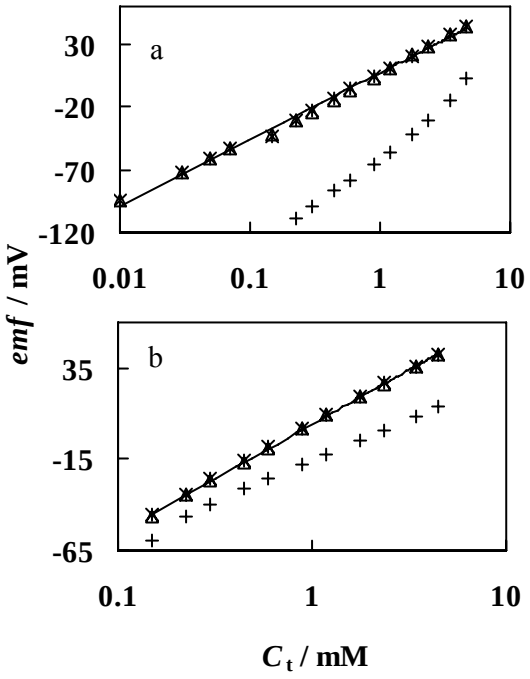

Figure 1. Potentiograms of (a) $C_{12}Py^+$-IHA system and (b) $C_{12}Py^+$-IFA system. (Δ); (∗) without FA or HA; (+) with FA or HA; pH = 9.18, I = 0.03, T = 25°C.

6.3.1. 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 [17]. 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 AHA system. These binding isotherms are replotted as Scatchard plots [19] to see the binding mode through the all binding degree. The Scatchard plots (Fig. 3) give the straight line with negative slope, suggesting the independent sites binding behavior [20] of surfactants to HAs. Applying the following equation:

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

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 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) also indicate that there is no significant differences among carbon and oxygen contents of these soil HAs, which is also agrees with our proposal that is no significant difference in hdrophobicity-hydrophilicity balance between these HAs.

The binding isotherm for 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 terrestrial 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 [17].

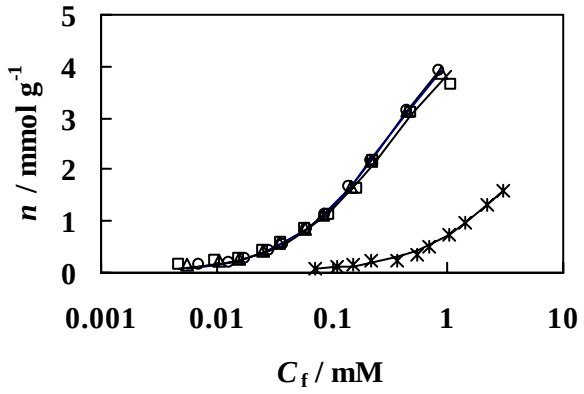

Figure 2. Binding isotherms of $C_{12}Py^+$ with HAs at 25°C. (o) AHA; (Δ) IHA; (□) DHA; (∗) BHA. Solid lines refer to the curves reproduced by using equation 1.

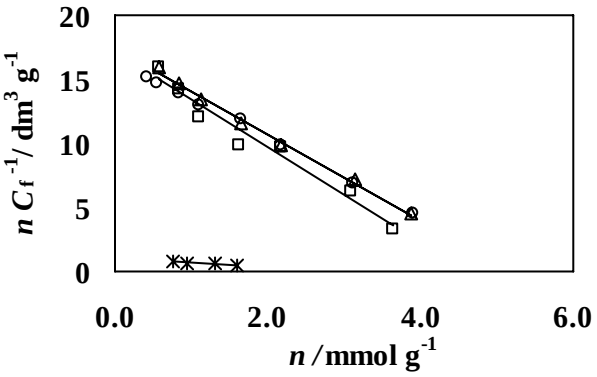

Figure 3. Scatchard plots for $C_{12}Py^+$-HA systems. (o) AHA; (Δ) IHA; (□) DHA; (∗) LHA.

6.3.2. CE Measurements for HA Systems

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 a comparable charge to size ratio. Differences in the intensity and electrophoretic mobility are established by structural and chemical differences resulting from the differing origins of HSs [7,21]. Fig.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 and migration times of first peak are almost the same for these three HAs and a little difference is observed in 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 well agreement with the results of potentiometric measurements.

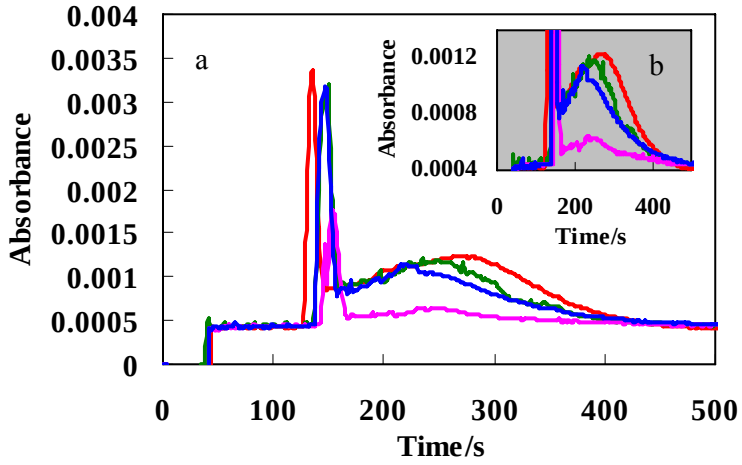

Figure 4. (A) Electropherograms of HAs analyzed with tetraborate buffer (pH 9.18) [20 kV, 25°C, detection at 200nm]. (—) AHA; (—) IHA; (—) DHA; (—) BHA. The inset (B) is the close view of the second peak of (A).

6.3.3. 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, 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 polyelectolytes [22-25]. In this concern, the binding isotherm can be empirically analyzed by Hill's equation [20]:

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

where n^* is the total number of binding sites expressed in meq 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 [24]. 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, (θ / (1-θ)) is plotted in Fig. 6 against with C_f. Then, the binding constant of a surfactant with an individual binding site, K can be calculated by using the equation:

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

The calculated h and K values for $C_{12}Py^+$ binding to FAs 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 studied FAs systems.

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 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.

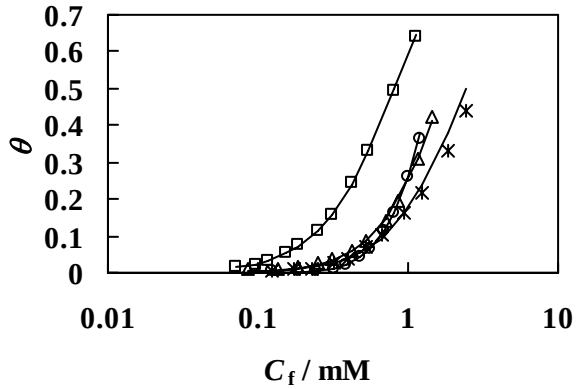

Figure 5. Binding isotherms for $C_{12}Py^{+}$-FA systems. (o) AFA; (Δ) IFA; (□) DFA; (∗) BFA. Solid lines refer to the curves reproduced by using equation 2.

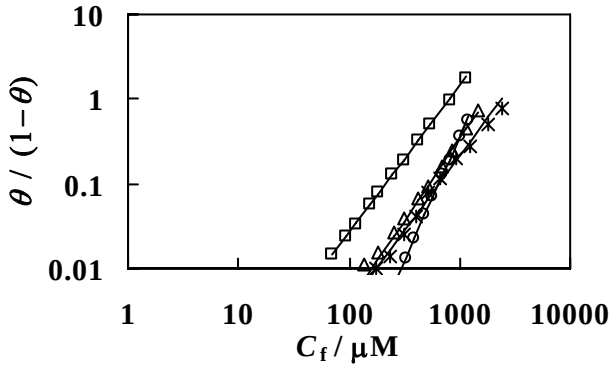

Figure 6. Hill's plots for $C_{12}Py^{+}$-FA systems. (o) AFA; (Δ) IFA; (□) DFA; (∗) BFA. Solid lines refer to the curves reproduced by Hill's equation.

Table 2. Number of binding sites (n^*) and binding constant (K) for $C_{12}Py^+$-HA systems and the number of binding sites (n^*), cooperative parameter (h), binding constant (K), for $C_{12}Py^+$-FA systems.

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

6.3.4. 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 very similar compositions 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.

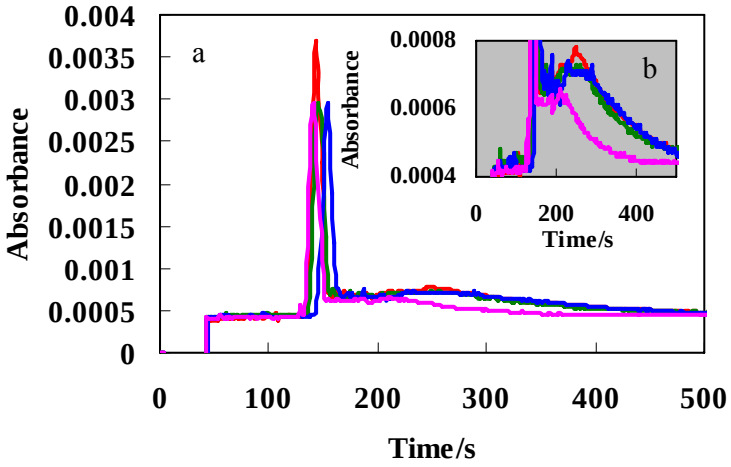

Figure 7. (A) Electropherograms of FAs analyzed with tetraborate buffer (pH 9.18) [20 kV, 25°C, detection at 200nm]. (—) AFA; (—) IFA; (—) DFA; (—) BFA. The inset (B) is the close view of the second peak of (A).

6.3.5. Comparison between the Binding Behavior of HAs and FAs Systems

Difference 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 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 systems. 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.

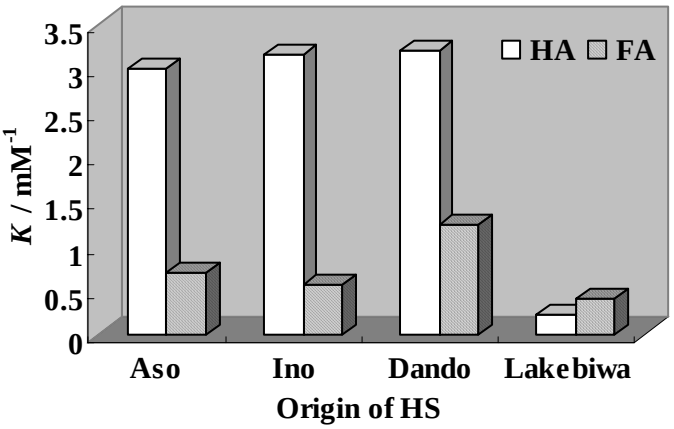

Figure 8. Binding constant (K) for $C_{12}Py^+$ binding with HS of different origins.

6.4. Conclusion

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 HS in addition to electrostatic interaction.

6.5. References

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Summary

In this work we attempted to advance the understanding of ionic surfactants-humic substances interaction based on a comprehensive study of their binding isotherms, solution physicochemistry, and a morphological change in their aggregates formation. Introduction of HSs, ionic surfactants, and their properties is desirable because so much knowledge of our understanding of their molecular interaction is based on these factors and is described in chapter 1.

In general, it is well known that the amphiphilic properties of HSs display the cricual role in the interaction with both inorganic and organic materials. In this regard, the amphiphilic properties of Aso fulvic acid (AFA) and Aso humic acid (AHA) have been evaluated through the alkylpyridinium binding (C_nPy^+) study. In AFA systems, the binding is highly cooperative and the binding constants and cooperative parameters are determined by Hill's binding theory. In AHA system, an independent site binding behavior is observed with C_nPy^+ ions, and the number of binding sites and binding constants are analyzed by Scatchard plot equation. Apart from the electrostatic interaction, two different hydrophobic interactions are involved in surfactant-humic substance interactions: one is hydrophobic interaction between the hydrocarbon tail of surfactant and the backbone of humic substances (C_nPy^+-AHA system) and another is the hydrophobic interaction among surfactants themselves (C_nPy^+-AFA system).

Study on the thermodynamic information of the surfactant-HSs interaction facilitates the better understanding in mechanism. The thermodynamic information of dodecylpyridinium ion ($C_{12}Py^+$) binding with FA and HA is described in chapter 3 and 4. The cooperative binding of $C_{12}Py^+$ with AFA is the endothermic process driven by the positive entropy resulting possibly from the dehydration of hydrophobically hydrated water molecules around the hydrocarbon chains of the bound $C_{12}Py^+$ ions. On the other hand, the enthalpy of $C_{12}Py^+$ ions binding with AHA is slightly negative. The entropy of binding ($\Delta S°$) in AFA and AHA systems is 95 and 61 J mol^{-1} K^{-1} respectively. This revealed that the magnitude of counterions screening is higher in AFA system than

in AHA system because of the greater charge density of FA molecules. The fact is coincident with the observations found in ionic strength effect.

It is substantial to determine the role of pH and ionic strength on the binding of $C_{12}Py^+$ ions with HS since the solution parameters have a profound effect on their binding. In this context, the effect of pH, ionic strength, and the concentration of HS on $C_{12}Py^+$ binding with AFA and AHA are included in chapter 3 and 4. In $C_{12}Py^+$-AFA system, different binding modes are observed at pH>7 and pH<7: cooperative binding at pH>7 and noncooperative binding at pH<7. The binding strength is most pronounced at pH 9.18 in both AFA and AHA systems since the carboxylate functional groups of AFA or AHA are fully ionized at this pH.

Apparently in both AFA and AHA systems, the binding strength decreases with increasing ionic strength due to the ion-screening effect. The sensitivity of binding to electrolyte concentration is higher in AHA system than that in AFA system, meaning that the binding strength is not so much changed in AFA system due to the changes of electrolyte concentration in comparison with AHA system. It suggests that, the more counterions, that is Na^+ ions, are condensed on the oppositely charged AFA chains at certain pH and ionic strength. Thus, relatively smaller change in binding can be observed with the additional changing of ionic strength. This observation is in consistent with the greater entropy of binding in AFA system.

The intermolecular interaction between $C_{12}Py^+$ with AFA or AHA is also probed by measuring the hydrodynamic diameters ($2R_h$) of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates using dynamic light scattering (DLS). The hydrodynamic diameter increases with increasing $C_{12}Py^+$ concentration in both systems while maintaining a constant pH, ionic strength, and AFA/AHA concentration at 9.18 and 0.03 mol dm^{-3}, 0.05g/L, respectively, due to the formation of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregates. Actually, the hydrodynamic diameter of AHA alone is unattainable within the experimental condition because of their inherent polydispersity.

In addition, the hydrodynamic diameters of $C_{12}Py^+$-AFA and $C_{12}Py^+$-AHA aggregate increase with increasing ionic strength, which is more pronounce in AHA system. This results is in agreement with the results of the binding isotherms where the sensitivity of binding to electrolyte concentration is much greater in $C_{12}Py^+$-AHA system than that of $C_{12}Py^+$- AFA system.

Moreover, the study of the interaction between anionic surfactant, sodium dodecyl sulfate (SDS) with AHA by potentiometric titration and dynamic light scattering (DLS) methods at pH 9.18 (ionic strength 0.03 mol dm^{-3}) and pH 3.98 (ionic strength 0.10 mol dm^{-3}) is reported in chapter 5. At pH 9.18 and low ionic strength no binding is observed between SDS and AHA, whereas some interaction is observed at pH 3.98 and high ionic strength by DLS measurement since electrostatic repulsion is suppressed by counterions at this solution condition.

The effect of cationic surfactant headgroup on the binding with HSs is also report in this chapter. The binding of dodecyltrimethylammonium ($DTMA^+$) ions with AFA or AHA is weaker than that of $C_{12}Py^+$ ions, due to steric hindrance of headgroup of $DTMA^+$ ions. On one way, the binding of $C_{12}Py^+$ ions with AFA or AHA is stronger than that of $DTMA^+$ due to stronger attractive force induced by resonance effect of benzene ring $C_{12}Py^+$ ions. From DLS measurements, it is found that the hydrodynamic diameter of $DTMA^+$-AFA/$DTMA^+$-AHA aggregates is smaller that of $C_{12}Py^+$-AFA/$C_{12}Py^+$-AHA aggregates and $DTMA^+$-AHA aggregates is smaller than $DTMA^+$-AFA aggregates. It indicates that the size of the aggregates might be affected by both HSs and cationic surfactants and may be the function of binding behavior, solution conditions, and morphological change in AFA and AHA molecules induced by surfactant binding.

It is well known that HSs are continuously subject to alterations in the biosphere. Already small changes of natural conditions are able to induce modifications of structural properties. Thus, the affinity of ionic surfactants to HSs appears to vary among HSs samples from different origins. We finally present the study of interaction between dodecylpyridinium ($C_{12}Py^+$) ions with FA and HA of different origins in chapter 6 and relate the binding affinity with the structural and chemical features of HSs. The binding strength of $C_{12}Py^+$ ions to HSs vary depending on their origins. In both FA and HA systems, $C_{12}Py^+$ binding is stronger with soil samples than that with aquatic samples. In addition, the binding affinity of $C_{12}Py^+$ ions is stronger with soil HA than with soil FA. In brief, hydrophobicity is one of the key factors in cationic surfactant – HS binding since soil HSs is more hydrophobic than aquatic one as well as HA is more hydrophobic than FA.

In conclusion, cationic surfactants are bound with HS in cooperatively as well as in independent sites binding behavior depending on the solution conditions and the type of HSs used. Not only the electrostatic interaction but also the hydrophobic interaction should be taken into account in their binding. The binding strength and the hydrodynamic diameter of ionic surfactant-HS aggregates are influenced by various factors such as pH, the added salt concentration, surfactant chain length, and temperature. On the whole, study of ionic surfactant-HS interaction comprehensively leads to new application of chemistry in other fields and in technology.

Resume

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Publications

1. “Evaluation of amphiphilic properties of fulvic acid and humic acid by alkylpyridinium binding study,” *Colloid Surf. A* : *Physicochem. Eng. Asp.*, **2006**, *272*, 182-188. Y. M. Min, T. Miyajima, N. Takisawa.
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5. “Study of ionic surfactants binding to humic acid and fulvic acid by potentiometric titration and dynamic light scattering”, *Colloid Surf. A* : *Physicochem. Eng. Asp., to be submitted,* Y. M. Min, T. Miyajima, N. Takisawa.