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Molecular Association of Water-Soluble Calixarenes with Several Stilbene Dyes and Its Application to the Facile Determination of Cationic Surfactant Concentrations

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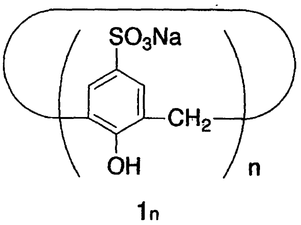
The molecular association of water-soluble *p*-sulfonatocalix[*n*]arenes ($\mathbf{1}_n$; $n=4$, 6, and 8) with several stilbene dyes (D) has been studied by spectrophotometric and ^{1}H NMR methods. It was found that $\mathbf{1}_n$ reacts in 1 : 1 stoichiometry with, D such as 4-[(4-dimethylamino)styryl]-1-methylpyridinium (St–4Me), 2-[(4-dimethylamino)styryl]-1-methylpyridinium (St–2Me) and 2-[(4-dimethylamino)styryl]-1-ethylpyridinium (St–2Et) iodides to form their complexes ($\mathbf{1}_n$D). The apparent association constants for $\mathbf{1}_8$-complexes are larger by about ten times than those of $\mathbf{1}_4$- and $\mathbf{1}_6$-complexes. Examinations of the CPK molecular models and the spectrophotometric studies suggested that $\mathbf{1}_4$ complexes D mainly with its 1-methyl- or 1-ethylpyridinium group, whereas $\mathbf{1}_6$ complexes D either with its pyridinium ring or with its protonated dimethylamino group in a similar probability, and in $\mathbf{1}_8$-complexes the molecule is wholly incorporated into the cavity of $\mathbf{1}_8$. The absorbance of D, which was decreased by association with $\mathbf{1}_n$, was regenerated by the addition of other colorless cationic surfactants (A) such as cetylethyldimethylammonium ion. Studies on the substitution reaction of $\mathbf{1}_n$D with A indicated that a 1 : 1 complex ($\mathbf{1}_n$A) is formed by releasing a free dye molecule (D). This was applied to a convenient determination of the cationic surfactant concentration in water.

Water-soluble *p*-sulfonatocalix[*n*]arenes ($\mathbf{1}_n$), which have a molecular cavity comprising benzene units arranged in the cyclic structure, as shown in Scheme 1, have been actively investigated during last two decades owing to their abilities to complex a variety of organic compounds[1] as well as inorganic ions in water.[1] We have been interested in the determination of their complexation ability with cationic dyes (D), which are relatively large in molecular size, in relation to the analytical determination of colorless alkylammonium ions (A) in water using a substitution reaction with colored dyes in the complex.

A spectroscopic change in the guest molecule induced by added calixarenes was first reported by Shinkai et al.[2] on phenol blue (PB) with host $\mathbf{1}_6$. They found that the absorption maximum of PB shifts to longer wavelength in the presence of $\mathbf{1}_6$; also absorption spectroscopic and NMR measurement studies have indicated that the spectral change can be attributed to complex formation between PB and $\mathbf{1}_6$.[2] They determined the binding ratio and the association constant of the complex to be 1 : 1 and 5.6×10^2 M^{-1}, respectively (1 M = 1 mol dm^{-3}), based on the spectral change of PB in the presence of various amounts of $\mathbf{1}_6$ in water at pH 6.30. According to their conclusion,[2] PB is incorporated into the center of the $\mathbf{1}_6$ cavity by an interaction between the cationic dimethylammonium group in PB and the anionic sulfonato groups in $\mathbf{1}_6$ and between the anionic phenoxy group in PB and the hydroxy groups in $\mathbf{1}_6$, forming a hydrogen bonded 1 : 1 complex.[3]



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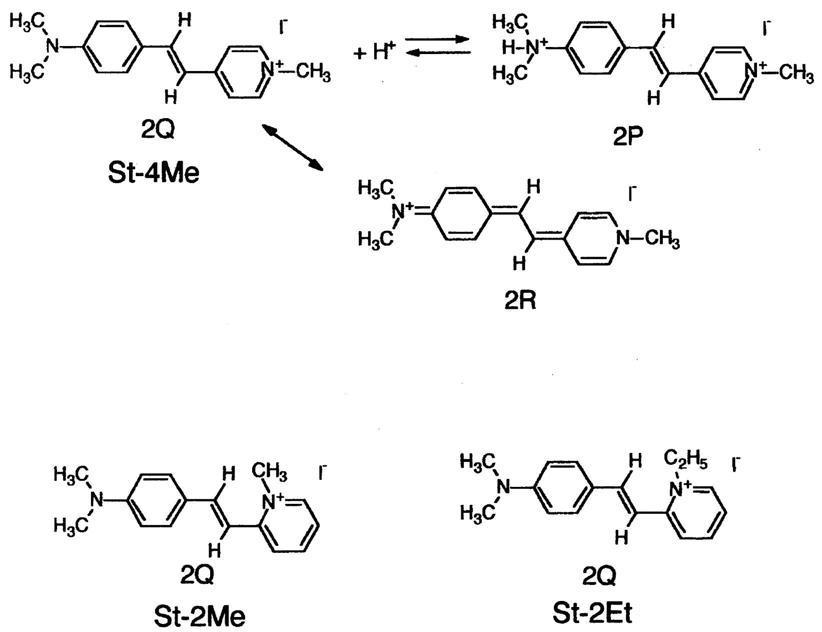
Scheme 1. Water-soluble calix[*n*]arene, $\mathbf{1}_n$ ($n=4$, 6, and 8).

We investigated a change in the absorption spectra of a variety of cationic dyes (D) in the presence of $\mathbf{1}_n$ in water. While all of these dyes investigated herein (16 different dyes) changed their absorption spectra more or less in the presence of $\mathbf{1}_n$, three of them, such as those given in Scheme 2, which showed a fairly large decrease in the absorbance and a remarkable shift of the absorption maximum to longer wavelength, were selected;[4] also, the detailed studies were performed to determine the binding ratio and the apparent association constants in water. ^{1}H NMR measurements were also carried out in order to study the structure of the complexes. It was found that the decreased absorbance and the shift of the absorption maximum of the dyes, which are caused by complex formation with $\mathbf{1}_n$, are recovered by the addition of colorless alkylammonium, A_m where $A_m = (CH_3)_3N^+\text{–}(CH_2)_m\text{–}H$, chlorides, such as dodecyltri-



Scheme 2. Stilbene dyes, D, and protonation equilibrium and electronic resonance for St–4Me.

methylammonium chloride, $A_{12}\cdot Cl$. The increase in the absorbance originated from the release of dye in the complex due to a replacement with alkylammonium ion. The binding ratio and the association constants of colorless complexes of $\mathbf{1}_n$ with a variety of alkylammonium ions, the estimation of which has been very problematic, can be easily determined by the spectrophotometric method. The novel reaction system was applied to the analytical determination of the cationic surfactant concentration in water.

Experimental

Reagents. Water-soluble calix[*n*]arenes $\mathbf{1}_n$ were obtained from Sugai Kagaku Co. and used after recrystallization from a water–methanol mixture. After drying at 70 °C at reduced pressure, sulfur and sodium were analyzed by the inductively coupled plasma atomic-emission spectroscopy (ICP-AES) in a 0.10 M (1 M = 1 mol dm^{-3}) hydrochloric acid solution. Anal. Found for $\mathrm{Na_4}\mathbf{1}_4$: S, 12.7; Na, 9.18%. Calcd for $(C_7H_5SO_4Na)_4\cdot 10H_2O$: S, 12.7; Na, 9.08%. Anal. Found for $\mathrm{Na_6}\mathbf{1}_6$: S, 11.7; Na, 8.05%. Calcd for $(C_7H_5SO_4Na)_6\cdot 23H_2O$: S, 11.6; Na, 8.21%. Anal. Found for $\mathrm{Na_8}\mathbf{1}_8$: S, 11.9; Na, 8.10%. Calcd for $(C_7H_5SO_4Na)_8\cdot 30H_2O$: S, 11.6; Na, 8.34%.

The standard 0.01 M $\mathbf{1}_n$ solutions were prepared as follows. Each 2.53 g of sodium salt of $\mathbf{1}_4$, 4.16 g of $\mathbf{1}_6$, and 5.51 g of $\mathbf{1}_8$ was dissolved in water and diluted to 250 ml, respectively. Since the first acid dissociation constant, $pK_{a_1} = 3.26$ for $\mathbf{1}_4$ is considerably separated from the second constant, $pK_{a_2} = 11.6$,[5,6] the concentration of $\mathbf{1}_4$ was standardized by the titration of the standard 0.01 M NaOH solution in the presence of phenolphthalein as an indicator. The concentration of $\mathbf{1}_6$ was also standardized in a manner similar to $\mathbf{1}_4$ by the titration of its two hydroxy groups, which dissociate in a weakly acidic solution, because both the first and second acid dissociation constants, $pK_{a_1} = 3.28$ and $pK_{a_2} = 5.10$, are greatly separated from the third one, $pK_{a_3} = 11.5$.[7] The concentration of $\mathbf{1}_8$ was easily determined by the titration of its two hydroxy groups in the presence of a bromophenol blue indicator discriminating, because they dissociate in a weakly acidic solution ($pK_{a_1} = 3.69$, $pK_{a_2} = 4.34$) and are separated from the higher dissociation ($pK_{a_3} = 8.04$).[7] The resultant solutions of $\mathbf{1}_n$ were used for spectrophotometric studies after appropriate dilution.

Stilbene dyes were obtained from Ardrich Chem. Co., and used after recrystallization from methanol and drying in a P_2O_5 desiccator at room temperature. A part of them was dried at 200 °C for 2 d, and based on the weight loss their purities were calculated to be: St–4Me, 98.2%; St–2Me, 97.4%, and St–2Et, 98.2%.

Ammonium chloride, alkylammonium chlorides or bromides, and amino acids were purchased from Wako Pure Chemicals Co., and alkylammonium chlorides were used after recrystallization from acetone or benzene. Tetramethylammonium chloride and other alkylammonium chlorides or bromides were titrated with a standard sodium tetraphenylborate solution.[8]

Apparatus. Absorption spectra were measured with a Shimadzu double-beam spectrophotometer UV-180 in a 1 cm light-pass length quartz cell at 20 °C under the circulation of thermostated water. ^{1}H NMR spectroscopy was measured by a Nippon Bunko EX-90 NMR apparatus in D_2O using sodium 3-(trimethylsilyl)-propionate-d4 (TSP) as an external standard. The pH was measured with a Toa HM-30S pH meter.

Results and Discussion

Absorption Spectra of Dyes D in the Presence and the Absence of $\mathbf{1}_n$. Figure 1 shows the absorption spectra of the solutions containing D alone, D+25 times of $\mathbf{1}_n$ in a molar ratio, and D+25 times of sodium 4-phenolsulfonate, PS, in a molar ratio at pH 4.6. The solution of $\mathbf{1}_n$ and PS are colorless and do not absorb visible light. Each curve for D alone can superpose each corresponding curve for D+PS. These facts indicate that D does not interact with PS. In contrast, the addition of $\mathbf{1}_n$ decreases the absorbance of D and induces a shift of the absorption maxima to a longer wavelength ($n = 6$ and 8).

Shinkai et al.[9] found that the absorption maximum of PB shifts to a longer wavelength upon complexation with

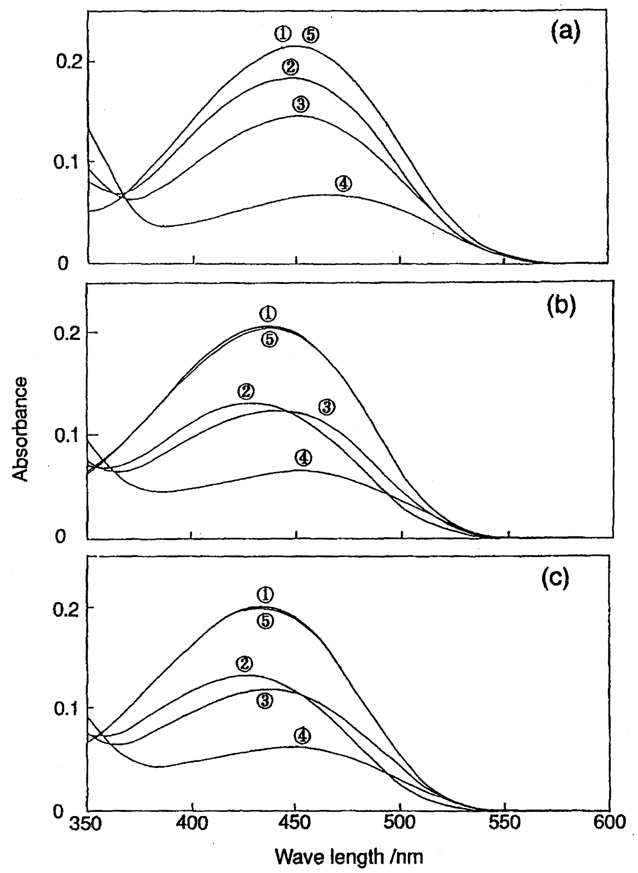


Fig. 1. Absorption spectra of dye solutions in the absence ①, and in the presence of $\mathbf{1}_n$ (②: [$\mathbf{1}_4$ = 1.96×10^{-4} M], ③: [$\mathbf{1}_6$ = 1.99×10^{-4} M], ④: [$\mathbf{1}_8$ = 2.04×10^{-4} M], and PS(⑤: [PS = 2.00×10^{-4} M]) at pH 4.6. (a)[St–4Me] = 7.87×10^{-6} M, (b)[St–2Me] = 7.90×10^{-6} M, (c)[St–2Et] = 8.00×10^{-6} M.

$\mathbf{1}_6$. The spectral changes observed in this study can also be attributed to the complex formation of D with $\mathbf{1}_n$, but not to the self-association of the dye; any spectral change induced by dye aggregation could not be observed in the concentration range from 1.0×10^{-7} M to 1.0×10^{-4} M.

Effect of the pH on the Absorbance of D in the Presence and Absence of $\mathbf{1}_n$. The changes in the absorbance of the dye solutions measured at each absorption maximum are given in Fig. 2 as a function of the pH in both the presence and absence of $\mathbf{1}_n$. The absorbance of a solution containing D alone increases along with an increase in the pH and becomes a constant value at pH > 5. These spectral changes indicate that the fraction of a colorless dicationic species (2P) which is formed by protonation of the amine nitrogen, decreases, whereas the fraction of a pyridinium monocatioic species (2Q) which can resonate to give a colored qunoid species (2R) increases with an increase in the pH.

The presence of $\mathbf{1}_n$ induces a shift of the curve for an absorbance–pH relationship to the higher pH region side (about 2 for $\mathbf{1}_4$, 3 for $\mathbf{1}_6$, and 4 for $\mathbf{1}_8$) and decreases the absorbance in the acidic solutions. The maximal decrease was observed at pH 4 to 5. These results suggest that the presence of $\mathbf{1}_n$ stabilizes the colorless dicationic species (2P) by forming the complex.

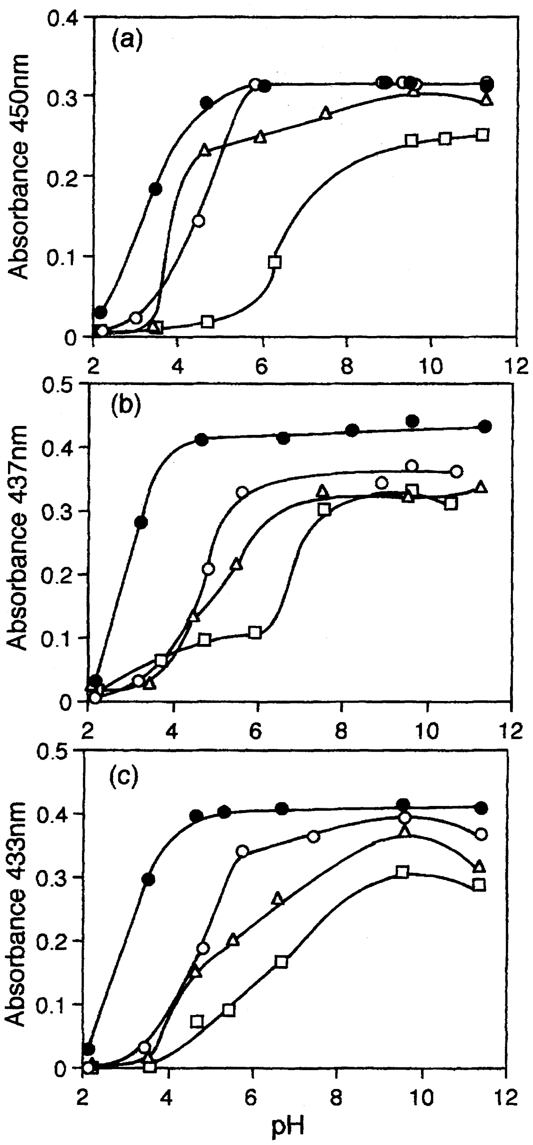


Fig. 2. Effect of pH on the absorbance of dye solutions in the absence (●) and in the presence of $\mathbf{1}_n$ (○: [$\mathbf{1}_4$] = 7.86×10^{-5} M, △: [$\mathbf{1}_6$] = 7.96×10^{-5} M, □: [$\mathbf{1}_8$] = 8.15×10^{-5} M) at pH 4.6. (a) [St–4Me] = 1.57×10^{-5} M, (b) [St–2Me] = 1.58×10^{-5} M, (c) [St–2Et] = 1.62×10^{-5} M.

Binding Ratio of the Complexes. The binding ratios of the complexes were measured by a continuous variation method. The absorbances (P_{M}) of a series of solutions containing D and $\mathbf{1}_n$ at a constant total concentration, d + c, but different concentration ratios, were measured. The observed values were subtracted from the absorbances (P_{D}) of the dye solutions at a corresponding concentration (d) in the absence of $\mathbf{1}_n$ to obtain the difference absorbance (ΔP),

$$\Delta P = P_{\mathrm{D}} - P_{\mathrm{M}}.$$

ΔP was plotted against the molar ratio, $d/(c+d)$. The maximum value for ΔP was clearly obtained at $d/(c+d)=0.5$ for each system, indicating the 1 : 1 binding ratio of D and $\mathbf{1}_n$ for a series of the complexes. Plots of the St–4Me systems

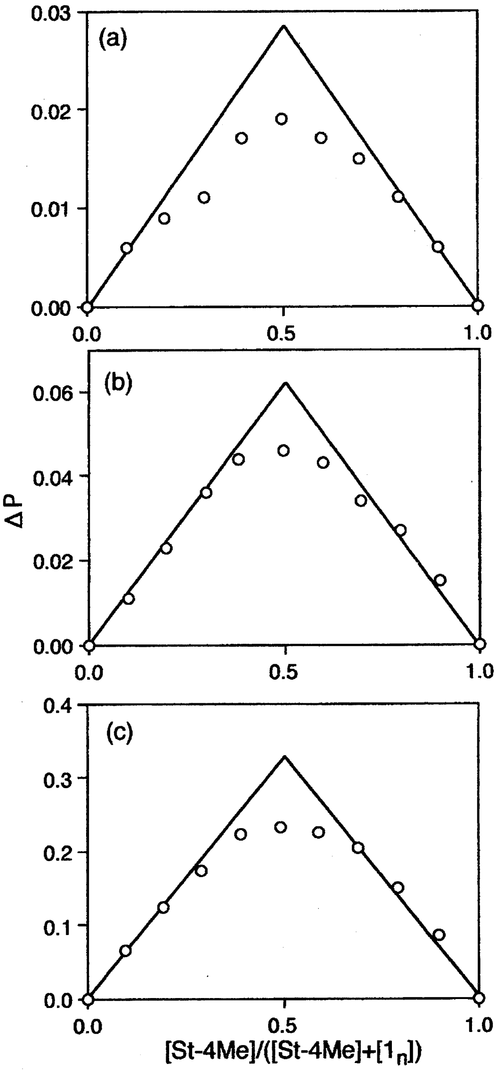


Fig. 3. Determination of binding ratio of $\mathbf{1}_n$-complexes with St–4Me at pH 4.6.
light-path length = 1 cm, wavelength = 450 nm, (a): $n = 4$, (b): $n = 6$, (c): $n = 8$, [St–4Me]+[$\mathbf{1}_n$] = 4.00×10^{-5} M.

are shown in Fig. 3.

Evaluation of Apparent Association Constants. The apparent association constant ($K_{1_n\mathrm{D}}$) for the 1 : 1 complex $\mathbf{1}_n$D, which is formed according to Eq. 1 from dye D and calixarene $\mathbf{1}_n$ is given by Eq. 2:

$$\mathbf{1}_n + \mathrm{D} = \mathbf{1}_n\mathrm{D}, \tag{1}$$

$$K_{1_n\mathrm{D}} = cd/c\cdot d. \tag{2}$$

Here, c, d, and cd are the equilibrium concentrations of $\mathbf{1}_n$, D, and $\mathbf{1}_n$D. Material balances for $\mathbf{1}_n$ and D are given by

$$c_0 = c + cd \tag{3}$$

and

$$d_0 = d + cd. \tag{4}$$

Here, c_0 and d_0 are the initial concentrations for $\mathbf{1}_n$ and D. The absorbance of the solution in a 1 cm light-pass length cell (P) can be given by

$$P = \varepsilon_{1_n}\cdot c + \varepsilon_{\mathrm{D}}\cdot d + \varepsilon_{1_n\mathrm{D}}\cdot cd, \tag{5}$$

where ε_{1n}, ε_{D}, and $\varepsilon_{1_n\mathrm{D}}$ are the molar absorptivities of $\mathbf{1}_n$, D, and $\mathbf{1}_n$D. From Eqs. 2, 3, 4, and 5, we obtain

$$c_0\cdot d_0/P^{\mathrm{E}} + P^{\mathrm{E}}/q^2 = 1/q\cdot K_{1_n\mathrm{D}} + (c_0 + d_0)/q, \tag{6}$$

where $P^{\mathrm{E}}=P-\varepsilon_{1_n}\cdot c_0-\varepsilon_{\mathrm{D}}\cdot d_0$ and $q=\varepsilon_{1_n\mathrm{D}}-\varepsilon_{1_n}-\varepsilon_{\mathrm{D}}$. A plot of ($c_0\cdot d_0/P^{\mathrm{E}}+P^{\mathrm{E}}/q^2$) against ($c_0$+$d_0$) according to Eq. 6 gives a straight line with a slope of $1/q$ and an intercept of $1/q\cdot K_{1_n\mathrm{D}}$. By dividing the slope ($1/q$) by the intercept ($1/q\cdot K_{1_n\mathrm{D}}$), the apparent association constant ($K_{1_n\mathrm{D}}$) can be determined.

The 1 : 1 complex $\mathbf{1}_n$D would be composed of a 1 : 1 acidic complex, $\mathbf{1}_n\mathbf{2P}$, and a 1 : 1 basic complex, $\mathbf{1}_n\mathbf{2Q}$, because two dye species, **2P** and **2Q**, which are a conjugate acid-base pair present in the mixture solution and both of them can be associated with $\mathbf{1}_n$. The following four equilibria Eqs. 7, 8, 9, and 10 can be written, and the corresponding equilibrium constants are defined in Eqs. 11, 12, 13, and 14:

$$\mathbf{2P} + \mathbf{1}_n = \mathbf{1}_n\mathbf{2P} \tag{7}$$

$$\mathbf{2Q} + \mathbf{1}_n = \mathbf{1}_n\mathbf{2Q} \tag{8}$$

$$\mathbf{2P} = \mathbf{2Q} + \mathrm{H}^+ \tag{9}$$

$$\mathbf{1}_n\mathbf{2P} = \mathbf{1}_n\mathbf{2Q} + \mathrm{H}^+ \tag{10}$$

$$K_{1_n\mathrm{2P}} = cp/p\cdot c \tag{11}$$

$$K_{1_n\mathrm{2Q}} = cq/q\cdot c \tag{12}$$

$$K_{\mathrm{a}} = q\cdot h/p \tag{13}$$

$$K_{\mathrm{a1}} = cq\cdot h/cp \tag{14}$$

Here, p, q, h, cp, and cq are the equilibrium concentrations of **2P**, **2Q**, proton, $\mathbf{1}_n\mathbf{2P}$, and $\mathbf{1}_n\mathbf{2Q}$, respectively. The material balances for free and complexed dyes are given by

$$d = p + q, \tag{15}$$

$$cd = cp + cq. \tag{16}$$

From these equations, $K_{1_n\mathrm{D}}$ can be expressed in the form of

$$K_{1_n\mathrm{D}} = (cp + cq)/c\cdot(p + q) = K_{1_n\mathrm{2P}}\cdot(K_{\mathrm{a1}} + h)/(K_{\mathrm{a}} + h). \tag{17}$$

Equation 17 indicates that $K_{1_n\mathrm{D}}$ is an apparent association constant which is dependent on the hydrogen-ion concentration.

Into a 25 ml measuring flask, 2 ml of a ca. 1×10^{-4} M dye solution (for St–4Me; d_0=9.84×10^{-5} M, St–2Me; d_0= 9.88×10^{-5} M, St–2Et; d_0=10.00×10^{-5} M) and 5 ml of a 1 M acetic acid–sodium acetate buffer solution (pH 4.60) were added. Then, a standard $\mathbf{1}_n$ solution was added to make reaction mixtures with different molar rations, c_0/d_0= 0—1000; and the resultant solution was diluted to the mark with water. After the mixture was kept in a water bath at 20 °C for 30 min, the absorbance (P) was measured at the absorption maximum for each dye (for St–4Me; 450 nm, St–2Me; 437 nm, St–2Et; 433 nm) in a 1 cm light-path length quartz cell.

As shown in Fig. 4, the P value decreases with an increase in the molar ratio (c_0/d_0) and becomes almost constant at $c_0/d_0 > 400$. From the P value at $c_0/d_0 = 1000$, $\varepsilon_{1_n\mathrm{D}}$ was calculated assuming $cd = d_0$ and $\varepsilon_{1_n} = 0$, since no absorption band due to $\mathbf{1}_n$ was observed at a wavelength longer than 350 nm. The ε_D value was obtained by separate experiments, i.e., from a plot of the absorbance and the concentration of D ($d_0 = 3.94\times10^{-6}$ M—4.00×10^{-5} M) at pH 4.6 in the absence of $\mathbf{1}_n$.

A typical plot for Eq. 6 is shown in Fig. 5 for the $\mathbf{1}_8$ and St–2Me system. Clearly, a good linear relationship is seen.

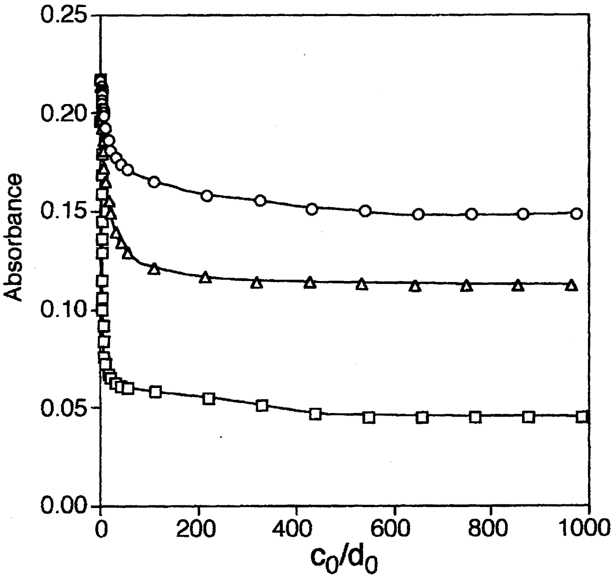


Fig. 4. Effect of molar ratio c_0/d_0 on the absorbance of St–4Me at pH 4.6.
$d_0 = 7.87\times10^{-6}$M, wavelength=450 nm. (○): [$\mathbf{1}_4$], (△): [$\mathbf{1}_6$], (□): [$\mathbf{1}_8$].

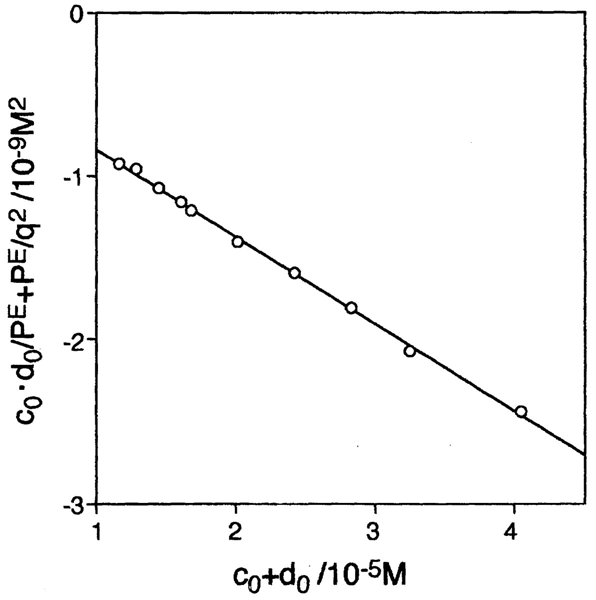


Fig. 5. Plot according to Eq. 6 for $\mathbf{1}_8$-complex with St–2Me at pH 4.6 wavelength = 437 nm, light-path length = 1 cm.

Eight other systems also gave a similar linear relationship. In Table 1, the apparent association constants ($K_{1_n\mathrm{D}}$) for thus-obtained 1 : 1 complexes are summarized. The $K_{18\mathrm{D}}$ values are larger by about ten times than the $K_{14\mathrm{D}}$ and $K_{16\mathrm{D}}$ values. This may be due to the large cavity size of $\mathbf{1}_8$ comparable to that of large dyes. Among these values, those for St–4Me complexes are the smallest. This may be attributed to the lessened cationic charge density of the 4-pyridinium nitrogen in St–4Me compared with that of the 2-pyridinium nitrogen in St–2Me and St–2Et because the former compound can enjoy a resonance structure (**2R**) more efficiently than the latter compounds. The fact that the $K_{1_n\mathrm{D}}$ values for the St–2Me and St–2Et complexes are not so different from each other indicates that the size difference between the methyl and ethyl groups scarcely affects the total stability of the complexes.

^{1}H NMR Studies in D$_2$O. Figure 6 shows ^{1}H NMR spectra of St–4Me in both the absence and presence of $\mathbf{1}_n$ in D_2O and 20 °C. The molar ratio of St–4Me to $\mathbf{1}_n$ was fixed at 0.5 in order for almost all of it to form a complex with $\mathbf{1}_n$. The singlet peak, A, at about 3.0 ppm and, B, at about 4.2 ppm for St–4Me in the absence of $\mathbf{1}_n$ will be assigned to the aminodimethyl and pyridiniummethyl protons, respectively. Both of them shift to a higher magnetic field in the presence of $\mathbf{1}_n$. Unfortunately, we cannot see peak B in the presence of $\mathbf{1}_4$. Probably, it ovelaps with a wider peak at 4.2 ppm due to the methylene protons in $\mathbf{1}_4$. The unknown singlet peak at a higher magnetic field in the spectrum for the $\mathbf{1}_4$–St–4Me system can be ascribed to the signal due to some impurity from $\mathbf{1}_4$, since it always appears in the spectra for $\mathbf{1}_4$.

The proton chemical shifts (δ) for methyl or ethyl groups attached to pyridinium nitrogen and for methyl groups attached to amino nitrogen of dyes determined in the presence and absence of $\mathbf{1}_n$ are summarized in Table 2 together with the difference between the proton chemical shift for D and that for $\mathbf{1}_n$ complexes ($\Delta\delta$). Table 2 indicates that the chemical shifts for the complex move to a higher magnetic field compared to those for free dye, as shown by Shinkai et al.[9] They concluded that the ring current of $\mathbf{1}_n$ induces a change in the chemical shift of the guest molecule to a higher magnetic field by binding to the center of the cavity of $\mathbf{1}_n$.

A gradual increase in the molar absorptivity was observed at a higher concentration than ca. 1.0×10^{-4} M for each dye. This suggests that the dye associates itself to a greater or lesser degree in the solutions for NMR measurements. Since ^{1}H NMR peaks for a self-associated dye can be expected to shift to a higher magnetic field,[10] the positive $\Delta\delta$ values, as

Table 1. Association Constants, $K_{1_n\mathrm{D}}$, for $\mathbf{1}_n$D Complexes at 20 °C

$\mathbf{1}_n$	$K_{1_n\mathrm{D}}\times10^{-4}/\mathrm{M}^{-1}$		
	St–4Me	St–2Me	St–2Et
$\mathbf{1}_4$	0.978	2.27	1.80
$\mathbf{1}_6$	1.25	2.00	1.81
$\mathbf{1}_8$	15.8	18.6	18.5

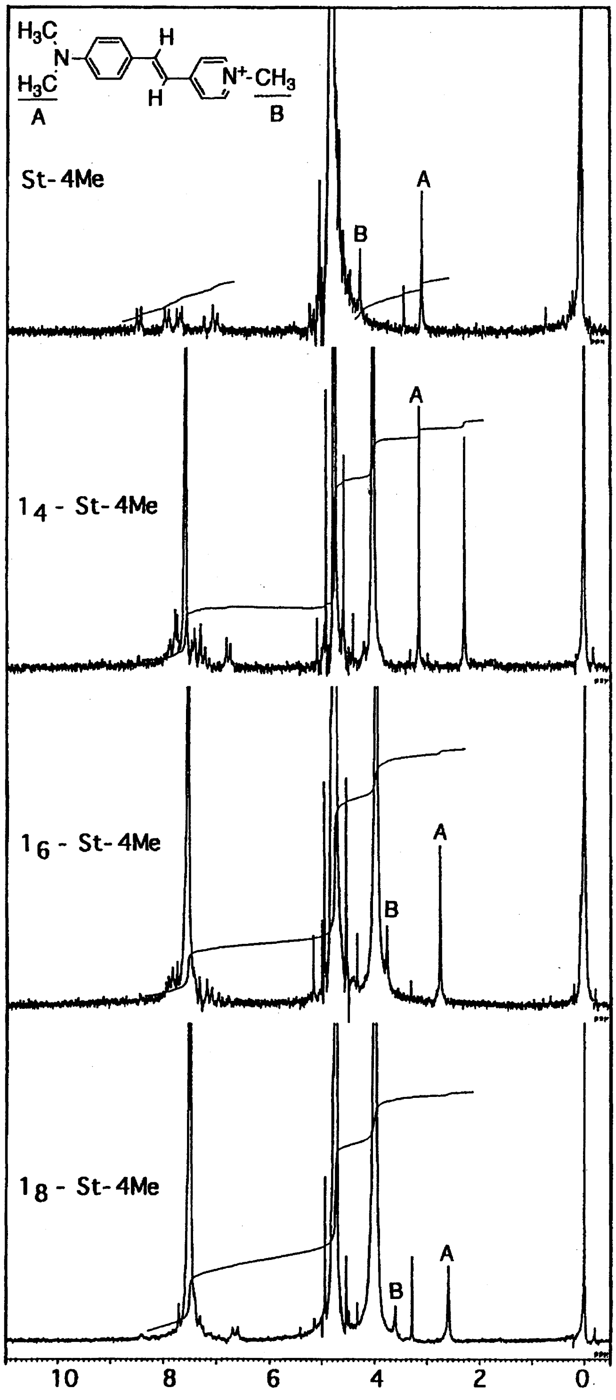


Fig. 6. ^{1}H NMR spectra of St–4Me in the absence and presence of $\mathbf{1}_n$ in D_2O and at pD=4.7.

shown in Table 2, conclusively indicate the formation of the dye–$\mathbf{1}_n$ complexes.

As shown in Table 2, the $\Delta\delta$ value increases in the order $\mathbf{1}_4 < \mathbf{1}_6 < \mathbf{1}_8$. This order may reflect the order of the association constant, and can be explained by the structural compatibility of the complex. Table 2 also indicates that the $\Delta\delta$ values for the amino protons of the $\mathbf{1}_4$-complexes are considerably smaller than those for the pyridinium protons. This suggests that the guest molecule D is mainly associated with the interaction of its pyridinium group with a cavity of $\mathbf{1}_4$. Since the size of D (ca. 16 Å in length) is larger than that of the cavity for $\mathbf{1}_4$ (6.4 Å at the upper sulfonato side, 2 Å at the lower hydroxy side), they can not be incorporated into the cavity of $\mathbf{1}_4$. The fact that the up-field shift is observed for the pyridinium protons, but not for the aminomethyl protons of the $\mathbf{1}_4$-complexes, indicates that $\mathbf{1}_4$ incorporates D mainly with the pyridinium group and stabilizes it as the colorless structure **2P**.

An examination of the CPK molecular model indicates that $\mathbf{1}_6$ has a cavity size of 11.7—5.6 Å at the upper sulfonato side, and 6—4.8 Å at the lower hydroxy side. Since the whole molecule of D connot enter into the $\mathbf{1}_6$ cavity, either the pyridinium group or the protonated aminomethyl group should be incorporated with a similar probability, and, as a result, the absorbance at visible region decreases because of the decrease in the contribution of colored structure **2R**. On the other hand, the $\Delta\delta$ values for $\mathbf{1}_8$-complexes are considerably larger than those for $\mathbf{1}_4$- and $\mathbf{1}_6$-complexes, and almost similar between the pyridinium and aminomethylprotons, unlike the corresponding $\mathbf{1}_4$-complexes. These facts indicate that two sites of D are included in the cavity of $\mathbf{1}_8$ at the same time. The likely structures are illustrated in Fig. 7.

Substitution Reaction of Dye Complex $\mathbf{1}_n$D with Various Ammonium Ions, A. Substitutions reaction of a series of complexes $\mathbf{1}_n$D with twelve different ammonium ions A, such as ammonium, tetramethylammonium, and alkylammonium (A_m) chlorides or bromides including several other alkylammonium ions and amino acids, has been studied by a spectrophotometric method using St–4Me complexes as a marker.

Into a 25 ml measuring flask containing each 5 ml of 2.50×10^{-4} M St–4Me, $\mathbf{1}_n$ ($\mathbf{1}_4=2.55\times10^{-4}$ M, $\mathbf{1}_6=2.52\times10^{-4}$ M, $\mathbf{1}_8=2.50\times10^{-4}$ M), and 1 M acetic acid–sodium acetate solutions (pH=4.6) were added then mixed. After 5 ml of various amounts of ammonium ion A were added and the mixture were diluted to the mark with water, and the absorbance of each resultant solution was measured. The spectral change of the $\mathbf{1}_8$–St–4Me complex induced by the addition of dodecylammonium chloride at pH 4.6 are shown in Fig. 8, and the changes in the absorbance at 450 nm for the St–4Me complex solutions caused by the addition of various ammonium ions are shown in Table 3.

The addition of cetyltrimethylammonium, cetylethyldimethylammonium, and stearyltrimethylammonium chloride and cetylpyridinium bromide to the solutions of $\mathbf{1}_6$–St–4Me and $\mathbf{1}_8$–St–4Me complexes increased the absorbance. However, no change was induced by the addition of ammonium chloride and amino acids. This suggests that amino acids having an anionic group and a hydrophilic ammonium ion cannot be accepted by $\mathbf{1}_n$. The addition of an alkylammonium ion to the solution of $\mathbf{1}_4$-complexes formed a white precipitate under the conditions recorded in Table 3. Although no precipitate was formed, based on experiments with the lower concentration of these ammonium ions, the clear so-

Table 2. ^{1}H NMR Chemical Shifts of Methyl Protons of Dye and $\mathbf{1}_n$–Dye Complex in D_2O

	N^+–CH_3		$N(CH_3)_2$	
$\mathbf{1}_n$–Dye Complex	δ/ppm	$\Delta\delta$/ppm	δ/ppm	$\Delta\delta$/ppm
St–4Me	4.168		2.997	
$\mathbf{1}_4$–St–4Me	3.9	0.3	2.966	0.031
$\mathbf{1}_6$–St–4Me	3.792	0.376	2.799	0.198
$\mathbf{1}_8$–St–4Me	3.690	0.478	2.399	0.598
St–2Me	4.125		3.000	
$\mathbf{1}_4$–St–2Me	3.742	0.387	2.918	0.082
$\mathbf{1}_6$–St–2Me	3.525	0.600	2.724	0.276
$\mathbf{1}_8$–St–2Me	3.562	0.567	2.437	0.563
St–2Et	1.556 (N^+–CH_2–CH_3)		3.035	
$\mathbf{1}_4$–St–2Et	1.209	0.357	2.906	0.109
$\mathbf{1}_6$–St–2Et	0.984	0.582	2.675	0.360
18–St–2Et	0.902	0.664	2.456	0.579

$\Delta\delta=\delta_D-\delta_{1_nD}$, $[D]=5.00\times10^{-3}$ M, $[\mathbf{1}_n]=0.010$ M, 26 °C, pD = 4.7 with 1 M CD_3COOD–CD_3COONa.

Table 3. Effect of the Presence of Ammonium, Alkylammonium, A_m[a] Compounds, and Amino Acids on the Absorbance of the Solution of $\mathbf{1}_n$–St–4Me Complexes[b]

Ammonium, A_m,		Absorbance at 450 mn		
amino acid	Conch × 10^4 M	$\mathbf{1}_4$	$\mathbf{1}_6$	$\mathbf{1}_8$
None		1.300	1.138	0.660
NH_4Cl	2.00	1.300	1.138	0.660
$CH_3NHCOOH$				
NH_2CH_2COOH				
$NH_2(CH_2)_2COOH$				
$NH_2(CH_2)_3COOH$				
A_1·Cl	2.18	1.364	1.260	0.742
A_8·Br	1.93	1.345	1.264	0.695
A_{12}·Cl	2.02	1.345	1.262	0.995
A_{16}·Cl	1.91	e)	1.300	0.940
c)	2.06	e)	1.310	0.953
A_{18}·Cl	1.86	e)	1.280	0.853
d)	2.10	e)	1.270	0.996

a) $A_m=(CH_3)_3N^+$–$(CH_2)_m$–H. b) pH=4.6, light-pass length=1 cm, reference=water, [St–4Me]=5.00×10^{-5} M, $[\mathbf{1}_4]=5.10\times10^{-5}$ M, $[\mathbf{1}_6]=5.04\times10^{-5}$ M, $[\mathbf{1}_8]=5.00\times10^{-5}$ M. c) $C_{16}H_{33}N(CH_3)_2C_2H_5Br$. d) $C_5H_5NC_{16}H_{33}Br$. e) Not measured due to the formation of precipitates.

lution simply increased the absorbance by the addition of ammonium ions.

Such an increase in the absorbance can be ascribed to the displacement of a dye complex $\mathbf{1}_n$D with an ammonium ion (A) to give a free dye molecule (D) and an ammonium complex ($\mathbf{1}_n$A),

$$\mathbf{1}_n\mathrm{D}+\mathrm{A}\rightarrow\mathbf{1}_n\mathrm{A}+\mathrm{D}. \tag{18}$$

The association constant for the ammonium complex ($\mathbf{1}_n$A) is defined as

$$K_{1_n\mathrm{A}}=ca/c{\cdot}a, \tag{19}$$

where a and ca are the concentrations of the ammonium ion (A) and its complex ($\mathbf{1}_n$A). The mass balance among $\mathbf{1}_n$, D, and A are given as follows:

$$c_0=c+cd+ca, \tag{20}$$

$$d_0=d+cd, \tag{21}$$

$$a_0=a+ca, \tag{22}$$

where a_0 is the total concentration of A. Since $\varepsilon_{1_n}=0$, $\varepsilon_\mathrm{A}=0$, and $\varepsilon_{1_n\mathrm{A}}=0$, the absorbance ($P$) measured in a 1 cm light-pass length cell is given by

$$P=\varepsilon_\mathrm{D}{\cdot}d+\varepsilon_{1_n\mathrm{D}}{\cdot}cd, \tag{23}$$

By defining Q

$$Q=(P-\varepsilon_{1_n}{\cdot}d_0)/(\varepsilon_\mathrm{D}{\cdot}d_0-P), \tag{24}$$

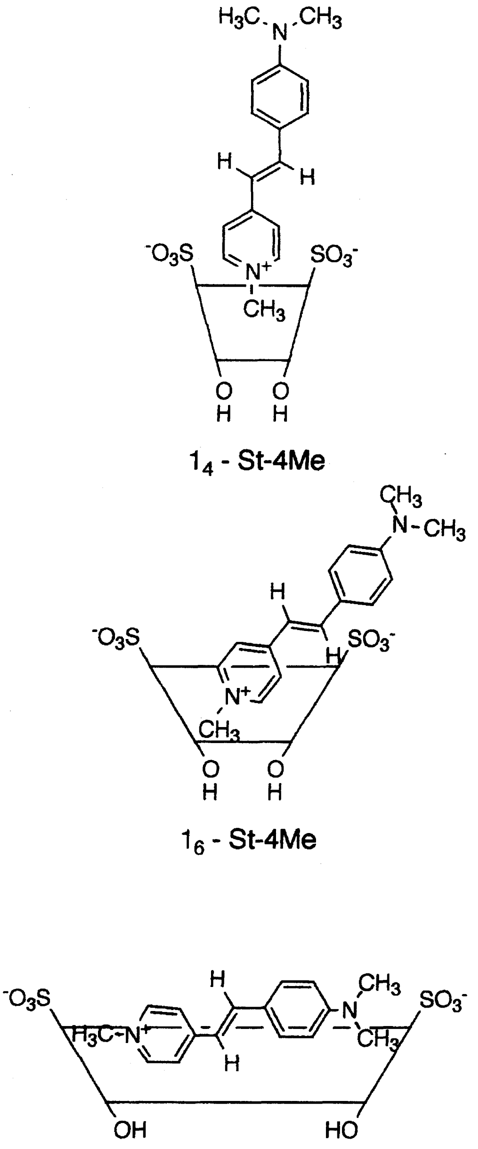
the concentration (ca) can be obtained from

$$ca=c_0-1/(Q{\cdot}K_{1_n\mathrm{D}})-d_0/(Q+1). \tag{25}$$

From Eqs. 24 and 25,

$$a_0/ca=(K_{1_n\mathrm{D}}/K_{1_n\mathrm{A}}){\cdot}Q+1 \tag{26}$$

is obtained. Equation 26 provides a graphical approach to determing $K_{1_n\mathrm{A}}$ by plotting a_0/ca against Q, where ca is obtained using Eq. 25. Figure 9 shows a plot of Eq. 26 in the competitive spectrophotometric determination of the



1_4 - St-4Me

1_6 - St-4Me

1_8 - St-4Me

Fig. 7. Estimated structures for 1_4-, 1_6-, and 1_8-complexes with St–4Me.

binding between dodecyltrimethylammonium chloride and 1_8–St–4Me complex. Similar good linear relationship between Q and a_0/ca with an intercept at 1 were obtained with other ammonium ion and St–4Me complexes. This indicates that the substitution reaction of ammonium ions with 1_8–St–4Me and 1_6–St–4Me complexes takes place with a 1 : 1 ratio to form complexes 1_nA ($n = 6$ and 8) and free St–4Me molecule. The association constants for various ammonium complexes ($K_{1_n\mathrm{A}}$) were evaluated from the slope of the line and the $K_{1_n\mathrm{D}}$ value. The results are summarized in Table 4.

Table 4 indicates that the $K_{1_n\mathrm{A}}$ values are smaller than those for $K_{1_n\mathrm{D}}$, and that 1_8 forms the most stable complex with the C12-trimethylammonium ion (A_{12}). Table 4 also shows that 1_6 forms the most stable complex with A_8, and that the best size of the alkyl group becomes smaller along with a decrease in the cavity size of 1_n.

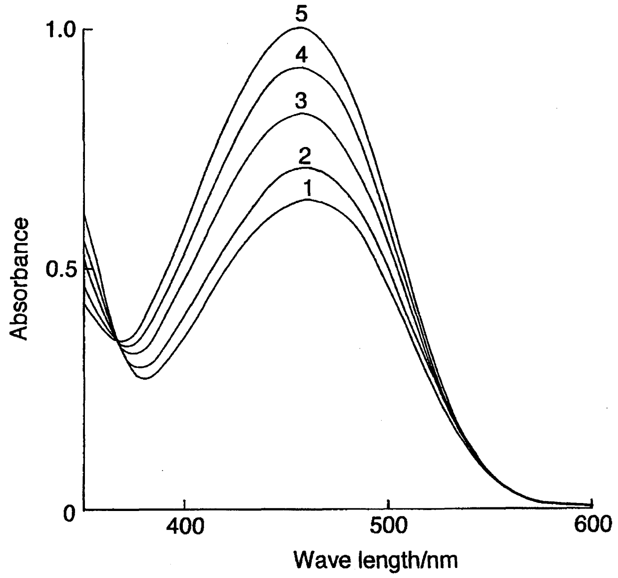


Fig. 8. Changes of absorption spectra of 1_8–St–4Me complex by the addition of dodecyltrimethylammonium chloride at pH 4.6.
[dodecyltrimethylammonium chloride] = 0 : 1, 2.02×10^{-5} M: 2, 6.06×10^{-5} M: 3, 1.21×10^{-4} M: 4, 2.02×10^{-4} M: 5, [1_8] = 5.00×10^{-6} M, [St–4Me] = 5.00×10^{-6} M, light-path length = 1 cm.

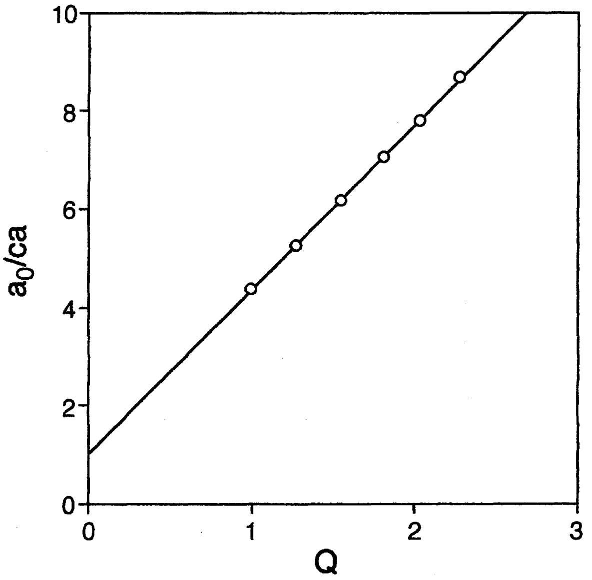


Fig. 9. Plot according to Eq. 26 for 1_8–St–4Me complex and dodecyltrimethylammonium chloride.
wavelength = 450 nm, light-path length = 1 cm.

^{1}H NMR of Alkylammonium Complexes in D_2O. The ^{1}H NMR spectra of alkylammonium ions (A_m ($m = 6$, 12, 16)) were measured in D_2O in both the absence and presence of 1_n ($n = 6$, 8) at pD = 4.6 ($CD_3COOD-CD_3COONa$). The molar ratio of 1_n to A_m was kept to 2.0 in order that more than 96% of A_m would form the complex (1_nA_m).

In Table 5, the proton chemical shifts, δ for A_m and 1_nA_m are summarized together with the difference between the proton chemical shifts for A_m and 1_nA_m, $\Delta\delta$. All of the

Table 4. Association Constants, $K_{1_n\mathrm{A}_m}$[a] and Those with Other Alkylammonium Ions[b,c] at 20 °C.

	$K_{1_n\mathrm{A}_m}/10^3\ \mathrm{M}^{-1}$	
m	$\mathbf{1}_6$	$\mathbf{1}_8$
1•Cl	5.14	1.18
6•Br	7.91	1.85
8•Br	9.77	5.15
10•Cl	6.94	23.1
12•Cl	5.87	45.8
14•Cl	5.34	39.3
16•Cl	d)	35.5
18•Cl	d)	18.4
b)	d)	30.1
c)	d)	39.7

a) A_m = $(\mathrm{CH_3})_3\mathrm{N^+}$–$(\mathrm{CH_2})_m$–H. b) $(\mathrm{CH_3})_2(\mathrm{C_2H_5})\mathrm{N^+}$–$\mathrm{C_{16}H_{33}}$-$\mathrm{Br^-}$. c) $\mathrm{C_5H_5N^+}$–$\mathrm{C_{16}H_{33}Br^-}$. d) Not determined due to the formation of precipitates.

chemical shifts for the complexed A_m move to a higher magnetic field compared to those for free A_m. This indicates that all of the protons of complexed A_m are influenced by the ring current of $\mathbf{1}_n$, and that, judging from the $\Delta\delta$ value, the greatest influence is observed for the $\mathrm{N^+}$–$(\mathrm{CH_3})_3$ group on each $\mathbf{1}_n\mathrm{A}_m$. It can therefore be concluded that A_m complexes $\mathbf{1}_n$ mainly with its $\mathrm{N^+}$–$(\mathrm{CH_3})_3$ group by a cation–π interaction[3,11] in the cavity, taking an appropriate position to produce the most stable complex.

In the $\mathbf{1}_6\mathrm{A}_m$ complexes, both $\Delta\delta$ values for –$\mathrm{CH_2}$– and terminal –$\mathrm{CH_3}$ protons are considerably smaller than those for $\mathrm{N^+}$–$(\mathrm{CH_3})_3$ and $\mathrm{N^+}$–$\mathrm{CH_2}$– protons of A_m, especially in the complexes $\mathbf{1}_6\mathrm{A}_{12}$ and $\mathbf{1}_6\mathrm{A}_{16}$. These facts indicate that both alkyl groups of A_{12} and A_{16} can barely enter into the cavity of $\mathbf{1}_6$, and that they complex $\mathbf{1}_6$ by their cationic nitrogen in a cavity comprising benzene rings. Thus, the value for association constant of $\mathbf{1}_6$ complexes results in the order $\mathbf{1}_6\mathrm{A}_6 > \mathbf{1}_6\mathrm{A}_{12}$. Unfortunately, the association constant for $\mathbf{1}_6\mathrm{A}_{16}$ could not be obtained due to its poor solubility.

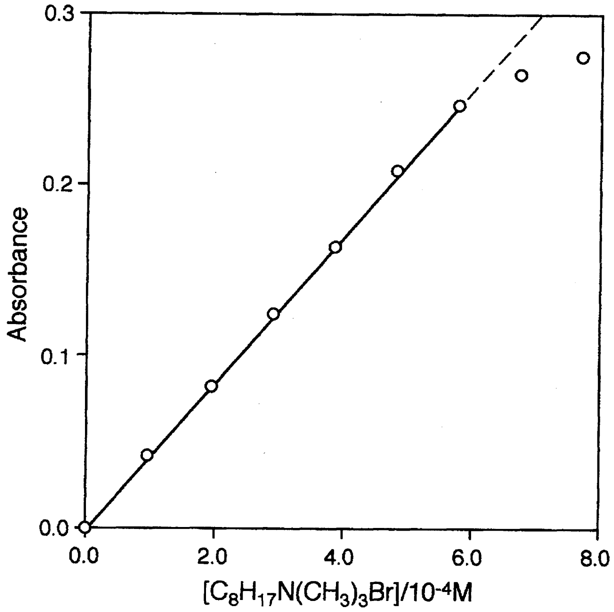


Fig. 10. Calibration line for octyltrimethylammonium bromide by the $\mathbf{1}_6$–St–4Me complex at pH 4.6. $[\mathbf{1}_6]=3.02\times10^{-4}$ M, [St–4Me]$=5.00\times10^{-5}$ M, wavelength =450 nm, light-path length=1 cm.

The smaller difference of the $\Delta\delta$ values for $\mathbf{1}_8\mathrm{A}_6$ than those for $\mathbf{1}_6\mathrm{A}_6$ (shown in Table 5) seem to indicate that A_6 having 11.8 Å in molecular size can be completely incorporated in the cavity of $\mathbf{1}_8$ (ca. 16 Å), and that all of the protons of A_6 are similarly influenced by the ring current of $\mathbf{1}_8$. While the $\Delta\delta$ value (0.557 ppm) for $\mathrm{N^+}$–$(\mathrm{CH_3})_3$ protons of $\mathbf{1}_8\mathrm{A}_{12}$ which has the greatest association constant among the complexes investigated is smaller than that for $\mathbf{1}_6\mathrm{A}_{12}$ (0.845 ppm), those for alkyl groups are in reverse order. This suggests that the $\mathrm{N^+}$–$(\mathrm{CH_3})_3$ groups moves from the appropriate position to a different place in the cavity to form a more stable complex as a whole by interacting with $\mathbf{1}_8$ by its longer alkyl group. This means that the hydro-

Table 5. ^{1}H NMR Chemical Shift, δ and the Difference of Chemical Shift, $\Delta\delta$,[a] for Methyl and Methylene Protons of A_m and $\mathbf{1}_n\mathrm{A}_m$[b]

	$\mathrm{N^+}$–$\mathrm{CH_3}$		$\mathrm{N^+}$–$\mathrm{CH_2}$		–$\mathrm{CH_2}$–		–$\mathrm{CH_3}$	
	δ/ppm	$\Delta\delta$/ppm	δ/ppm	$\Delta\delta$/ppm	δ/ppm	$\Delta\delta$/ppm	δ/ppm	$\Delta\delta$/ppm
A_6[c]	3.076		3.190		1.330		0.853	
$\mathbf{1}_6\mathrm{A}_6$[c]	1.919	1.157	2.417	0.773	1.058	0.272	0.732	0.121
$\mathbf{1}_8\mathrm{A}_6$[c]	2.232	0.844	2.370	0.820	0.508	0.822	0.340	0.513
A_{12}[c]	3.077		3.190		1.265		0.839	
$\mathbf{1}_6\mathrm{A}_{12}$[c]	2.232	0.845	2.604	0.586	1.175	0.090	0.737	0.102
$\mathbf{1}_8\mathrm{A}_{12}$[c]	2.520	0.557	e)		1.045 0.840	0.220 0.425	0.463	0.376
A_{16}[d]	3.019		3.210		1.288		0.875	
$\mathbf{1}_6\mathrm{A}_{16}$[d]	2.804	0.215	e)		1.220	0.068	0.807	0.068
$\mathbf{1}_8\mathrm{A}_{16}$[d]	2.753	0.266	e)		1.185	0.103	0.770	0.105

a) $\Delta\delta=\delta_{\mathrm{A}_m}-\delta_{1_n\mathrm{A}_m}$. b) 26 °C, pD=4.6 with $\mathrm{CD_3COOD}$–$\mathrm{CD_3COONa}$ buffer. c) $[\mathrm{A}_6]=[\mathrm{A}_{12}]=0.010$ M, $[\mathbf{1}_6]=[\mathbf{1}_8]=0.020$ M. d) $[\mathrm{A}_{16}]=0.0010$ M, $[\mathbf{1}_6]=[\mathbf{1}_8]=0.0020$ M. e) No independent peak was observed by overlapping with the peak for $\mathrm{N^+}$–$\mathrm{CH_3}$.

phobic interaction between $\mathbf{1}_n$ and the alkyl group of A_m is also important when the latter is incorporated in the cavity of the former. The size of the alkyl group of A_{16} (21.2 Å) seems too big to enter completely even into the cavity of $\mathbf{1}_8$, however, its association constant is still larger than those for $\mathbf{1}_6A_m$.

These results indicate that the association of $\mathbf{1}_n$ and A_m is under the control of both the cation–π and hydrophobic interactions of $\mathbf{1}_n$ and A_m when A_m complexes $\mathbf{1}_n$ in its cavity. Thus, we can understand why A_6 forms a less-stable complex with $\mathbf{1}_8$ than with $\mathbf{1}_6$, and why the tetramethylammonium ion A_1 forms complexes having the lowest association constant in each series of $\mathbf{1}_6A_m$ complexes. Furthermore, this understanding will resolve the reason why the dye in $\mathbf{1}_n$ complexes cannot be replaced by an ammonium ion.

Application of the Substitution Reaction to the Determination of Cationic Surfactants. The spectrophotometric determination of the cationic surfactant concentration using $\mathbf{1}_n$D ($n = 6$ and 8, D = St–4Me) complexes has been studied in water. Into 25 ml of a measuring flask, each 5 ml of 2.50×10^{-4} M of St–4Me, a $\mathbf{1}_n$ ($\mathbf{1}_6 = 1.51\times10^{-3}$ M, $\mathbf{1}_8 = 2.50\times10^{-4}$ M), and a 1 M acetic acid–sodium acetate solutions (pH = 4.6) was taken and mixed, and then 0—0.02 mmol of cationic surfactant in water was added and diluted to the mark with water. After 30 min, the absorbances was measured using the reagent blank as a reference.

A calibration line for octyltrimethylammonium bromide with $\mathbf{1}_6$D is shown in Fig. 10. A linear line was obtained up to 5.78×10^{-4} M. The molar absorptivity is 430 $M^{-1}\,cm^{-1}$ for the surfactant. Although the proposed method is not very high in sensitivity, it can be used to determine the cationic surfactant concentration in water without using any organic solvents, as in the well-known extraction-spectrophotometric determination.[12,13]

Although a fairly high sensitivity (about 2000 $M^{-1}\,cm^{-1}$ in molar absorptivity) could be obtained when the $\mathbf{1}_8$D complex was used to be displaced, the calibration line was not linear. A theoretical consideration concerning the relationship between the absorbance and the concentration of the surfactant indicated that the calibration line curves when the value for $K_{1_n\mathrm{D}}$ is considerably larger then that for $K_{1_n\mathrm{A}_m}$. It can thus be concluded that in order to determine an alkylammonium ion with a high sensitivity by the proposed method, we must select a dye having a high molar absorptivity under a free molecule, and which forms a colorless complex of a comparable association constant to the alkylammonium complex.

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