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ORIGINAL CONTRIBUTION

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# Distribution of fluorocarbon quencher among micelles via pyrene fluorescence probe method

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**Abstract** Fluorescence-quenching of pyrene in micellar system has been investigated using 1,1,2,2-tetrahydroheptadecafluorodecylpyridinium chloride (HFDePC). The new fluorocarbon quencher has a similar quenching ability as hexadecylpyridinium chloride (CPC) towards pyrene in hydrocarbon micelles if only a quencher molecule is solubilized in a micelle. The fluorocarbon quencher randomly distributed among micelles if the average occupancy number of probes per a micelle was small enough. The fluorescence behavior of pyrene was examined for hexadecyltrimethylammonium chloride (CTAC) and HFDePC mixtures. The variation of fluorescence intensity gave second cmc, reflecting the micellar immiscibility of fluorocarbon and hydrocarbon surfactants. The second cmc can be simulated by material balances of both surfactants supposing the coexistence of two kinds of mixed micelles. The fluorescence-quenching behavior suggested the enhanced micellar immiscibility probably due to nonrandom distribution of fluorocarbon quenchers among micelles.

**Key words** Cationic surfactant mixture – fluorocarbon quencher – micropolarity – micellar immiscibility – pyrene fluorescence quenching

## Introduction

There has been a great volume of work on fluorescence quenching of pyrene in micellar systems [1–4]. The hydrophobic fluorescence probes such as pyrene have a residence time inside a micelle much longer than its fluorescence lifetime. The mechanism of quenching process depends on the nature of quenchers. The fluorescence quenching using hexadecylpyridinium chloride (CPC) has been widely used as the so-called "immobile" quencher in a micelle [5]. The micellar aggregation number can be determined by fluorescence quenching assuming a Poissonian distribution of probes among micelles [6].

Recently, we have focused on a new quencher having a fluorocarbon chain (HFDePC) in mixed micelles [7]. The quenching behavior of pyrene reveals the less miscible nature between fluorocarbon and hydrocarbon species, i.e. pyrene was found to prefer hydrocarbon micelles, while the fluorocarbon quencher was partitioned in fluorocarbon micelles if two kinds of mixed micelles coexist in an aqueous solution. The depression of fluorescence quenching was closely related to the micellar immiscibility.

In this paper, we present the fluorescence-quenching behavior of pyrene in CTAC micelles by HFDePC in comparsion with CPC quencher. The effect of probe concentration on quenching ability was examined in order to get the information about distribution of probes among micelles and/or perturbations of micellar structure by probes. The intensity ratio of the first and third peaks of pyrene fluorescence was used for sensitive parameter characterizing the polarity of the probe's environment in micelles. The variation of fluorescence intensity for CTAC–HFDePC mixtures was measured and discussed in terms of solubilization behavior of pyrene and quenchers.

## Experimental

$[C_8F_{17}CH_2CH_2NC_5H_5]^+Cl^-$(HFDePC), $[C_{16}H_{33}NC_5H_5]^+Cl^-$(CPC) and $[C_{16}H_{33}N(CH_3)_3]^+Cl^-$(CTAC) were prepared by the same procedures reported previously [8]. The solubilization of pyrene in micelles was performed by sonicating for 10 min and shaking overnight at 25 °C. Then, the quencher (CPC or HFDePC) was accurately added to a part of pyrene-solubilized micellar solutions. Steady-state fluorescence spectra of pyrene were recorded with a Hitachi F-3010 spectrometer using a thermostated cell. The typical pyrene monomer fluorescence spectra were observed by excitation at 335 nm (excitation slit width 5 nm, emission slit width 1.5 nm). The spectra were used to determine the ratios ($I_1/I_3$) of the fluorescence intensities of the first ($I_1$, 373 nm) and third ($I_3$, 384 nm) vibronic peaks of monomeric pyrene. The fluorescence-quenching ratios ($I/I_0$) were calculated by using the fluorescence intensities at 384 nm in the absence of quencher ($I_0$) and in the presence of it ($I$). All experiments were performed at 25 °C.

## Results and discussion

HFDePC have a hydrophobic chain and a hydrophilic group, and thus they will associate to form micellar aggregates with increasing concentration. First, we examined the properties of micelles formed by HFDePC surfactant which act as a quencher. The $I_1/I_3$ ratio obtained from pyrene emission spectra has been shown to be very sensitive to the polarity of its environment [9, 10]. Under our experimental conditions, $I_1/I_3$ values in water, hexane and perfluorohexane were 1.87, 0.65 and 0.57, respectively, i.e., the decrease in $I_1/I_3$ value is an indication of the solubilization into a more hydrophobic environment of micelles. Thus, we could determine the cmc of HFDePC by monitoring the $I_1/I_3$ value of pyrene. The change in $I_1/I_3$ as a function of CPC and HFDePC surfactant concentrations are given in Fig. 1. Pyrene concentration was kept low enough in order to minimize the disturbance of micellar characteristics by the incorporation of pyrene. The slight but steady decreases in $I_1/I_3$ value were observed for both surfactants (quenchers). The plots of $I_1/I_3$ against surfactant concentrations gave their cmc's (1.0 mM for CPC, 2.7 mM for HFDePC) in good agreement with the conductivity cmc's (0.9 mM for CPC, 2.5 mM for HDePC). The difference in $I_1/I_3$ value between CPC and HFDePC would come from the affinity between pyrene and hydrophobic chains. The significant difference above cmc can be ascribed to the difference in solubilization amount of pyrene by micelles. From solubility and partitioning studies, pyrene was found to prefer a hydrocarbon micelle over a fluorocarbon micelle by a factor of 15 [11]. The $I_1/I_3$ value in hexadecyltrimethylammonium micelle was 1.35 [10]. The considerable drop in $I_1/I_3$ value for CPC (about 0.7) might be related to the solubilization site of pyrene, i.e., the pyrene solubilized in the palisade layer of micelles can be effectively quenched by the pyridinium head group of surfactant, while the pyrene solubilized in micelle core may have fewer chances of encounter with it. The unquenched fluorescence spectra would give the information of microenvironment of micelle core. The $I_1/I_3 = 0.7$ for CPC became similar to that of hexane.

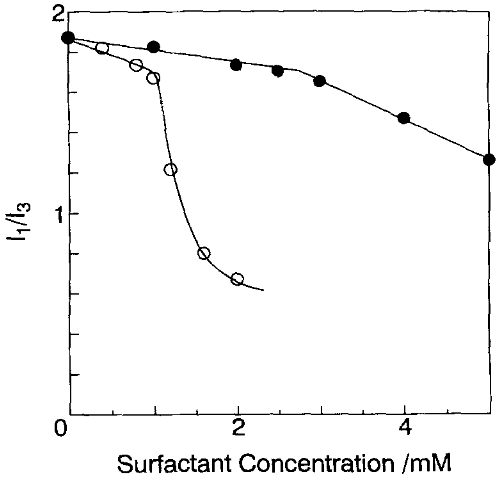

**Fig. 1** Variation of pyrene fluorescence intensity ratios $I_1/I_3$ as a function of surfactant concentration. Pyrene concentration was fixed at $1.0 \times 10^{-7}$ M. CPC (○), HFDePC (●)

The quenching of pyrene emission is due to the pyridinium group, and the hexadecyl group and $Cl^-$ are not known to quench pyrene emission [12]. In order to clarify the difference in quenching ability between CPC and HFDePC surfactants, the fluorescence-quenching ratios were shown as Stern–Volmer plots in Fig. 2. The plots gave the cmc's more precisely due to the sharp changes in $I_0/I$ values (cmc 0.9 mM for CPC, 2.5 mM for HFDePC). The effective quenching comes from dramatically increasing concentration of probes by micelle formation from the microscopic viewpoint. The difference in $I_0/I$ value between CPC and HFDePC would be due to the affinity between pyrene and hydrophobic chains. This might suggest the association of pyrene and CPC prior to the micelle

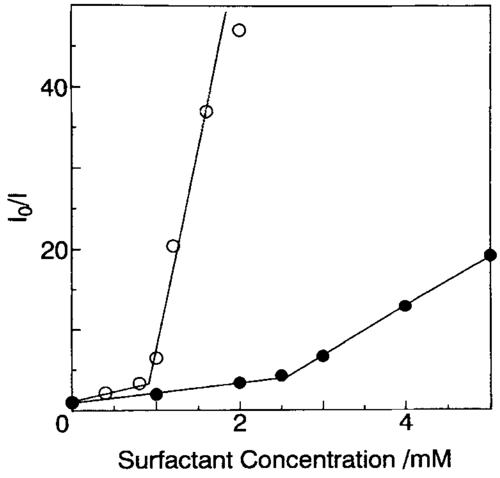

**Fig. 2** Fluorescence-quenching of pyrene in aqueous surfactant solutions. Pyrene concentration was fixed at $1.0 \times 10^{-7}$ M. CPC (○), HFDePC (●)

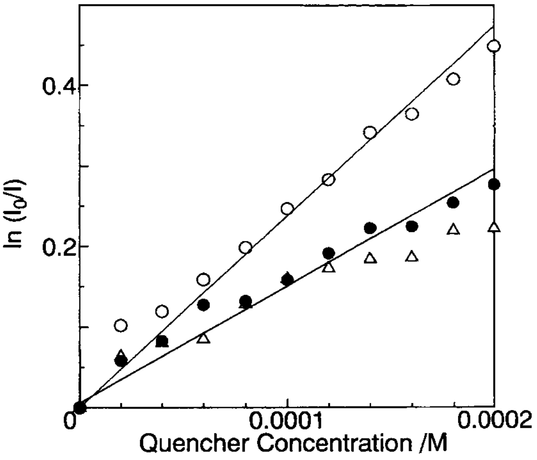

**Fig. 3** Plot of $\ln(I_0/I)$ vs. quencher concentration in 30 mM CTAC aqueous solution. Pyrene concentration was fixed at $2.0 \times 10^{-5}$ M. CPC (○), DPC (△), HFDePC (●)

formation. The significant difference above cmc can be ascribed to the difference in solubilization amount of pyrene by micelles.

Next, we used HFDePC surfactant as a quencher toward pyrene emission. The pyrene fluorescence quenching using CPC was presented for the determination of micellar aggregation numbers [6]. The residence time of probes in micelles is much longer than the pyrene fluorescence lifetime. Assuming the quencher has a Poissonian distribution among micelles, the measured $I_0/I$ is given by the equation,

$$\ln(I_0/I) = N_{\mathrm{agg}}[Q]/(C - \mathrm{cmc}) ,$$

where $N_{\mathrm{agg}}$, $[Q]$ and $(C - \mathrm{cmc})$ are micellar aggregation number, quencher and micelle concentrations, respectively. The plots of $\ln(I_0/I)$ vs $[Q]$ for CPC gave a linear dependence and the aggregation number of CTAC micelles as shown in Fig. 3. The obtained aggregation number of CTAC was 70 which would be reasonable value in the case of CPC. The apparent aggregation number led to about 40 using DPC and HFDePC. The apparent aggregation number using DPC was smaller than that using CPC, because the quenchers will distribute among micelles and bulk water phase due to high cmc value. The small apparent aggregation number using HFDePC might come from the less miscible nature between fluorocarbon and hydrocarbon chains. The distribution of pyrene and quencher may be complex, and the micelles solubilizing pyrene may have a less solubilization ability toward fluorocarbon quenchers.

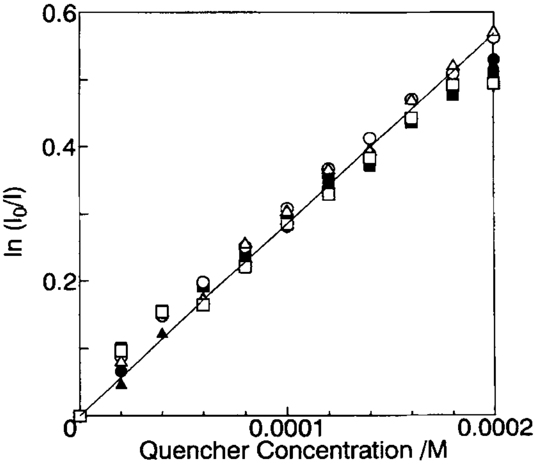

**Fig. 4** Plot of $\ln(I_0/I)$ vs. quencher concentration in 30 mM CTAC 1.0 M NaCl at various concentrations of pyrene. CPC (○), HFDePC (●) in $2.0 \times 10^{-5}$ M pyrene-solubilized micelles, CPC (△), HFDePC (▲) in $2.0 \times 10^{-6}$ M pyrene-solubilized micelles, CPC (□), HFDePC (■) in $2.0 \times 10^{-7}$ M pyrene-solubilized micelles

An addition of salt is well known to suppress the concentration of monomeric surfactant in equilibrium with its micelles. The quencher can be almost completely partitioned in micelles with sufficient addition of salt. Figure 4 shows the effect of quencher and pyrene concentrations toward quenching in 30 mM CTAC 1.0 M NaCl. The plots of $\ln(I_0/I)$ vs. $[Q]$ gave linear dependences with various concentrations of pyrene. There are no differences among the kinds of quencher within experimental error. CTAC micelles would effectively solubilize not only dodecylpyridinium chloride (DPC) but also HFDePC with sufficient addition of salt. The average occupancy numbers of pyrene and quencher gave no differences in quenching behavior under such a low average occupancy number (about 0.06 for $2.0 \times 10^{-5}$ M pyrene, about 0.6 for

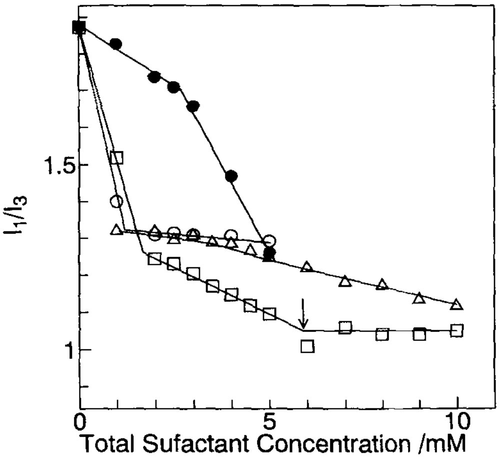

**Fig. 5** Variation of pyrene fluorescence intensity ratios $I_1/I_3$ in CTAC-HFDePC mixtures as a function of total surfactant concentration. Pyrene concentration was fixed at $1.0 \times 10^{-7}$ M. CTAC (○), $\alpha = 0.2$ (△), $\alpha = 0.5$ (□), HFDePC (●), $\alpha$ is mole fraction of HFDePC for binary system

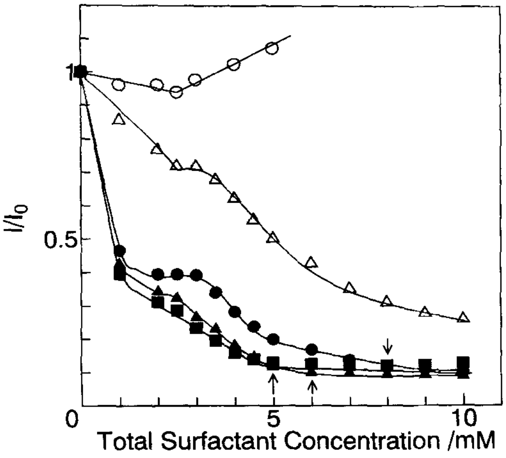

**Fig. 6** Fluorescence quenching ratios in CTAC-HFDePC mixtures as a function of total surfactant concentration. Pyrene concentration was fixed at $1.0 \times 10^{-7}$ M: CTAC (○), $\alpha = 0.2$ (△), $\alpha = 0.3$ (●), $\alpha = 0.4$ (▲), $\alpha = 0.5$ (■)

$2.0 \times 10^{-4}$ M quencher). Therefore, the effect of nonrandom distribution of probes on micelles can be neglected under these experimental conditions. The quenching ability of HFDePC was found to be equivalent to that of CPC in hydrocarbon micelles. The difference in the diffusion rate of both quenchers could be neglected in such an intramicellar quenching considering that its mechanism was known to be a diffusion-controlled encounter between pyrene and quenchers. Therefore, there is no significant difference between hydrocarbon (CPC) and fluorocarbon (HFDePC) quenchers if only a quencher molecule is solubilized in a micelle.

Next, we focused on the fluorescence behavior in the mixed micelles composed of CTAC and HFDePC. Figure 5 shows the plots of ratio $I_1/I_3$ against total surfactant concentrations in CTAC and HFDePC mixtures. The $I_1/I_3$ value for CTAC abruptly decreased with micelle formation and remained almost constant above cmc indicating similar values (about 1.3) with ref. [10]. The $I_1/I_3$ value for mixtures gradually decreased with total surfactant concentration above the cmc of the mixture. For equimolar mixture ($\alpha = 0.5$), the $I_1/I_3$ value reached a constant far above the cmc of the mixture. The $I_1/I_3$ values for the mixed system were considerably low compared to CTAC pure micelles. The pyrene emission was effectively quenched by HFDePC surfactant in these mixed systems. Therefore, the unquenched fluorescence spectra would give the micropolarity in rather micellar core as mentioned for CPC in Fig. 1. The constant value ($I_1/I_3 = 1.05$) between 6 and 10 mM for $\alpha = 0.5$ might suggest that CTAC micelles were saturated with HFDePC indicating a constant micropolarity. Therefore, the inflection point (indicated as arrow in Fig. 5) can be assigned to the second cmc at which two kinds of mixed micelles begin to coexist [13–20].

Figure 6 shows the quenching behavior in CTAC and HFDePC mixtures. The $I/I_0$ values for CTAC pure system were slightly changed along with micelle formation, reflecting the change in environment around pyrene molecule. The $I/I_0$ values for CTAC–HFDePC mixed systems significantly decreased with increasing total surfactant concentrations. The inflection points of $I/I_0$ curves corresponded to the mixture's cmc's. The $I/I_0$ values gradually decreased with increasing concentration above the cmc of the mixture and then became almost constant far above the cmc of the mixture. The residual fluorescence intensity might come from the pyrene-solubilized CTAC micelles with no occupancy of HFDePC molecule, i.e., the constancy in $I/I_0$ value could be ascribed to the micellar demixing due to the less miscibility between fluorocarbon and hydrocarbon chains. The inflection points far above the cmc of the mixture can be assigned to the second cmc. The second cmc decreased with increasing mole fraction of HFDePC.

When we reconsider the results of equimolar ($\alpha = 0.5$) CTAC–HFDePC systems in Fig. 6, the fluorescence quenching experimental conditions can be considered as follows. Pyrene concentration was kept at $10^{-7}$ M without disturbance of micellar characteristics. Pyrene preferred to be solubilized in hydrocarbon micelles. If we assume the ideal mixing for CTAC–HFDePC system, the average occupancy number of quencher (HFDePC) per micelle

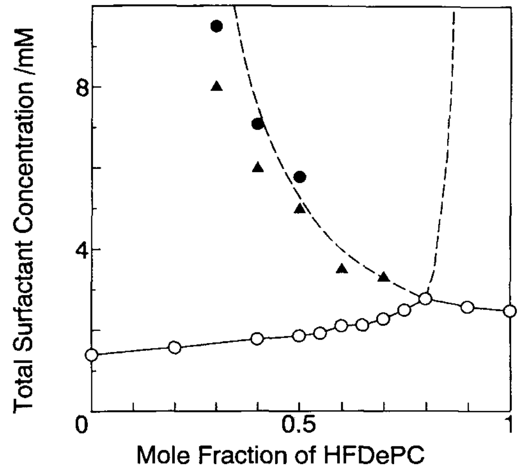

**Fig. 7** Micellar pseudophase diagram of CTAC-HFDePC mixture. The dashed lines are calculated second cmc curves by material balances with $X_H = 0.17$, $X_F = 0.89$, $X_{AZ} = 0.80$ and $C_{AZ} = 2.75$ mM. $X_H$, $X_F$, and $X_{AZ}$ are the compositions of HFDePC in hydrocarbon-rich and fluorocarbon-rich micelles, and azeotropic composition, respectively. $C_{AZ}$ is monomer concentration at azeotropic composition: (○) mixture cmc's by conductivity method; (●) second cmc's by micropolarity parameter $I_1/I_3$; (▲) second cmc's by quenching ratio $I/I_0$

would became to about 30 at 10 mM total surfactant concentration using the cmc of the mixture (1.86 mM) at $\alpha = 0.5$ and $N_{agg} = 70$. Such a large occupancy of quencher in micelles will lead to an effective quenching of pyrene emission by HFDePC having similar quenching ability of CPC. Infelta reported that the relation between $I/I_0$ value and average number of quencher per micelle [21]. The value of $I/I_0 = 0.11$ corresponded only to about 2 or 3 quenchers per micelle with fast quenching, i.e., the constant $I/I_0$ values at high surfactant concentration suggest that micelle composition would become constant due to the micellar demixing.

Based on pyrene fluorescence data, the micellar pseudophase diagram of CTAC–HFDePC system is shown in Fig. 7. The CTAC-rich micelles first appear at the cmc's of the mixture up to $\alpha = 0.8$ due to the low cmc of CTAC. As the total surfactant concentration increases, the monomeric HFDePC increases until the second cmc, at which the HFDePC-rich micelles appear due to the immiscibility between fluorocarbon and hydrocarbon chains. The observed second cmc's were simulated by the material balance of both surfactants with the coexistence of two kinds of mixed micelles. The micellar demixing region with HFDePC mole fraction of 0.17 and 0.89 was predicted by the group contribution method [8], i.e., CTAC-rich micelles contain about 12 HFDePC molecule per micelle. Such a large occupancy of quencher must lead to sufficient fluorescence quenching considering fluorocarbon quencher has a similar quenching ability with CPC. This might come from preferred solubilization of fluorocarbon quencher toward pyrene-nonsolubilized micelles due to the less miscible nature between pyrene and fluorocarbon chain. Such a nonrandom effect must be solved in the future. Nevertheless, the fluorescence quenching behavior suggests that fluorocarbon and hydrocarbon micelles have significant limited mutual solubilities in microscopic viewpoints.

## Conclusions

We presented the micro information of nonideal mixed micelles composed of fluorocarbon and hydrocarbon surfactants. The pyrene fluorescence quenching method provided useful information of changes in microenvironment which obtained by other simple methods. The micellar pseudophase separation region was in good agreement with the predicted one by the group contribution method. The fluorescence quenching behavior suggested that the enhanced micellar immiscibility as probably due to nonrandom distribution of fluorocarbon quenchers among micelles. However, there is no significant difference between fluorocarbon and hydrocarbon quenchers if the average occupancy number of probes per micelle was low enough. The fluorescence probe method using a new fluorocarbon quencher will be useful to demonstrate the nonideal behavior of mixtures with regard to the microscopic aspects.

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