# Fluorescence Studies on Morphological Change of Oil-in-Water Microemulsions upon **Dilution with Water**

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The morphological change of oil-in-water microemulsions (ME) has been studied by steady-state and time-resolved fluorimetry. The standard ME (ME1) consisted of sodium hexadecyl sulfate (7.2% wt/wt), pentanol (10.8%), hexadecane (2.7%), and water (79.3%). The fluorescence quenching of pyrene by N,N-dihexylaniline was used as a probe reaction. Both nonlinear Stern-Volmer relationship and nonexponential fluorescence decay curves of the pyrene monomer in ME containing the quencher were analyzed by Poisson statistics, which revealed that ME is subdivided upon dilution with water.

#### Introduction

Oil-in-water microemulsions (ME) are transparent dispersions which are regarded as swollen micelles.<sup>2</sup> A strongly hydrophobic environment of ME is provided by hydrocarbon oil droplets which are stabilized by surfactant and cosurfactant. One of the remarkable characteristics of microemulsions is their ability to solubilize large amounts of hydrophobic molecules. For example, we know experimentally that it is very difficult to solubilize tetraphenylporphine in water by using surfactant micelles and/or liposomes, while ME can solubilize the porphyrin very easily.<sup>3-5</sup> The aqueous ME dispersions consist of bulk water, surfactantcosurfactant, and oil phases. A recent review has demonstrated that such multiphase dispersions are profitable for charge separation in artificial photosynthetic systems.<sup>6</sup> Indeed, there are several studies on the charge separation in ME systems.<sup>5,7-11</sup> Besides these studies, the function of ME for ester hydrolyses<sup>12</sup> and metal complex formation<sup>13</sup> has been investigated. In general, however, we feel that a detailed discussion on the function of ME has not been presented for these reactions, which should be ascribed to the lack of study on the characterization of ME.

The present study deals with the characterization of ME by using the fluorescence technique. In particular, this paper deals with the morphological change of ME which occurs upon dilution with water. We believe that such a study can provide fundamental information on ME as a reaction field.

#### **Experimental Section**

Materials. Sodium hexadecyl sulfate (SHS) was prepared according to the procedures described in the literature.<sup>14</sup> Sodium dodecyl sulfate (SDS, Nakarai, 99%) and Triton X-100 (Ishizu) were purchased and used without purification. Hexadecyltrimethylammonium bromide (HTAB, Tokyo Kasei) and chloride (HTAC, Tokyo Kasei) were recrystallized from water and aqueous methanol, respectively. Dimyristoyldimethylammonium chloride (2C14NC) was kindly provided by Professor Toyoki Kunitake of this university. Dipalmitoyl- (DPPC) and dimyristoyl-L- $\alpha$ phosphatidylcholines (DMPC) were purchased (Sigma) and used without purification. The reagent grades of pentanol ( $C_5OH$ ), hexadecane  $(C_{16})$ , and 2-methylanthracene (2-MA) were commercially obtained (Wako) and used without purification. Pyrene

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(Nakarai) was carefully purified by silica gel column chromatography with cyclohexane as an eluent. Proflavin (3,6-diaminoacridine hemisulfate, PF, Wako) was recrystallized from aqueous methanol. N,N-Dihexylaniline (DHA) was prepared by refluxing the ethanolic solution (30 mL) of a mixture of aniline (0.032 mol) and 1-hexyl bromide (0.079 mol) in the presence of potassium carbonate (3 g) for 10 h. The crude DHA was purified by means of silica gel column chromatography with cyclohaxane-chloroform (1:1). 1-Ethyl-4-carbomethoxypyridinium iodide (ECPI) was prepared by alkylation of methyl isonicotinate with ethyl iodide and purified by recrystallization from acetone. 1,3-Bis(1-pyrenyl)propane (P(3)P) and N,N-dimethyl-4-[3-(1pyrenyl)propyl]aniline (PA) were prepared and purified by Hideyuki Goto of this laboratory.

Preparation of ME. A standard solution of oil-in-water ME (ME<sub>1</sub>) was prepared by mixing SHS (7.2% wt/wt), C<sub>5</sub>OH (10.8%),  $C_{16}$  (2.7%), and water (79.3%). After the solution was mixed, it was sonicated for several minutes by a bath-type sonicator (Bransonic 12, 50 W) to accelerate the formation of the transparent ME. Pyrene- and DHA-embedded ME was prepared by injecting stock solutions of pyrene and DHA in acetone into the aqueous ME solution. The concentration of pyrene was adjusted to  $5 \times 10^{-6}$  M. The maximum acetone content was less than 1% vol/vol.

Optical Measurements. The absorption and fluorescence spectra were taken on a Jasco UVIDEC 505 spectrophotometer and a Hitachi 650-10S spectrofluorimeter, respectively, whose cell compartments were thermostated. The measurements were undertaken at 25 °C unless otherwise noted. Fluorescence decay curves were obtained by using a single-photon counting apparatus described in the previous paper.<sup>15</sup> The sample solution was placed in a quartz cell (1-cm optical path) and deaerated by evacuating the system and admitting nitrogen gas at room temperature (10 cycles). The freeze-pump-thaw technique could not be applied to the present system because irreversible destruction of ME was observed sometimes when the solution was frozen and thawed.

## **Results and Discussion**

Microscopic Viscosity of the Oil Core of ME. P(3)P has been used as a fluorescent probe for evaluating microscopic viscosities  $(\eta_{\text{micro}})$  of micelles and liposomes.<sup>16-19</sup> If intramolecular excimer formation is affected only by the viscosity of the medium,  $\eta_{\text{micro}}$ can be determined from a calibration curve which correlates the ratio of the fluorescence intensity of locally excited P(3)P(M)to that of the intramolecular excimer (E)  $(I_M/I_E)$  with viscosity.

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TABLE I: Fluidity Change in the Oil Core of ME upon Dilution of ME, with Water

	oil, % wt/wt <sup>a</sup>	$\frac{I_{\mathbf{M}}/I_{\mathbf{E}}}{(\mathbf{P}(3)\mathbf{P})^{b}}$	<i>P</i> (2-MA) <sup><i>c</i></sup>
ME,	20.7	0.58	0.0034
ME <sub>0.4</sub>	8.3	0.59	0.0025
ME <sub>0</sub>	4.1	0.59	0.0037
ME <sub>0.1</sub>	2.1	0.59	0.0026

<sup>a</sup> (oil, % wt/wt) = (SHS +  $C_{16} + C_5 OH$ )/(SHS +  $C_{16} + C_5 OH$  +  $H_2O$ ). The standard ME (ME<sub>1</sub>) solution was diluted by factors 2.5 (ME<sub>0,4</sub>), 5.0 (ME<sub>0,2</sub>), and 10 (ME<sub>0,1</sub>) with water. <sup>b</sup> The ratio of the monomer fluorescence intensity to the intramolecular excimer fluorescence intensity  $(I_M/I_E)$  was obtained by exciting P(3)P  $(1 \times 10^{-6} \text{ M})$  at 348 nm and following  $I_{M}$  at 377 nm and  $I_{E}$  at 485 nm. <sup>c</sup> 2-MA  $(2 \times 10^{-5} \text{ M})$  was excited at 350 nm and the degree of the fluorescence polarization (P) was followed at 387 nm.

The recent study by Henderson et al., however, suggested that not only the viscosity but also the polarity of the medium affects the  $I_M/I_E$  value.<sup>19</sup> Then we compared  $I_M/I_E$  of P(3)P in ME with those in C<sub>16</sub> at various temperatures to estimate  $\eta_{micro}$  of the oil core of ME. P(3)P is so hydrophobic that the probe molecules seem to be located in the oil cores of the ME particles which are composed of C<sub>16</sub>.

The  $I_M/I_E$  value in ME<sub>1</sub> (0.58) at 25 °C corresponded to that in C<sub>16</sub> at 26 °C, suggesting that  $\eta_{\text{micro}}$  of the oil core of ME<sub>1</sub> is about 3 cP. The estimated  $\eta_{\text{micro}}$  value is in good agreement with those reported by Gregoritch and Thomas (<3 cP)<sup>9</sup> and Tricot et al. (3.6 cP at 20 °C).20

The fluidity of the oil core of ME can be estimated also by measuring the fluorescence polarization of 2-MA. The degree of the fluorescence polarization (P) in  $ME_1$  (0.0034) is the same as that in  $C_{16}$  (0.0040), within experimental error (see Table I). It can be concluded, therefore, that the oil core of ME is very fluid and can be regarded as a hydrocarbon-like droplet.

The fluidity of the oil core of ME was not affected by dilution of ME with water. Table I shows the changes in the  $I_M/I_E$  values of P(3)P and the P values of 2-MA upon dilution of  $ME_1$ . No significant changes in these values were observed upon dilution.

Microscopic Polarity of ME. We have demonstrated that PA is a sensitive fluorescent probe for estimating the polarity of hydrophobic environments provided by micelles, liposomes, and ME.<sup>21</sup> The dipole moment of the intramolecular exciplex of PA is so large that the fluorescence maximum of the exciplex shifted to longer wavelength with increasing solvent polarity.<sup>22</sup> A linear relationship between the energies of the intramolecular exciplex emission band ( $\nu_{\text{exciplex}}$ ) and the solvent polarity parameter ( $f(\epsilon, n)$ ) can be applied to estimate the microscopic dielectric constant  $(\epsilon_{micro})$  of ME.<sup>21</sup> The viscosity of the medium affects the yield of the intramolecular exciplex emission but not the position of the exciplex emission band.

Table II shows the fluorescence maximum of the intramolecular exciplex of PA ( $\lambda_{max}^{F}$ ) and  $\epsilon_{micro}$  derived from  $\lambda_{max}^{F}$ . The  $\lambda_{max}^{F}$  and  $\epsilon_{micro}$  values for various micelles, liposomes, and ME are also listed in Table II for comparison. We assumed 1.3 as the refractive index to calculate  $\epsilon_{max}$ . Since PA is insoluble in water, it is probably located in the oillike core of the SHS ME. The estimated  $\epsilon_{micro}$ value for ME<sub>1</sub> is 4.6 which is larger than that for C<sub>16</sub> ( $\epsilon = 2$ ). The higher value of  $\epsilon$  may be due to the dissolution of a certain amount of C<sub>5</sub>OH ( $\epsilon = 14$ ) in the C<sub>16</sub> oil core thus increasing the polarity. In any event, it is clear that the oillike core of  $ME_1$  is a very apolar environment. Zachariasse et al. measured the Dimroth  $E_{T}(30)$  values of the bulk water-ME interfaces of the SHS, SDS, and HTAB oil-in-water ME to be 53.7, 54.5, and 52.2, respectively.<sup>23</sup> Judging from the  $E_{\rm T}(30)$  values of ethanol (51.9,

TABLE II: Wavelengths at Fluorescence Maximum of PA ( $\lambda_{max}^{F}$ ) and Estimated Microscopic Dielectric Constants ( $\epsilon_{
m micro}$ ) for Various Media at 25 °Ca

medium	$\lambda_{\max}^{F}$ , nm	$\epsilon_{ m micro}{}^{b}$
SDS (0.1 M) micelles	с	>40
HTAB (0.01 M) micelles	С	>40
HTAC (0.01 M) micelles	с	>40
Triton X-100 (20 $\mu$ L/mL) micelles	515	5.5
DPPC (5 $\times$ 10 <sup>-3</sup> M) sonicated liposomes	483	2.4
DMPC (5 $\times$ 10 <sup>-3</sup> M) sonicated liposomes	495	3.2
$2C_{14}NC (4.8 \times 10^{-2} \text{ M})$ bilayer membranes	512	5.0
SHS ME $(ME_1)$	508	4.6
SDS ME <sup>d</sup>	510	4.7
HTAB ME <sup>e</sup>	513	52

<sup>a</sup> Typically, PA ( $5.0 \times 10^{-6}$  M) was excited at 346 nm to measure the fluorescence spectrum. <sup>b</sup> The method for determining  $\epsilon_{\text{micro}}$  has been reported in ref 21. <sup>c</sup> No fluorescence emission from the intramolecular exciplex was observed in the micellar systems except for Triton X-100. d SDS (7.3% wt/wt)-C<sub>s</sub>OH  $(10.0\%)-C_{16}$  (2.7%)-H<sub>2</sub>O (80.0%) oil-in-water ME. <sup>e</sup> HTAB (17.8% wt/wt)-butyl alcohol (18.2%)-C<sub>16</sub> (4.0%)-H<sub>2</sub>O (40%) oilin-water ME.

 $\epsilon = 24.3$ ) and methanol (55.5,  $\epsilon = 32.6$ ), one can conclude that the interface of ME is very polar.

Fluorescence Quenching of Pyrene by DHA. Determination of the ME Concentration. Determination of the ME concentration ([ME]) was performed by using a method developed by Atik and Thomas.<sup>24-27</sup> In aqueous micellar or ME solutions, quencher molecules are distributed among micelles or microemulsions according to Poisson statistics. Under such circumstances, the time-resolved fluorescence intensity (I(t)) can be represented as<sup>28,29</sup>

$$I(t) = I(0) \exp[\bar{n}(e^{-k_1 t} - 1) - k_0 t]$$
(1)

where I(0) is the fluorescence intensity at t = 0,  $k_0$  is the first-order rate constant for fluorescence decay in the absence of quencher, and  $k_1$  is the first-order rate constant for intraemulsion fluorescence quenching in ME containing one quencher molecule. It should be noted that the rate of fluorescence quenching in a microemulsion which contains one quencher molecule increases with decreasing size of ME and with decreasing viscosity of the medium. Therefore,  $k_1$  also increases with decreasing size and viscosity of ME. The average number of quencher per ME  $(\bar{n})$  is expressed bν

$$\bar{n} = [quencher] / [ME]$$
 (2)

The  $\bar{n}$  and  $k_1$  values can be determined by fitting the experimentally obtained fluorescence decay curve with the theoretical one calculated from eq 1 and  $\bar{n}$  and  $k_1$  as the parameters. The concentration of ME can be determined from  $\bar{n}$  and eq 2. Equation 1 can be applied only for the case where no quencher molecule is distributed in the aqueous bulk phase. We used, therefore, the hydrophobic quencher, DHA. Integration of eq 1 affords a correlation between the fluorescence intensity obtained from a steady-state fluorescence measurement and [quencher]:

$$I/I_0 = \sum_{n=0}^{\infty} \frac{\bar{n}^n (e^{-\bar{n}}/n!)}{1 + n(k_1/k_0)}$$
(3)

where  $I_0$  and I are the fluorescence intensities in the absence and in prescence of quencher, respectively.

Figure 1 shows plots of  $I_0/I$  vs. [DHA] when the pyrene monomer fluorescence was quenched by DHA in ME<sub>1</sub> and the

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Figure 1. Fluorescence quenching of the pyrene monomer by DHA in SHS ME at 25 °C. Pyrene  $(5 \times 10^{-6} \text{ M})$  in the ME solutions (2 mL) was excited at 337 nm and the fluorescence intensities were monitored at 385 nm. ME<sub>1</sub> consisted of SHS (7.2% wt/wt), C<sub>16</sub> (2.7%), C<sub>5</sub>OH (10.8%), and water (79.3%) and was diluted with water by factors of 2.5 (ME<sub>0.4</sub>), 5.0 (ME<sub>0.2</sub>), and 10 (ME<sub>0.1</sub>).



**Figure 2.** Fluorescence decay curves of pyrene monomer in ME<sub>1</sub> with and without DHA at 25 °C.  $10^{3}$ [DHA] = 0, 0.5, 1.0, 1.5, and 2.0 M (from the top). The solid lines are the decay curves calculated by using eq 1 and the  $k_{1}$  and  $\bar{n}$  values listed in Table III.

microemulsions which were prepared by dilution of ME<sub>1</sub> with water. ME<sub>0.4</sub>, ME<sub>0.2</sub>, and ME<sub>0.1</sub> represent the microemulsions which were prepared by the 2.5-, 5-, and 10-times dilution of ME<sub>1</sub>, respectively. As Figure 1 indicates, the fluorescence quenching was accelerated as ME<sub>1</sub> is diluted. The accelerated quenching in diluted ME is interpreted in terms of the concentration of the quencher molecules in the ME particles. The fluorescence quenching of pyrene by DHA in ME did not obey a simple Stern–Volmer linear relationship, i.e., plots of  $I_0/I$  vs. [DHA] deviated upward from straight lines.

Figure 2 shows the fluorescence decay curves of the pyrene monomer in ME<sub>1</sub> containing various amounts of DHA. Nonexponential decay curves were obtained in the presence of DHA. The nonexponential fluorescence decay as well as the nonlinear Stern-Volmer relationship suggests that the statistical distribution of the quencher molecules among emulsions should be considered for fluorescence quenching in ME. The curved Stern-Volmer plots are predicted from eq 3. The solid lines in Figure 2 are the theoretical fluorescence decay curves calculated by using eq 1 and  $k_1$  and  $\bar{n}$  as parameters. The  $k_0$  value was obtained from the single-exponential decay curve of the pyrene monomer fluorescence in ME in the absence of DHA ( $k_0 = 2.7 \times 10^6 \text{ s}^{-1}$ ). The experimentally obtained decay curves are well fitted with the theoretical ones. The  $\bar{n}$  and  $k_1$  values for ME<sub>1</sub> determined by the fitting method are listed in Table III. Table III also shows [ME] calculated from eq 2 and the aggregation number of SHS (AN = [SHS]/[ME]). Almost identical  $k_1$  and [ME] were obtained

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TABLE III: First-Order Rate Constants for Intraemulsion Fluorescence Quenching of Pyrene by DHA  $(k_1)$ , Number of DHA per ME  $(\overline{n})$ , ME Concentrations ([ME]), and Aggregation Number of SHS (AN) Obtained from Analyses of the Fluorescence Decay Curves of Pyrene in ME<sub>1</sub> Containing Various Amounts of DHA

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10 <sup>3</sup> [DHA], M	$10^{-7}k_1,^a$	$\overline{n}a$	10 <sup>4</sup> [ME], <sup>b</sup> M	ANC	
0.5	1.1	0.6	8.3	241	
1.0	1.1	1.1	9.1	220	
1.5	1.1	1.7	8.8	227	
2.0	1.1	2.2	9.1	220	

<sup>a</sup> The  $k_1$  and  $\overline{n}$  values were determined by fitting the fluorescence decay curves of the pyrene monomer with the calculated curves obtained by using eq 1 (see Figure 2). <sup>b</sup> [ME] was calculated by using eq 2. <sup>c</sup> AN = [SHS]/[ME].

TABLE IV: Variation in  $k_1$ , [ME], AN, and Radius of the Oil Core (r) upon Dilution of ME<sub>1</sub> with Water at 25 °C

	$10^{-7}k$	10 <sup>4</sup> . [ME].	AN			
	s <sup>-1</sup>	M	SHS	С₅ОН	C 16	r, <sup>a</sup> Å
ME <sub>1</sub>	1.1	8.8	230	1320	130	25
ME <sub>04</sub>	2.4	6.7	120	690	70	20
ME <sub>0</sub> ,	2.2	4.2	100	550	50	19
ME <sub>0.1</sub>	1.2	2.4	80	480	50	18

<sup>*a*</sup> The *r* values were calculated by assuming that the specific gravities of the oil cores of the microemulsions are the same as that of  $C_{16}$  and using the aggregation numbers of  $C_{16}$ .

even if  $ME_1$  contains different amounts of DHA. The results in Table III show the accuracy of this method for determining [ME].

According to the same procedures,  $k_1$ , [ME], and AN for ME<sub>0.4</sub>, ME<sub>0.2</sub>, and ME<sub>0.1</sub> were determined and the results are summarized in Table IV. The concentration as well as the aggregation numbers of the components of ME decreased continuously with dilution. An optimum  $k_1$  value was obtained for ME<sub>0.4</sub>. If no morphological change of ME occurs during dilution, both  $k_1$  and AN should remain constant. The reduction of AN clearly indicates that ME was subdivided upon dilution. If we assume that the oillike core of ME has the same specific gravity as C<sub>16</sub> (0.773) and is spherical, the radius of the oillike core for each ME can be calculated. The calculated radius for each ME is shown in Table IV. The radius of the oillike core decreases upon dilution.

By measuring the fluorescence bands of pyrene which are sensitive to the solvent polarity, Atik and Thomas have concluded that pyrene molecules reside in the oillike core of ME.<sup>26</sup> We have reproduced their observation and confirmed that pyrene is located in the oillike core of the SHS ME. If both pyrene and DHA are located in the oillike core of ME,  $k_1$  should increase with decreasing size of the oillike core (vide supra). Apparently, this is the case for the dilution of  $ME_1$  to  $ME_{0.4}$ . In spite of the decrease in the ME size, the  $k_1$  values decreased upon further dilution. This cannot be interpreted in terms of the morphological change of ME. As mentioned above, the dilution does not lead to a viscosity change of the oillike core of ME. The reduction of the  $k_1$  values for  $ME_{0,2}$  and  $ME_{0,1}$ , therefore, is not ascribed to the increase of the viscosities of the oillike cores. Binding site and diffusional behavior of DHA in ME should be considered to explain the effect of dilution on  $k_1$ .

Binding Site of DHA. Effect of Dilution with Water on  $k_1$ . DHA itself fluoreses in  $C_{16}$  at  $\lambda_{max}^F = 338$  nm which shifts to longer wavelength with increasing polarity of the solvent ( $\lambda_{max}^F$  in  $C_5$ OH = 348 nm). Figures 3 and 4 show the effects of dilution of ME<sub>1</sub> on both  $\lambda_{max}^F$  and the degree of the fluorescence polarization (P) of DHA, respectively. The fluorescence maximum of DHA was observed at 345 nm in ME<sub>1</sub>, which suggests that DHA is located in a polar environment, i.e., in the surfactant-cosurfactant phase of ME<sub>1</sub>. Upon dilution of ME<sub>1</sub>,  $\lambda_{max}^F$  shifted to shorter wavelength ( $\lambda_{max}^F$  in ME<sub>0.1</sub> = 340 nm), indicating that the dilution causes a decrease in the polarity of the environment around DHA. If DHA the mean distance between DHA and pyrene should be shortened



Figure 3. Effects of dilution of ME on the fluorescence spectrum of DHA and absorption spectrum of ECPI. The fluorescence spectrum (uncorrected) of DHA ( $4 \times 10^{-4}$  M) was measured by exciting DHA at 310 nm. The Kosower Z values were obtained from the wavelength at the absorption maximum of ECPI which binds electrostatically with the surfaces of the microemulsions. The experiments were carried out by using ME<sub>1</sub>, ME<sub>0.4</sub>, ME<sub>0.2</sub>, and ME<sub>0.1</sub>.



Figure 4. Changes in the degree of fluorescence polarization (P) of DHA  $(4 \times 10^{-4} \text{ M})$  and PF  $(2 \times 10^{-5} \text{ M})$  upon dilution of microemulsions with water at 25 °C. The P values were determined by exciting DHA at 318 nm and PF at 470 nm and following the emission intensities at 348 nm for DHA and 510 nm for PF. The experiments were carried out by using ME<sub>1</sub>, ME<sub>0.4</sub>, ME<sub>0.2</sub>, and ME<sub>0.1</sub>.

to result in an increase of  $k_1$ . This is inconsistent with the experimental results. As Figure 4 shows, the *P* value of DHA in ME increased upon dilution, which suggests that the restriction of DHA mobility is enhanced in diluted ME. Since the dilution does not lead to the fluidity change of the oillike core of ME (vide supra), DHA is probably located in the surfactant-cosurfactant phase even if ME is diluted with water.

To confirm the polarity and fluidity changes of the surfactant-cosurfactant phase of ME, we measured the absorption spectrum of 1-ethyl-4-carbomethoxypyridinium iodide (ECPI) and the flurescence polarization of proflavin (PF) in  $ME_1$  and the diluted microemulsions. These cationic dye molecules bind electrostatically with the negatively charged surfaces of ME. It was very difficult to find a probe which is located predominantly in the surfactant-cosurfactant phase of ME. It may be reasonable to assume, however, that the nature of the interface of ME reflects the nature of the surfactant-cosurfactant phase of ME. The apparent Kosower Z value, which is obtained from the wavelength of the charge-transfer (CT) absorption band of ECPI, and the P value of PF for each ME are shown in Figures 3 and 4, respectively. No appreciable CT band of ECPI was observed in  $ME_1$ , suggesting that the water-ME interface of  $ME_1$  is very polar. The CT band appeared and shifted to longer wavelength upon dilution of  $ME_1$  (340, 347, and 347 nm for  $ME_{0.4}$ ,  $ME_{0.2}$ , and  $ME_{0.1}$ , respectively). This means that the polarity of the interface of ME decreases upon dilution. This is consistent with the result obtained from DHA fluorescence measurements in ME. On the other hand, the P value of PF in ME increased with dilution of  $ME_1$  (Figure 4), indicating that the water-ME interface become less fluid upon dilution. This also agrees with the result obtained from the P-value measurements of DHA in ME. The fluorescence behavior of DHA and PF and the absorption spectrum of ECPI indicate that both the surfactant-cosurfactant phase and the ME-water interface become less fluid and apolar when ME is diluted with water.

A remarkable reduction in the size of ME was found in the case of the dilution of  $ME_1$  to  $ME_{0.4}$  (see the r values in Table IV), whereas the mobility of DHA is scarcely altered by this dilution (see the P values of DHA in Figure 4). It may be reasonable to conclude, therefore, that the increase of  $k_1$  in  $ME_{0.4}$  is ascribed to the decrease in the ME size, which leads to a reduction of the mean distance between pyrene and DHA. The decrease of  $k_1$  in the further diluted microemulsions ( $ME_{0.2}$  and  $ME_{0.1}$ ) is probably due to a reduction of the diffusional mobility of DHA molecules located at the surfactant-cosurfactant phase of these diluted microemulsions.

### **Concluding Remarks**

The fluorescence technique has been applied for characterizing oil-in-water ME. According to the phase diagram presented by Mackay et al.,<sup>30</sup> we prepared the oil-in-water ME composed of SHS,  $C_5OH$ , and  $C_{16}$ . The intramolecular excimer and exciplex emissions were used as probes for evaluating the viscosities and polarities of the microemulsions, respectively. These fluorescent probes indicated that the core of ME is very hydrophobic and fluid and can be regarded as an oillike droplet.

The fluorescence quenching of pyrene by DHA has been examined with respect to the morphological change of ME which occurs upon dilution with water. This technique revealed that the size of ME and the fluidity and the polarity of the surfactant-cosurfactant phase of ME decrease upon dilution.

(30) Mackay, R. A.; Letts, K.; Jones, C. "Micellization, Solubilization and Microemulsions"; Mittal, K. L., Ed.; Plenum: New York, 1977; Vol. II, p 801.