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SHORT COMMUNICATION

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Fast transfer process of pyrene between oil-in-water miniemulsion droplets

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Abstract Recently phase formation mechanisms have been estimated by using various fluorescent probes. In this report, the mixing process between internal phases of oil-in-water miniemulsions is discussed for two-dimensional color graphics data (two-dimensional fluorescence images) based on the excimer formation of pyrene as a hydrophobic fluorescent probe. Just after miniemulsion solution B (water, oil, and nonionic surfactant) was gradually added to miniemulsion A (water, oil, surfactant, and trace amount of pyrene) with gentle and careful stirring, the fluorescence spectra and the two-dimensional image of pyrene were measured. The decreasing of the excimer peak of pyrene was observed as soon as miniemulsion solution B was added. The result showed that pyrene initially located in miniemulsion droplets was smoothly diluted by the addition of miniemulsion droplets which contain only oil in the internal phase. The internal phases of miniemulsion droplets are miscible without changing the droplet diameter, and it is declared that pyrene transfers smoothly to the interface between droplets stabilized by the nonionic surfactant because the droplet diameter showed no significant difference throughout this mixing process.

Key words Miniemulsion · Miscibility of the internal phase · Pyrene excimer formation · Fluorescence image processing · Nonionic surfactant

Introduction

A thermal procedure, which allowed us to produce a translucent oil-in-water (O/W) emulsion consisting of nanosphere droplets, was proposed [1, 2]. In spite of the fact that these droplets had a very small diameter (about 30 nm), this phase is not a microemulsion. It was observed that the O/W emulsion approached a thermodynamically equilibrium state (in this case it was of Winsor I type) with time [1]. Hence we consider the O/W emulsion obtained as a miniemulsion.

Miniemulsions are thermodynamically unstable and in general separate into two phases over a period of time [3]. At the initial time, the miniemulsion droplets have hard-sphere interaction in terms of the Percus–Yevick approximation [2]. The droplet diameter of the miniemulsion obtained was about 20 nm and the ratio of the hydrodynamic diameter to the hard-sphere diameter was about 1.2. In the destabilization process of the miniemulsion, the droplet size distribution developed gradually from monodisperse to polydisperse over 8 days. By plotting the normalized droplet number as a function of time, it was considered that the growth of the miniemulsion droplets obeyed a diffusion-controlled process [4]. The *z*-average radius of the miniemulsion droplets, r_z^3, is proportional to time [5]. This suggests that Ostwald ripening is the growth mechanism of the droplets [3, 5]. Contrary to List (LSW) theory proposed as a mechanism of Ostwald ripening [6, 7], two distinct stages of the growth process of the droplets were observed [5]. At the earlier stage, the droplet size distribution became broad as time passed. Then the distribution approached a time-independent form after about 8 days. The distribution after 12 days was too broad compared with the one predicted by LSW theory. At the later stage, r_z^3 varied linearly with time and the droplet size distribution approached the predicted time-independent distribution of LSW theory.

In order to clarify the growth mechanism of miniemulsion droplets, it is desirable to investigate the dynamical behavior of the internal phase at earlier stages. In this work, the exchange of pyrene molecules located in the inside of droplets was examined using two different fluorescent probe methods: steady-state fluorescence spectroscopy and two-dimensional fluorescence image processing as a novel method. Pyrene is widely used as a hydrophobic probe in order to investigate the properties of aqueous colloidal solutions [8–12]. In general, the fluorescence quenching method is applied for the investigation of exchange processes of probe molecules between droplets in the steady state [13]. On the other hand, the method presented, the two-dimensional fluorescence image, enables us to observe the transient process on the mixing of the internal phases between the miniemulsion droplets. This method may be useful for investigating the transfer of probe molecules between metastable droplets, such as miniemulsions.

Experimental

Mergital LT7 (main component is heptaethylene glycol mono-*n*-dodecyl ether) was obtained from Sidobre Sinnova, *n*-hexadecane was purchased from Fluka, and pyrene was purchased from Tokyo Kasei (TCI). All chemicals were used as received. Referring to the phase inversion temperature (PIT) method proposed by Shinoda and Saito [14, 15], the miniemulsions were prepared following a thermal procedure under gentle stirring. The role of PIT for the preparation of miniemulsions was reported earlier [1]. The volume ratio of water to oil was 9, and the surfactant mass fraction was fixed at 0.12. All experiments were performed at room temperature (about 25 °C). The hydrodynamic diameter of the miniemulsion obtained was about 16 nm [1].

Fluorescence spectra were measured using an FP-777 spectrofluorometer (Jasco) using 1.5-nm slits for the excitation and the emission monochrometers. Two miniemulsions, A and B, with the same composition were prepared separately. A contained 3.2×10^{-3} M pyrene in oil. B was emulsified with pure hexadecane. These miniemulsions were mixed before the fluorescence spectra were measured. The concentration of pyrene in solution was of the order of 10^{-6} M.

The two-dimensional fluorescence images were observed using a DCR VX-1000 digital video camera (Sony) with excited light (about 350 nm) from an FL68L-B black light (National). Then, the recorded video pictures were converted into a bitmap file by a Video Commander 32 as a video capture board (Canopus). The fluorescence image processing was performed by a program written in Delphi (Borland).

The droplet diameter was estimated by dynamic light scattering techniques. Dynamic light scattering measurements were carried out with a DLS-7000 (Otsuka). The *z*-averaged hydrodynamic diameter was obtained by second-order cumulant analysis [16].

Results and discussion

The emission spectra of pyrene were obtained as follows. At first, 0.02 ml miniemulsion A was diluted with 2.0 ml water. After miniemulsion B had been added with gentle stirring, the emission spectrum was measured.

The change in the emission spectra of pyrene on adding the miniemulsion is shown in Fig. 1. An isoemission wavelength is observed at 425 nm because

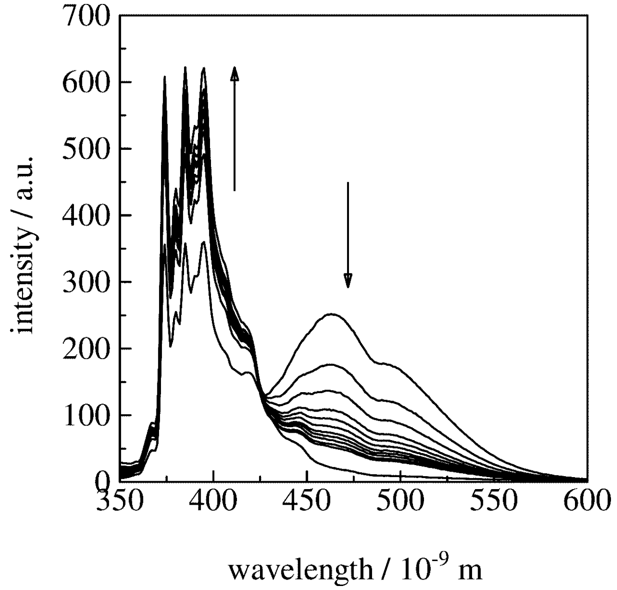

Fig. 1 Fluorescence spectra as a function of the volume fraction of the added miniemulsion droplets (miniemulsion B). At lower volume fraction of miniemulsion B, the excimer peak was clearly observed. At higher volume fraction, the excimer peak disappears gradually. The *arrows* indicate the changes with increasing volume fraction of B (from 0 to 0.2061)

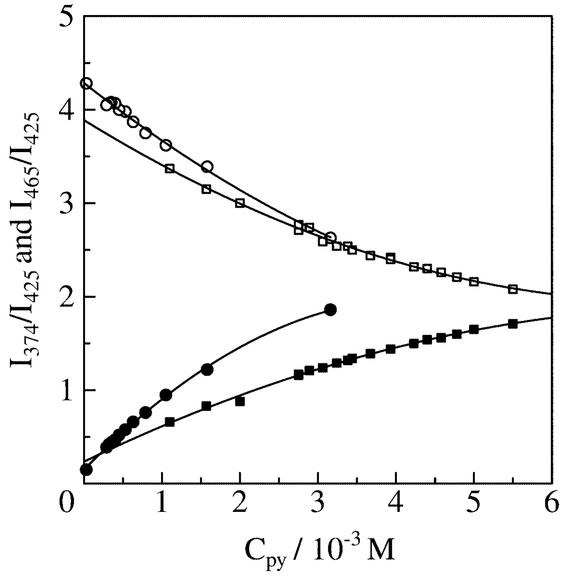

Fig. 2 Variations of I_{374}/I_{425} (*open symbols*) and I_{465}/I_{425} (*filled symbols*) as a function of pyrene concentration in hexadecane. The data represented by the *squares* and *circles* were measured in the bulk of hexadecane and in the miniemulsion, respectively

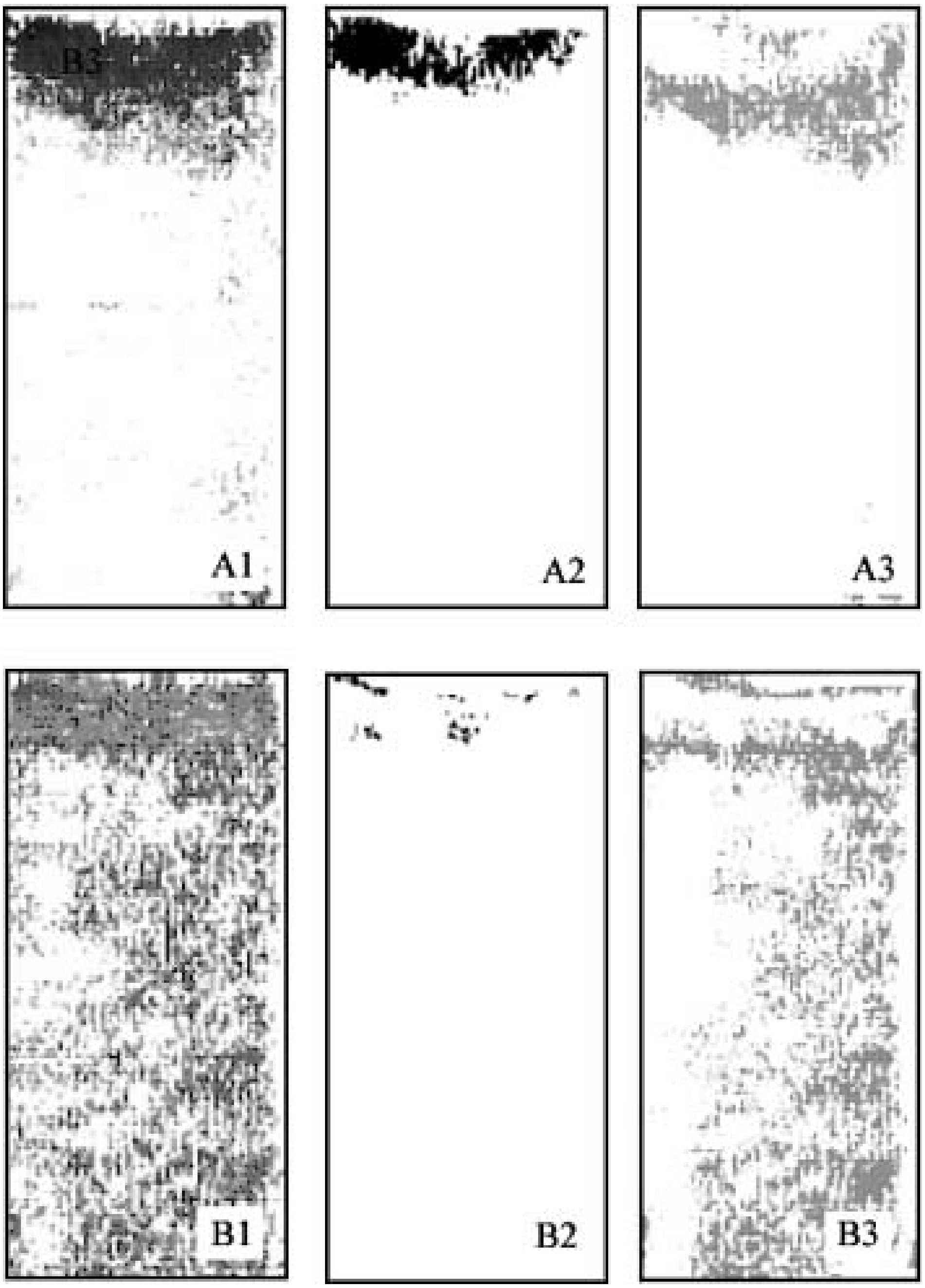

Fig. 3 Examples for the two-dimensional fluorescence image processing. *A1* and *B1* were obtained at 0 and 1.26×10^4 s. These figures were drawn after the elimination of background. *A2* and *B2* were drawn with "green" pixels (red, green, and blue values on the bitmap color image file with 256-color resolution are about 80, 210, 200). A3 and B3 are those for "blue" pixels (red, green, and blue values are about 80, 140, 200)

the amount of pyrene in solution was constant (6.01×10^{-6} M). When miniemulsion A was diluted with pure water, two regions in the spectrum were observed. Vibrational fine structure was seen between 360 and 430 nm. At longer wavelengths, there is a broad, structureless band with a maximum at 465 nm. This broad band is due to the excimer formation in the miniemulsion droplets. As pyrene is a hydrophobic molecule with a very low water solubility, most of the pyrene molecules are situated in the internal phase of the miniemulsion, i.e. the density of pyrene is so high that excimer formation is observed. In general, excimer formation of pyrene in nonpolar solvents requires a concentration of the order of 10^{-3} M.

The intensity ratio of the first (374 nm) to the third peak (385 nm) in the fluorescence emission spectrum of pyrene, I_1/I_3, is a useful indicator of the polarity of the probe environment [17–19]. In water, I_1/I_3 is 1.7, whereas in nonpolar solvents it has values well below 1 (e.g., in cyclohexane, $I_1/I_3 = 0.58$ [20]). In *n*-hexadecane, I_1/I_3

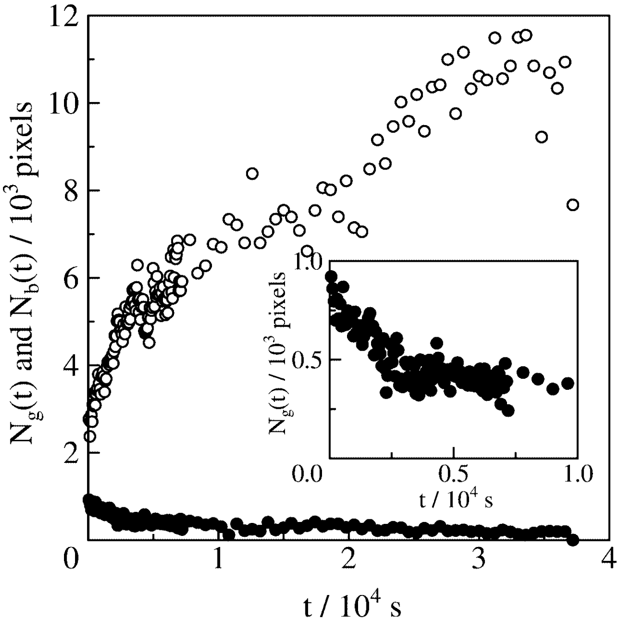

Fig. 4 Temporal changes in $N_g(t)$ (*filled circle*) and $N_b(t)$ (*open circle*)

was 0.58; on the other hand, 0.99 was obtained in the miniemulsions. This indicates that the probe environment is hydrophobic but that some contact with water still remains. The value is close to that found for pyrene in micelles of block copolymer of poly(ethylene oxide) end-capped with hexadecyl alkyl chains [18] and nonionic surfactant micelles [20]. These results prove that the probe molecules are associated with the miniemulsion droplets. However, there is always a fraction in the water phase that may be in dynamic equilibrium with the probe in the miniemulsion droplets.

I_{374}/I_{425} and I_{465}/I_{425} are shown as a function of the concentration of pyrene in hexadecane, c_{py}, in Fig. 2. For miniemulsions, c_{py} was calculated as the concentration of pyrene in the internal phase. Excimer formation in the miniemulsions and *n*-hexadecane shows the same tendency. I_1/I_3 did not change on the addition of miniemulsion B (Fig. 1). The environment around pyrene was not changed by the addition of miniemulsion B, i.e. pyrene resides in the same region of the internal phase of the miniemulsions. When miniemulsion B was added to the aqueous solution of miniemulsion A, the excimer peak decreased instantaneously as the pyrene solution was diluted. The emission spectra did not change over a period of 2 h after mixing.

In a previous report [1], the miniemulsion droplets were considered as hard spheres in terms of the Percus–Yevick approximation. The *z*-average hydrodynamic diameter of the miniemulsion droplets did not change significantly over a period of 12 h after preparation, and the characteristic time of the diffusion-controlled growth process was of the order of 10^4 s [4, 5]. In spite of the strong repulsion between the droplets, the result obtained here implies that pyrene in the internal phase of miniemulsion A is diluted instantaneously by the addition of miniemulsion B.

In order to observe the diffusion and dilution process of pyrene molecules originally located in miniemulsion A, fluorescence image processing was performed. At first, a glass tube was filled with miniemulsion B, then trace amounts of miniemulsion A were injected. Owing to the emitted light, a two-dimensional fluorescence image was obtained, which supplies information on the position and the emitted light of the probe molecules. Emitted light from excimers was observed as a "green" color in the recorded video pictures and that from monomers was "blue". On the bitmap color image file, the color of the excimer emission was represented by the following values for red (R), green (G), and blue (B) with 256-color resolution: $R \cong 80$, $G \cong 200$, and $B \cong 210$. For the monomer emission, $R \cong 80$, $G \cong 140$, and $B \cong 210$. The two-dimensional fluorescence images at 60 and 1.26×10^4 s are shown in Fig. 3. A1 and B1 show the images after the background correction. A2 and B2 show the images for the "green" pixels ($R \cong 80$, $G \cong 200$, and $B \cong 210$) and A3 and B3 are those for the "blue" pixels ($R \cong 80$, $G \cong 140$, and $B \cong 210$). As shown in A2 and A3, the areas of green and blue are well separated. On the other hand, a small number of green pixels were observed on B2, while in B3 the number of blue pixels increased significantly and the area of the blue pixels was expanded compared with A3. The green pixels were observed in the same region over the whole time range. Even when trace amounts of miniemulsion A were diluted with water, only a green color was observed and the image did not show a significant change over a period of 10 h (3.6×10^4 s).

The temporal changes in the total number of blue and green pixels on the bitmap color image, $N_b(t)$ and $N_g(t)$, are shown in Fig. 4. $N_g(t)$ decreased rapidly within 0.3×10^4 s (inset in Fig. 4). On the other hand, $N_b(t)$ kept increasing continuously. The half time of $N_g(t)$ is about 1.5×10^3 s. The result suggests that the pyrene located originally in miniemulsion A was diluted smoothly by the internal phase of miniemulsion B; that is, the number of droplets containing pyrene increased with time by the transfer of pyrene from the internal phase of A to B. Regarding the position of the green pixels (Fig. 3, A2, B2), emitted light from the monomers was not observed before droplet diffusion. This may suggest that droplets of miniemulsion A diffuse in the solution by exchanging a part of their internal phase. This partial exchange of the internal phase may not be accompanied by a significant change in the droplet diameter because the hydrodynamic diameter of the miniemulsion droplets did not change in this period, as indicated previously [1]. In the present state, it is difficult to discuss in detail the data obtained by fluorescence image processing techniques; however, the half time seems to be too short when the reported rate for the growth process of miniemulsion droplets is considered (about 1.6×10^5 s [5]). It seems probable that pyrene molecules can transfer smoothly from the internal phase of miniemulsion A to that of miniemulsion B throughout the interface stabilized by surfactant molecules. This may be a very curious suggestion to understand the dynamical property of miniemulsion droplets. In order to confirm this novel property, further investigations are being carried out.

Conclusions

The miscibility of the internal phase of miniemulsion droplets was investigated by fluorescence probe methods. Pyrene initially located in miniemulsion droplets is smoothly diluted by the addition of miniemulsion droplets which only contain pure oil as the internal phase. This exchange does not change the droplet diameters. With this novel method, the dilution and diffusion processes of the internal phase of dispersed droplets can be measured.

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