In Situ Measurement of Individual W/O Microemulsions of Aerosol OT in Dodecane/Water Extraction System by Total Internal Reflection Laser Light Scattering Microscopy

Satoshi Tsukahara,*[1] Haruyasu Kitaguchi,[2] and Hitoshi Watarai[2]
[1]*Department of Chemistry, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526*
[2]*Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043*

(Received October 10, 2006; CL-061189; E-mail: tsuka@sci.hiroshima-u.ac.jp)

The laser light scattering microscopy under the total internal reflection condition allowed one to do in situ measurements of individual W/O microemulsions of aerosol OT (AOT) formed spontaneously at a dodecane/water interface in a thin-layer two-phase microcell. The instrument can detect the AOT microemulsions being larger than 50 nm in hydrodynamic radius at the interface and in the dodecane phase without any probe molecules.

Commonly, reverse micelle is artificially made in organic solutions containing surfactants by injection of water. The reverse micelle possesses a water pool, and it can incorporate hydrophilic compounds in the water pool. Its general size is in a range of 4–20 nm, depending on organic solvents, water content, and surfactant concentration.[1] On the other hand, solvent extraction is one of the essential techniques for separation and concentration of metal ions or organic compounds of lower molecular weight. Recently, it has been found that some biological macromolecules, such as proteins or DNAs, are extracted into organic phase by surfactants that form reverse micelles.[2,3] The reverse micelle formation and the incorporation of macromolecules in the extraction systems should occur in the liquid/liquid interfacial region. The behaviors of reverse micelles at a heptane/water interface were discussed with interfacial tension changes,[4] but there have been no reports on in situ microscopic measurement of behaviors of individual reverse micelles near the interface. The reason would be that reverse micelle at the interface is believed to be too small to be detected by optical microscopy.

A liquid/liquid interface is easy to disturb physically and hard to fix spatially. Thus, microscopic measurements for the interface have been hard to do. A thin-layer two-phase microcell shown in Figure 1a is a recent breakthrough to overcome the difficulties;[5] a stable and flat liquid/liquid interface is prepared in it, and it enables one to measure the interface by in situ optical microscopy with high magnification.

Aerosol OT (AOT; sodium diisooctyl sulfosuccinate) is one of the typical surfactants that form reverse micelle in some organic solvents, and it can extract proteins into organic solvents.[2] The present study focuses on the detection of individual AOT reverse micelles near the dodecane/water interface by in situ optical microscopy without any probe molecules for the first time.

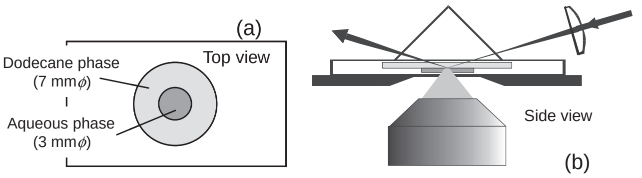

Figure 1. Schematic illustration of (a) the thin-layer two-phase microcell and (b) total internal reflection laser light scattering microscopy at the interface. The upper phase is dodecane and the lower one is water.

AOT (purity ≥ 99%) was purchased from Fluka. Dodecane (extra pure grade, Nacalai) was used after purification by passing through a silica-gel column. Water was purified with a Milli-Q system (Milli-Q SP. TOC., Millipore). The initial AOT concentration in dodecane solutions ($[\mathrm{AOT}]_{\mathrm{dod,i}}$) was in a range of 8.0×10^{-6}–1.0×10^{-4} M ($1\,\mathrm{M} = 1\,\mathrm{mol\,dm^{-3}}$). No water was added to the AOT solutions.

Figure 1b shows a schematic illustration of the used instrument system that includes an inversed microscope (IX-51, Olympus) with a 40× objective (SLCPlanFl, NA 0.55, Olympus) and a CCD camera (WAT-100N, Watec). The thin-layer two-phase microcell was made by a similar way to the previous study.[5] After a lower depression (1 mm in depth) of the microcell was filled with pure water, an upper one (1 mm in depth) was filled with the AOT dodecane solution quietly, and then the microcell was closed with a coverslip. In case of laser irradiation, a rectangle glass prism (BK-7, $1 \times 1 \times 1$ cm) was placed on it through index matching oil. The interface was irradiated with the second-harmonic light (532 nm, 5 mW; JST-5, Light Vision) of cw-Nd:YAG laser after it was focused by a lens (12 mm in focal length), which narrowed the width of the laser beam to about 10 μm at the interface. The incident angle was set to 73°, which was larger than the critical angle of the dodecane/water interface (69°). All the experiments were carried out at 20 ± 1 °C.

The spatial resolution of an optical microscope is restricted by the diffraction limit of light (Δx), which is calculated with $\Delta x = \lambda/(2\mathrm{NA})$, where λ is the wavelength of probe light and NA is the numerical aperture. With a 500 nm light, Δx is about 450 nm with the objective (NA 0.55). First, the dodecane/water interface containing AOT was observed by the microscope with illumination light (in normal bright field mode). An almost homogeneous picture was obtained at and near the interface, meaning that there were no substances larger than Δx. On the other hand, Figure 2a was obtained with scattered laser light; there were many particles scattering laser light. The spatial resolution of the laser light scattering microscopy is also restricted by Δx, but substances smaller than Δx are detectable. These facts mean that the scattering particles in Figure 2a were smaller than Δx, and thus their size was not determined from the image directly. Several small bright circles in Figure 2a corresponded to particles in focus, whereas larger circular images with interference fringes corresponded to those out of focus. The focal depth

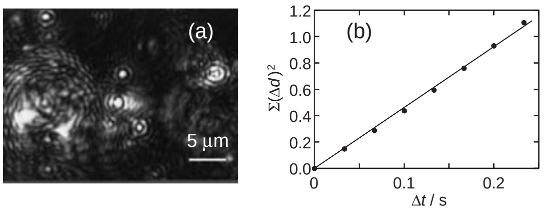

Figure 2. (a) Microscopic picture in the dodecane phase at 28 μm above the interface at 100 min after the interface preparation with scattered laser light. $[\mathrm{AOT}]_{\mathrm{dod,i}} = 1.0 \times 10^{-4}$ M. (b) A plot between $\Sigma(\Delta d)^2$ and Δt for one AOT microemulsion in the dodecane phase. $[\mathrm{AOT}]_{\mathrm{dod,i}} = 1.0 \times 10^{-4}$ M.

was about 1 μm for the present microscopic system. By changing the position of the focal plane, the existence of scattering particles was examined. At the beginning the particles were found only at the interface, but later they also existed in the dodecane phase near the interface.

No scatterers were observed at a bear dodecane/water interface without AOT or in a dry dodecane solution of 1.0×10^{-4} M AOT. Some batch experiments on a large scale by Karl–Fisher titration showed that the water concentration in the AOT dodecane phase increased with time after contact with water, indicating that the scattering particles contained water. The following sections will show that the particle size is 50–250 nm, and thus the particle is usually named water-in-oil (W/O) microemulsion. These AOT microemulsions were formed at the interface spontaneously and diffused away into the dodecane phase.

The microemulsions showed three-dimensional Brownian motion in the dodecane phase. Their hydrodynamic radius (r_{h}) was evaluated from the analysis of the Brownian motion.[6] The displacement (Δd) of individual microemulsions in the dodecane phase at about 10–60 μm above the interface during one video flame period (33 ms) was obtained, and the sum of squared moving displacement ($\Sigma(\Delta d)^2$) was calculated as a function of time interval (Δt). An example of plot between $\Sigma(\Delta d)^2$ and Δt is displayed in Figure 2b. The plots of other microemulsions also show proportional relationships with high correlation coefficients ($\geq$0.99), from which their diffusion coefficient (D) was determined with $\Sigma(\Delta d)^2 = 4D(\Delta t)$.[6] The Einstein–Stokes relation is $D = kT/(6\pi\eta r_{\mathrm{h}})$, where k is Boltzmann constant, T temperature, and η dodecane viscosity. The detection limit of the microemulsion for the present microscopic system is about 50 nm in r_{h}.

At $[\mathrm{AOT}]_{\mathrm{dod,i}} = 1.0 \times 10^{-4}$ M, r_{h} of the microemulsions was determined to be 140 ± 60 nm (mean ± standard deviation; data number 18), which barely depended on time in a range of 30–100 min. The microemulsions were formed at $[\mathrm{AOT}]_{\mathrm{dod,i}} \geq 2.0 \times 10^{-5}$ M, and the decrease in $[\mathrm{AOT}]_{\mathrm{dod,i}}$ resulted in smaller size and lower number density of the microemulsion. No microemulsions were observed at $[\mathrm{AOT}]_{\mathrm{dod,i}} \leq 1.0 \times 10^{-5}$ M at any time, but reverse micelles smaller than the detection limit would be formed. The present results mean that the size of the microemulsions formed at the dodecane/water interface spontaneously is determined naturally, which would depend on the AOT concentration in the dodecane phase.[7] In situ measurements of the individual W/O microemulsions formed in the extraction system have not been reported so far.

Many microemulsions were formed just after the interface preparation, but the moment of their formation could not be observed. Some interfacial convections occurred at the same time, which was recognized by a movement of microemulsions in one direction. The frequency of the formation and the degree of the convection decreased with the elapse of time. These facts suggest that the convection can cause some interfacial fluctuation, leading to the formation of microemulsions, as shown in Figure 3. Also, the convection may hinder the observation of the moment of AOT microemulsion formation.

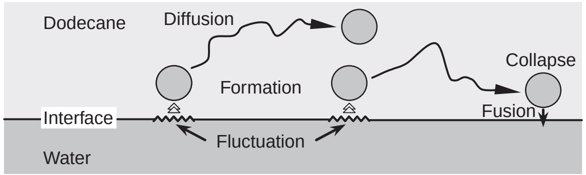

Figure 3. Schematic illustration of the formation and collapse of the W/O microemulsions of AOT in the dodecane/water interfacial region.

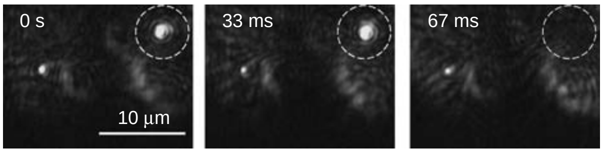

Figure 4. Continuous microscopic pictures with scattered laser light at the dodecane/water interface at 20 min after the interface preparation. The upper-right microemulsion existed in the first and second pictures, but it did not exist in the third picture. $[\mathrm{AOT}]_{\mathrm{dod,i}}$ was 5.0×10^{-5} M.

The microemulsions did not shrink, nor were they fused each other in the dodecane phase; they only diffused. However, several microemulsions vanished at the interface within one video flame (33 ms) as shown in Figure 4. This process was the only manner that the microemulsions vanished. The total internal reflection technique selectively catches the phenomena in the dodecane phase and at the interface. Thus, the sudden vanishing corresponds to physical collapse, that is, a fusion with the interface, as shown in Figure 3.

The extraction of proteins with AOT was commonly done at a high AOT concentration, such as 0.2 M,[2] and the microemulsions should be formed in such an extraction system. Therefore, the extraction of biological macromolecules would be deeply related to the microemulsions formed at the liquid/liquid interface found in the present study. Further investigations on their formation mechanisms or incorporation of biological macromolecules at the liquid/liquid interface are now done from a microscopic viewpoint.

This study was financially supported by a Grant-in-Aid for Scientific Research (B) (No. 16350046) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1 M. Adachi, M. Harada, A. Shioi, Y. Sato, *J. Phys. Chem.* **1991**, *95*, 7925.
2 M. Goto, A. Momota, T. Ono, *J. Chem. Eng. Jpn.* **2004**, *37*, 662.
3 K. Shiomori, Y. Kawano, R. Kuboi, I. Komasawa, *J. Chem. Eng. Jpn.* **2000**, *33*, 800.
4 M. Takahashi, H. Yui, Y. Ikezoe, T. Sawada, *Chem. Phys. Lett.* **2004**, *390*, 104.
5 F. Hashimoto, S. Tsukahara, H. Watarai, *Anal. Sci.* **2001**, *17*, Suppl., i81; F. Hashimoto, S. Tsukahara, H. Watarai, *Langmuir* **2003**, *19*, 4197.
6 P. C. Ke, C. A. Naumann, *Langmuir* **2001**, *17*, 3727.
7 C. Caillet, M. Hebrant, C. Tondre, *Colloids Surf., A* **2000**, *161*, 461.

Published on the web (Advance View) December 16, 2006; doi:10.1246/cl.2007.148