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Characteristics of a Liquid/Liquid Optical Waveguide Using Sheath Flow and Its Application to Detect Molecules at a Liquid/Liquid Interface

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The formation conditions and characteristics of a liquid/liquid optical waveguide (LLW) were studied using a two-phase sheath flow, where the inner organic phase flow acted as the core and the outer aqueous flow acted as the clad. In immiscible solvent systems, *i.e*., toluene/water and diethyl ether/water systems, the LLWs were formed in the range of higher than *ca*. 600 of the Reynolds number (*Re*), where the linear velocity of the organic solvent was much higher than that of the aqueous solution. On the other hand, in a miscible solvent system, *i.e*., a tetrahydrofuran/water system, a stable LLW was formed in the range of a much lower *Re* than in immiscible systems. Moreover, the molecules at the toluene/water interface of the LLW were observed with both fluorescence and absorbance measurement systems. In particular, the change in the fluorescence spectrum of 1-anilino-8-naphthalenesulfonate (ANS) at the interface within 1 ms was observed by this method, indicating the usefulness of the LLW for a fast kinetic study of a liquid/liquid interface.

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Introduction

Chemical reactions and mass-transfer phenomena at a liquid/liquid interface have interested researchers of various fields, such as separation chemistry, organic syntheses, and biology. Although it is generally difficult to study chemical reactions at a liquid/liquid interface, mainly because of the influence of the bulk phase on the measurements, several methods have been applied to this subject. Electrochemical methods are among the most important approaches.[1,2] Total-internal-reflection spectroscopy,[3–6] such as fluorescence spectroscopy (TIRF)[3–5] and Raman spectroscopy[6], are the most frequently applied techniques on this subject; *e.g*., time-resolved TIRF was applied to observe the microenvironments of fluorescence molecules at the heptane/water interface where molecules around the interface had a low polarity or an extremely high viscosity of the microenvironment;[3] Ishizaka *et al*. applied TIRF to calculate the thickness of the interface of carbon tetrachloride/water and 1,2-dichloroethane/water and so on, and suggested that the shape and thickness of the liquid/liquid interfaces were largely varied with the kind of solvents.[4,5] Second-harmonic generation (SHG) spectroscopy[7–10] is another technique that is highly sensitive and selective for molecules at a liquid/liquid interface; *e.g*., Nochi *et al*. exhibited that SHG was applicable to alkali metal-ion recognition with the ionophore at the heptane/water, interface; also, the orientation change of the molecules at the interface was observed.[8] The polarities of water/1,2-dichloroethane and water/chlorobenzene interfaces were investigated by measuring the SH intensity of the polarity indicator molecules, which were found to be influenced by the polarity of the bulk phases.[9] Recently, the time-resolved quasi-elastic laser scattering (QELS) method has been applied to study the behavior of a phase-transfer catalyst at the interface.[11,12] These techniques have been applied to study the phenomena in the equilibrium state or a slow reaction process at a static liquid/liquid interface. However, it is difficult to apply these techniques to measure the fast kinetics of reactions at the interface. To monitor such fast reactions, the use of a two-phase flow system, in which a stable liquid/liquid interface is formed, may be one of the promising approaches.

A two-phase sheath flow has often been applied for a low-background optical cell in ultratrace analyses, *e.g*., chemiluminescent and fluorescent detectors for capillary electrophoresis and a fluorescence detector for a DNA sequencer.[13–16] Moreover, the sheath flow has a stable liquid/liquid interface in a micro space, and may have a lot of possibilities for liquid/liquid interfacial analysis. A jet recycling reactor (LJRR) has been reported, which has a cylindrical flow of an organic solvent in an aqueous solution, to study interfacial reactions in two-phase flow systems.[17,18] Recently, Tokimoto *et al*. studied the kinetic process of metal-complexation at liquid/liquid interfaces using a two-phase sheath flow, where a laser-induced fluorescence measurement system with a confocal microscopy system was applied.[19–21] Although the method has been found to be useful for kinetic studies of interfacial reactions, it is not strictly surface-selective because the space-resolution of the method is about 1 μm. We have thus been proposing the use of this sheath flow as a

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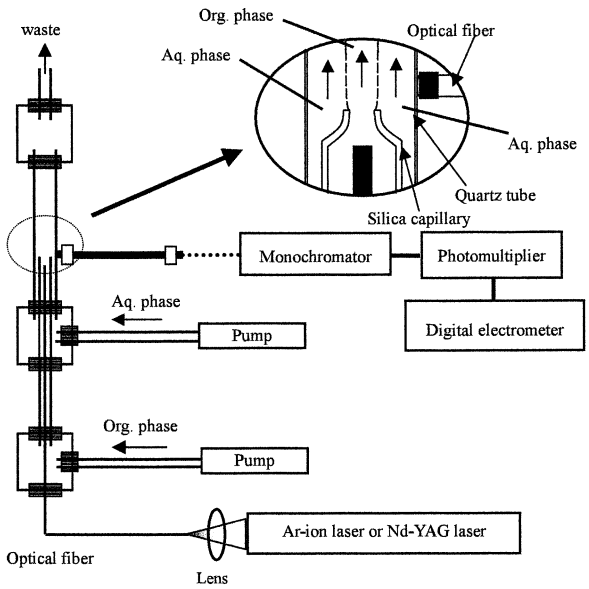

Fig. 1 Liquid/liquid optical waveguide setup. The detection lens can move along the outer silica capillary. The outer capillary was 1.1 mm i.d. and the inner capillary was 0.53 mm i.d. and 0.66 mm o.d.; the tip of the capillary was tapered to about 0.3 mm. To monitor ANS fluorescence, a Nd-YAG laser was used as a light source, and a CCD multi-channel detector was used to monitor the fluorescence spectrum.

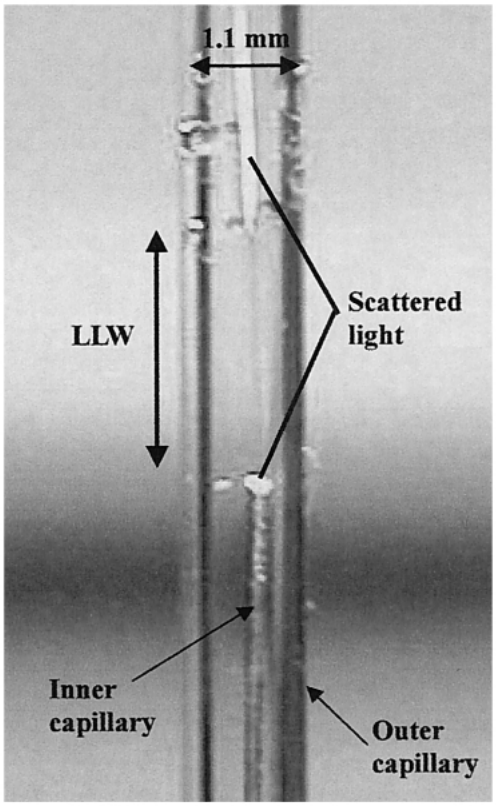

Fig. 2 Photograph of the LLW liquid/liquid optical waveguide. The inner phase was toluene and the outer phase was water, and the flow rates were 5.8 and 3.5 cm^3 min^{-1}, respectively.

liquid/liquid optical waveguide (LLW), in which the inner flow, into which source light is introduced, acts as the core and the outer flow as the clad. This waveguide makes it possible to measure interfacial phenomena with high sensitivity by the evanescent wave of guiding light, *i.e.*, by TIRF spectrometry. In a previous paper,[22] we preliminarily demonstrated that LLW is applicable to both miscible and immiscible liquid/liquid interfaces, and can be used to monitor the fluorescence of molecules at the interface.

In this paper, the formation conditions of LLWs with both miscible and immiscible solvents systems were considered in detail, and the behavior of rhodamine B and ANS, which is known as a solvatochromic fluorophore, at the interface was observed with LLWs. Moreover, LLWs were also applied to absorbance measurements of molecules at the interface.

Experimental

Reagents

Rhodamine B (RB, Wako Pure Chemical Industries, Japan), 1-anilino-8-naphtalene sulfonate (ANS, ICN Biochemical Inc.) and sunset yellow (SY; Tokyo Kasei Co., Ltd.) were used without further purification. Toluene, diethyl ether, ethanol and tetrahydrofuran (THF) were purchased from Wako Pure Chemical Industries, Japan. All of the aqueous solutions were prepared with water purified by a Milli-Q II system (Millipore, USA).

Apparatus

The experimental set up was almost the same as that reported in a previous paper,[10] as shown in Fig. 1. A photograph of the LLW is shown in Fig. 2. The outer capillary was a quartz tube (1.1 mm i.d.) and the inner capillary was a fused-silica capillary (0.150 mm i.d., 0.375 mm o.d. or 0.200 mm i.d., 0.320 mm o.d.) coated with polyimides. The outer and inner capillaries were assembled with T-joints, and the inner capillary was partially inserted into the outer capillary. Toluene (n_D = 1.49), diethyl ether (n_D = 1.38) or THF (n_D = 1.41) flowed into the inner capillary as an organic phase, while water (n_D = 1.33) or an aqueous solution flowed into the outer capillary as the aqueous phase. Toluene and diethyl ether are immiscible with water, while THF is miscible with water at any ratio. Two syringe pumps were used to send the organic solvent and the aqueous solution, respectively. An optical fiber (SF-112, Sumita Optical Grass, Inc., Japan, 0.175 mm o.d., UV transmitted) was inserted into the inner capillary. The source light, which was provided by an argon-ion laser (CW: 488 and 514.5 nm, 100 mW) or an Nd-YAG laser (15 Hz, 355 nm, 4 mJ), was introduced into the inner flow through the optical fiber. An optical fiber (O-OPCL-SMF-011, Nippon Sheet Glass, Japan) was used to collect light signals from the LLW, which was placed in the vertical direction along the outer capillary. The light signals were sent to a photomultiplier tube (R928, Hamamatsu Photonics Co., Japan)) or a multichannel CCD detector (PMA-11, Hamamatsu Photonics Co., Japan).

Measurement

Conditions for the formation of LLW. As shown in Fig. 2, we defined the LLW as the region where stable sheath flow was formed and the minimum scattering light was observed. Although the inner capillary was not located in the exact center of the outer quartz tube in the figure, the stability of the LLWs was scarcely influenced unless the inner capillary and the outer tube did not contact with each other. The dependence of the length of the LLW upon the flow rates of the organic and

aqueous phases was investigated by changing the flow rates of the organic phase at several fixed flow rates of the aqueous phase.

Fluorescence measurement at liquid/liquid interface. Fluorophores of RB and ANS were excited by an argon-ion laser and a Nd-YAG laser, respectively, and their fluorescence spectra from the LLW were monitored by moving the optical fiber for light collection from the tip of the inner capillary to the end of the LLW along with the outer capillary.

Absorbance measurement using rhodamine B as a fluorescent probe. In order to measure the absorbance of molecules at the liquid/liquid interface, we need to know the attenuation of the guiding light due to the absorption. However, it is not possible to measure the guiding light intensity directly by the present system. Thus, a small amount of fluorescent molecules were added as a probe to monitor the intensity of the guiding light, because we could assume that the fluorescence intensity is proportional to the guiding light intensity. That is, we added RB to the aqueous phase to measure the absorption of SY in the aqueous phase, which is a strong absorber of the guiding light. The fluorescence of RB was not quenched nor absorbed by SY. In this absorbance measurement, a thinner capillary (0.030 mm i.d., 0.150 mm o.d.) was attached to the tip of the inner capillary to enhance the sensitivity, which was achieved by an increase in the reflection number of the guiding light at the interface per unit length due to the thinner core.

Result and Discussion

Formation of a liquid/liquid optical waveguide

Figure 3a shows the dependence of the stable length of a liquid/liquid optical waveguide (LLW) upon the flow rate of the organic phase, where toluene or diethyl ether was used as the organic phase and water as the aqueous phase. The flow rate of the aqueous phase was fixed at 3.5 or 4.0 cm^3 min^{-1} for toluene and at 3.0 or 3.5 cm^3 min^{-1} for diethyl ether, respectively. In the ranges of the lower flow rates of the organic phases than 3.2 cm^3 min^{-1} for toluene and 1.5 cm^3 min^{-1} for diethyl ether, respectively, the LLW could not be formed due to the formation of droplets of the organic solvents. In the ranges of higher flow rates, on the other hand, the stable length of the LLW increased along with an increase in the flow rate of the organic phases in both cases. Moreover, the length of the LLW was not influenced by the flow rates of the aqueous phase in the region of a relatively low flow rate of the organic phase, as shown in Fig. 3a. However, the maximum possible length of a LLW was related to the aqueous flow rate, *i.e*., the higher flow rate of the aqueous phase gave the longer LLW. When toluene was used for the organic phase, the LLW was formed in the range of *ca*. 300 to 550 cm s^{-1} of the linear velocity of toluene (flow rate: 3.2 – 5.8 cm^3 min^{-1}) at a linear velocity of 6.3 cm s^{-1} for the aqueous phase (3.5 cm^3 min^{-1}). On the other hand, it was *ca*. 140 to 450 cm s^{-1} (1.5 – 4.8 cm^3 min^{-1}) for diethyl ether under the same flow condition of the aqueous phase. The difference in the linear velocity between toluene and diethyl ether may be caused by the difference in the viscosity of the two organic solvents. The Reynolds number (*Re*) is an important parameter in viscous flow to decide the state of the flow. *Re* is defined by

$$Re = \frac{\rho VD}{\mu}, \quad (1)$$

where ρ, μ, V and D stand for the fluidic density, viscosity, characteristic velocity and characteristic length, respectively. In the case of the LLW, we can regard V as the linear velocity and D as the i.d. of the inner capillary for the organic phase and the difference between the i.d.s of the inner and outer capillaries for the aqueous phase. The region of *Re* where the LLW was formed was *ca*. 660 to 1200 for toluene or *ca*. 610 to 2000 for diethyl ether from Fig. 3a, when the Re for water is *ca*. 63 at 3.5 cm^3 min^{-1}. As described in a textbook of fluid mechanics, *Re* is the ratio of the inertial force and the viscous drag of the flow: in an immiscible two-phase flow system, the inertial force has to exceed the viscous drag to achieve laminar flow, *i.e*., a larger value of *Re* is necessary; when the viscous drag becomes dominant, the formation of droplets occurs and laminar flow cannot be maintained anymore.[23] Our observation on the LLW coincides with such a textbook description. That is, in the region greater than *ca*. 600 of *Re*, the LLWs, *i.e*., laminar flows, were formed in both toluene and ethyl ether. On the other hand, in the region smaller than *ca*. 600 of *Re*, the LLWs could not be formed because of droplet formation. Since the viscosity of diethyl ether (0.24 mPa s) was much smaller than that of toluene (0.59 mPa s), the LLW in diethyl ether was formed in the region of a lower linear velocity than in toluene. Moreover, the slowdown of the inner flow caused a decrease in *Re*, resulting in the formation of droplets, which limited the length of the LLW. The slowdown of the inner flow may have been caused by friction with the outer flow, whose flow rate was much lower than that of the inner flow. On the other hand, it may be difficult to know the factors for determining the upper limit of the flow rates for the formation of the LLWs. As mentioned above, the upper limit of the *Re* is different between toluene (1200) and diethyl ether (2000). Unstable pumping of the solvents at a higher flow rate causes an increase in the friction between the two phases, and thus may cause fluctuation and convection in the flow, which disturb the formation of a laminar flow.

Figure 3b shows the dependence of the stable length of the LLW upon the flow rate of THF and water as the aqueous phase, where THF and water are miscible at any rate. As shown in the figure, a stable LLW of more than 32 mm (the length of the observation window) was obtained in the range of the inner

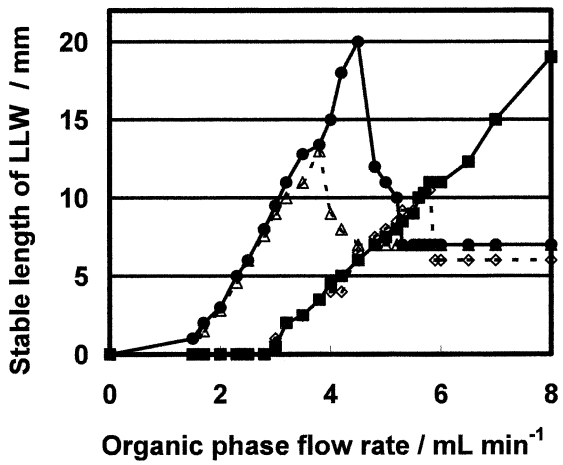

Fig. 3a Stable length of the LLW with immiscible solvent systems. A fused-silica capillary (0.150 mm i.d., 0.375 mm o.d.,) and a quartz tube (1.1 mm i.d.) were used for the inner and outer capillaries, respectively. The organic phase were toluene (■, aqueous flow rate of 4.0 cm^3 min^{-1}; ◇, 3.5 cm^3 min^{-1}) and diethyl ether (●, 3.5 cm^3 min^{-1}; △, 3.0 cm^3 min^{-1}). The region where scattered light was not observed was defined as the stable length of the LLW.

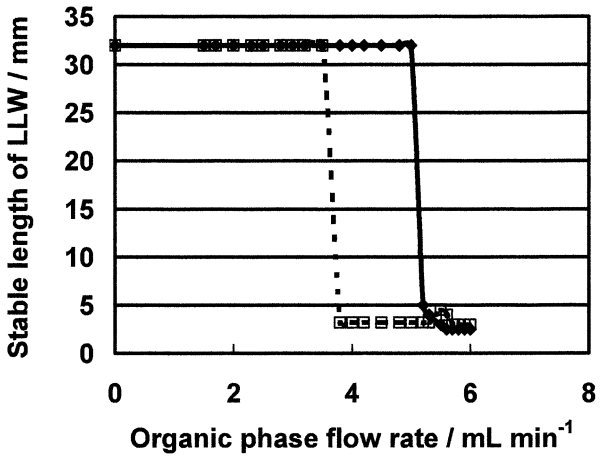

Fig. 3b Stable length of the LLW with a miscible solvent system. The organic phase was THF (aqueous flow rate: ◆, 3.5 cm^3 min^{-1}; □, 3.0 cm^3 min^{-1}) and the other measurement conditions were the same as those of Fig. 3a.

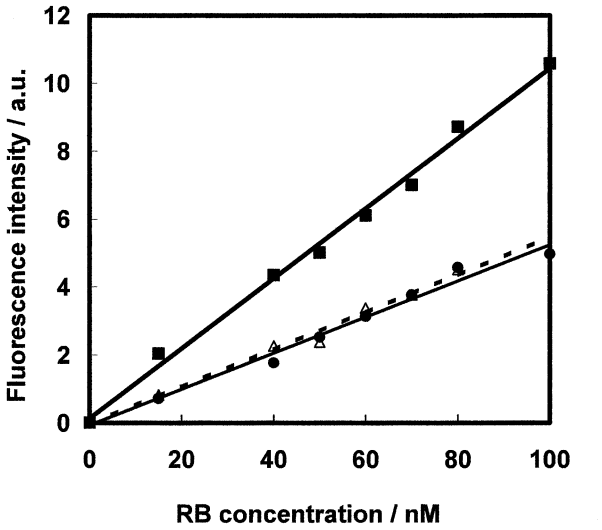

Fig. 4 Calibration curve for RB with the LLW. The outer capillary was 1.1 mm i.d. and the inner capillary was 0.53 mm i.d. and 0.66 mm o.d. and the tip of the capillary was tapered to *ca.* 0.3 mm. Toluene (■, aqueous flow rate of 5.8 cm^3 min^{-1}; organic phase flow rate, 3.5 cm^3 min^{-1}), diethyl ether (●, 3.0 cm^3 min^{-1}, 3.5 cm^3 min^{-1}) and THF (△, 2.5 cm^3 min^{-1}, 3.2 cm^3 min^{-1}) were used as the organic phase. The detection position was 2 mm from the tip of the inner capillary.

flow rate from 0.3 to 5.2 cm^3 min^{-1} (28 to 490 cm s^{-1} of the linear velocity) at 3.5 cm^3 min^{-1} of the outer flow rate (6.3 cm s^{-1} of the linear velocity), and from 0.2 to 3.8 cm^3 min^{-1} (19 to 360 cm s^{-1} of the linear velocity) at 3.0 cm^3 min^{-1} of the outer flow rate (5.4 cm s^{-1} of the linear velocity), respectively. The regions correspond to *Re* values of *ca.* 81 to 1400 for THF at a *Re* value of 63 for water, and *Re* values of *ca.* 55 to 1000 at a *Re* value of 54 for water, respectively. That is, a stable LLW was formed in the region of a much lower flow rate of the inner flow in a miscible solvent system than in an immiscible solvent system. When the flow rate of the inner flow exceeded the upper limit, a stable LLW could no longer be formed. A textbook on fluid mechanics mentions that a stable laminar flow is formed in the range of a lower *Re* value in a miscible solvent system, because droplet formation does not occur in this case, and an increase in the *Re* value, *i.e.*, an increase in the inertial force in the flow, brings about both fluctuation and convection of the flow, resulting in the formation of turbulent flow.[23] Our observation on the LLW with a miscible solvent system also corresponded to the description in the textbook.

As mentioned above, in the immiscible solvent system, the LLW was formed when the linear velocity of the inner flow was much higher than that of the outer flow. These conditions were similar to those of LJRR as well as those by Tokimoto *et al.*[17-21] On the other hand, in the miscible solvent system, a stable LLW, *i.e.*, laminar flow, was formed when the linear velocity of the inner flow was much lower than that in the immiscible solvent system, or close to that of the outer flow.

Fluorescence measurement at a liquid/liquid interface

Figure 4 shows the calibration curve of rhodamine B (RB) in the aqueous phase with the LLW apparatus. In the case of this measurement, 11 mm of the LLW was formed and the RB fluorescence was monitored at the point of 2 mm from the tip of the inner capillary. As shown in the figure, the fluorescence intensity was found to depend largely on the kind of organic solvent for the inner flow; *i.e.*, when toluene (n_D = 1.49) was used, stronger RB fluorescence was obtained when compared with diethyl ether (n_D = 1.38) or THF (n_D = 1.41). Although the reason has not been yet clarified, our present explanation is as follows:

Since RB dissolves in water, but not in diethyl ether and toluene, RB molecules are considered to exist only in the aqueous phase. Moreover, since the excitation light introduced into the LLW was considered to propagate only within the organic phase, only the RB molecules at the region of the interface were excited by the evanescent wave of the guiding light. The guiding light intensity is strongly dependent upon the difference in the RIs between the core and the clad, *i.e.*, the greater is the RI difference, the stronger is the guiding light intensity, because of the greater aperture number. Thus, this difference in the intensity of the guiding light may bring about the difference in the RB fluorescence. In spite of the RI difference between diethyl ether and THF, however, they gave almost the same intensity in RB fluorescence. Although the reason is not yet clear, it might be due to the difference in the diffusion process of RB at the interfaces, since THF/water is miscible; on the other hand, diethyl ether/water is immiscible.

Figure 5 shows the fluorescent spectra of ANS with the LLW with various linear velocities of the organic phase (112 to 250 cm s^{-1}) at a detection position of 1 mm from the tip of the inner capillary. ANS is well-known as a solvatochromic fluorescent reagent, and the peak wavelength of the fluorescent spectrum is 532 nm in an aqueous solution, 525 nm in 10% ethanol, 470 nm in 95% ethanol, or 460 nm in heptane;[12] *i.e.*, the ANS spectrum shows a strong blue shift in a hydrophobic solvent. Thus, we could use this compound as a probe to examine the hydrophobicity of the medium. As shown in Fig. 5, the peak wavelength of the ANS spectrum at the toluene/water interface in the LLW was just between those in water and an organic solvent. This result indicates that ANS molecules at the interface were mainly observed by the LLW system. Moreover, a greater blue shift of the ANS spectrum was observed at a lower linear velocity of the organic phase, *e.g.*, 515 nm at 250 cm s^{-1} to 496 nm at 112 cm s^{-1}. This result means that a longer period (*ca.* 0.2 to 0.9 ms) of contact between the organic phase and the aqueous phase brought about a change in the hydrophobicity of the solvent surrounding ANS molecules at

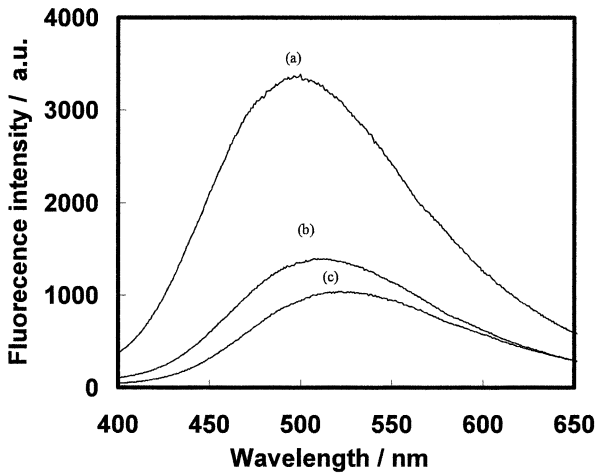

Fig. 5 Fluorescence spectrum of ANS (10 μmol dm^{-3}) at a toluene/water interface with the LLW having various linear velocities of the organic phase. The linear velocities of the organic phase were 112 cm s^{-1} (0.048 cm^3 min^{-1}) (a), 180 cm s^{-1} (0.076 cm^3 min^{-1}) (b) and 250 cm s^{-1} (0.105 cm^3 min^{-1}) (c). The inner capillary was 0.20 mm i.d. and 0.32 mm o.d., and a thinner capillary (0.030 mm i.d., 0.150 mm o.d.) was attached to the tip of the inner capillary. The detection position was 1 mm from the tip of the attached capillary.

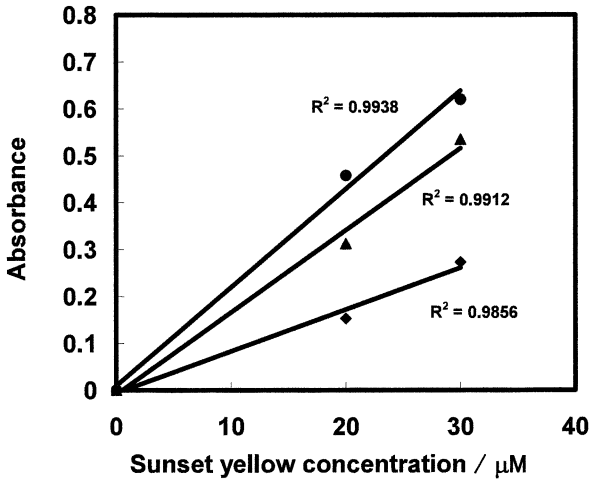

Fig. 6 Dependence of absorbance upon the concentration of SY. The inner capillary was the same as that of Fig. 5. The flow rates of the aqueous phase and the organic phase (toluene) were 0.5 cm^3 min^{-1} and 2.5 cm^3 min^{-1}, respectively. The detection positions were 0.5 mm (◆), 1.0 mm (▲) and 1.5 mm (●). All of the aqueous phases contained 2.0 μmol dm^{-3} of RB.

the interface. The peak wavelengths of ANS at 515 nm and 496 nm correspond to those of ANS in *ca*. 10% ethanol and *ca*. 50% ethanol, respectively. The quantum yield of ANS in *ca*. 50% ethanol is *ca*. 100-times greater than that of ANS in *ca*. 10% ethanol. As shown in Fig. 5, however, the fluorescence intensity of ANS at a linear velocity of 112 cm s^{-1} was only several-times higher than that at 250 cm s^{-1}. Considering these facts, we might have observed the diffusion process of a small part of ANS molecules at the interface of the LLW toward the higher concentration area of toluene during this period. Moreover, the spectra in Fig. 5 may be the sum of the ANS spectra in the media of various hydrophobicities. This result indicates that the LLW may be useful for observing the fast kinetic behavior of molecules at an interface.

Absorbance measurement using a fluorescent probe

Figures 6 and 7 are calibration curves for SY in the aqueous phase with the absorbance measurement using the LLW, where RB was used as a fluorescence probe (see above). When guided light (the argon-ion laser light) irradiates RB in the aqueous phase, it excites RB and progresses with the minimum attenuation. In the same way, when the aqueous phase contains SY in addition to RB, the guided light is further attenuated by SY, because SY is a strong absorber of guided light. That is, the situation is expressed by the following equation:

$$A = -\log\frac{F}{F_0} = -\log\frac{I}{I_0} = \varepsilon cl, \quad (2)$$

where F and I are the intensities of RB fluorescence and the guided light with the presence of an absorber at the measurement position, respectively; F_0 and I_0 are the intensities of the RB fluorescence and the guided light with the absence of an absorber, respectively. Thus, we tried to conduct absorbance measurements by the LLW system using a fluorescent probe. Figures 6 and 7 show a SY solution containing 2.0 μmol dm^{-3} RB, which was used as the aqueous phase at a flow rate of 2.5 cm^3 min^{-1} (4.3 cm s^{-1}). Toluene was used as the organic phase

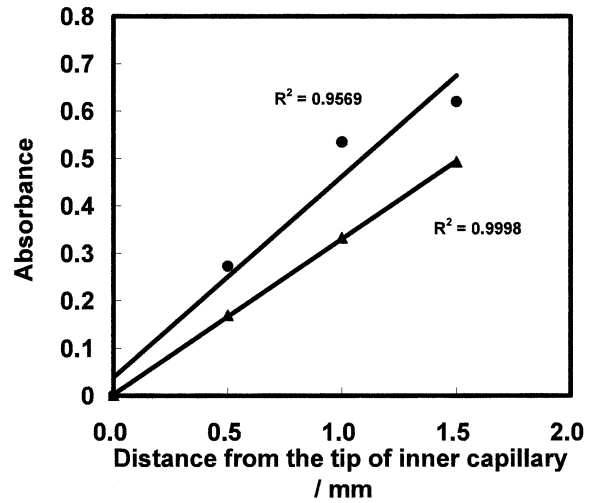

Fig. 7 Dependence of the absorbance for SY at the position of detection. The concentrations of SY in aqueous phase were 20 μmol dm^{-3} (▲) and 30 μmol dm^{-3} (●). The other conditions were the same as those of Fig. 6.

(0.5 cm^3 min^{-1}, 1180 cm s^{-1}). At each detection point, the absorbance was in proportion to the concentration of SY, as shown in Fig. 6. Moreover, Fig. 7 shows the dependence of the absorbance of SY on the detection point, where the transverse axis is the distance of the detection point from the tip of the inner capillary. As shown in the figure, at each SY concentration, the absorbance is in proportion to the distance. Since these results satisfy Lambert-Beer's law well, we can conclude that the absorbance measurement using a fluorescent probe can be useful in optical measurements with the LLW system.

Conclusions

An LLW apparatus was constructed by both immiscible and

miscible solvents with water, and molecules at the interface were successfully detected with both fluorescence and absorbance measurement modes. In particular, changes in the fluorescence spectrum of ANS at the interface within 1 ms were observed by this method. These results may show that this novel system can be useful for fast kinetic studies of a liquid/liquid interface.

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