

Kinetic Study of Fast Complexation of Zinc(Ⅱ) with 8-Quinolinol and 5-Octyloxymethyl-8-Quinolinol at 1-Butanol/Water Interface by Two-Phase Sheath Flow/Laser-Induced Fluorescence Microscopy

Takahira Tokimoto, Satoshi Tsukahara, and Hitoshi Watarai*

Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043

(Received January 8, 2003)

The kinetics of the fast complexation of zinc(Ⅱ) with 8-quinolinol (Hqn) and 5-octyloxymethyl-8-quinolinol (Hocqn) at the 1-butanol/water interface was investigated with two-phase sheath flow/laser-induced fluorescence microscopy. The micrometer-sized two-phase sheath flow system was constructed in a flow cell, in which the 1-butanol phase was introduced as an inner flow and the aqueous phase as an outer flow. The fluorescence emitted from the zinc(Ⅱ) complex was corrected by an objective lens, and detected with a CCD camera. It was revealed that the fluorescence intensity had a maximum value at the interfacial region, and increased with the reaction time up to 2 ms after the formation of the interface. The complexation rate increased with the increase of the initial ligand concentration in the organic phase, giving a saturation curve. The complexation rate of Hqn with zinc(Ⅱ) was three times faster than that of Hocqn at equivalent conditions. A digital simulation taking into account the interfacial formation rate of 1:1 complex, the interfacial adsorption of the ligand and complex, and the diffusion of each species reproduced accurately the observed results. It was suggested that the interfacial adsorption of ligands was delayed and depressed by the preferential occupancy of 1-butanol molecules at the interface.

The liquid/liquid interface is an attractive subject in various fields, such as solvent extraction, organic synthesis, and biological technology.[1] It is essential to elucidate the interfacial complexation mechanisms in the solvent extraction of metal ions for the sake of the design of effective extraction systems. It has been reported that the complexation at the liquid/liquid interface plays an important role in the extraction kinetics.[2] It is, however, generally difficult to measure interfacial reactions directly, because of the large contribution of bulk phase reactions. Therefore, only a limited number of methods have been developed so far to study interfacial reactions at the liquid/liquid interface.[2–6] In addition, the shortest measurable time of interfacial reactions in the reported methods was longer than 10 ms.[4] There has been no method to be able to measure rapid reactions of several milliseconds.

We have recently reported a novel method called a two-phase sheath flow method for the measurement of fast interfacial complexation in a solvent extraction system.[7] When an organic solution was introduced from a micro-capillary tip into a flowing aqueous phase, a fresh liquid/liquid interface was generated. In the chamber of the sheath flow cell, a micrometer-sized cylindrical organic phase flow is formed in the aqueous sheath flow with the same linear flow velocity as that of the organic phase flow. Therefore, the length of the organic phase flow from the capillary tip is converted to the reaction time. It makes possible the measurement of the reaction at the interface for several milliseconds just after the contact of the two phases. It is also possible to integrate the signal, such as weak fluorescence or Raman scattering, from the interfacial species at a fixed position of the flow, which corresponds to an interfacial reaction time. Therefore, we could attain a high S/N ratio in the measurement of the fast interfacial reaction that was difficult to measure by conventional methods.

The kinetic measurement of fast complexation of zinc(Ⅱ) with 5-octyloxymethyl-8-quinolinol (Hocqn) in the 1-butanol/water system was demonstrated by the two-phase sheath flow method coupled with laser-induced fluorescence microscopy.[8] In the 1-butanol/water system, a stable cylindrical flow of 1-butanol was formed because of its extremely low interfacial tension of 1.8 mN m^{-1}. The diameter of the 1-butanol cylindrical flow was 20 μm.

In the present study, the complexation kinetics of zinc(Ⅱ) with 8-quinolinol (Hqn) and 5-octyloxymethy-8-quinolinol in the 1-butanol/water system was investigated by means of the two-phase micro-sheath flow/laser-induced fluorescence microscopy. The spatial resolution of the quasi-confocal fluorescence microscope system was improved to 2 μm, and the fluorescence intensity profile of the organic phase flow was measured. The dependence of the interfacial complexation rates on each reactant concentration was examined. From the rate law of the interfacial complexation, the kinetic mechanisms were discussed in comparison with the digital simulation of the interfacial reaction.

Experimental

Reagents. 8-Quinolinol and 5-octyloxymethyl-8-quinolinol were purchased from Wako Chemical Inc. and were used without further purification. They were dissolved in 1-butanol (GR, Nacalai Tesque), in a concentration from 1.1×10^{-5} to 4.5×10^{-3} mol dm^{-3}. A zinc(Ⅱ) stock solution was prepared by dissolving metal

Published on the web August 15, 2003; DOI 10.1246/bcsj.76.1569

zinc (Wako) in hydrochloric acid and then diluted for the preparation of the aqueous phase solutions. The aqueous phase solutions used in the kinetic experiments contained 1.1×10^{-2} mol dm^{-3} zinc(II) and 1.0×10^{-3} mol dm^{-3} 2-morpholinoethanesulfonic acid (MES, Dojindo) as a buffer, and its pH was maintained at 6.0 ± 0.1. An aqueous solution of sodium hydroxide (GR, Nacalai Tesque) was used to adjust the pH of the aqueous phase. The aqueous phase solutions used for the flow experiments were saturated with 1-butanol. The organic phase solutions were not saturated with water in order to avoid the increase in the background scattering. All the aqueous solutions were prepared with water distilled and deionized through a Milli-Q system (Milli-Q SP. TOC, Millipore). The kinetic measurements were carried out in a thermostated room at 298 ± 2 K.

Fluorescence Spectrum Measurement of Extracted Zinc(II) Complex. The extractions of zinc(II) with Hqn and Hocqn were performed as follows. Five cubic centimeters of an aqueous solution (pH 6.0), including 1.1×10^{-5} mol dm^{-3} zinc(II), 1.0×10^{-3} mol dm^{-3} MES, and 0.01 mol dm^{-3} sodium chloride, and the same volume of a 1.0×10^{-3} mol dm^{-3} Hqn or 1.1×10^{-3} mol dm^{-3} Hocqn 1-butanol solution were shaken for 2 hours. After the phases were allowed to separate, the fluorescence spectrum of the separated organic phase was measured by a luminescence spectrometer (LS50B, Perkin Elmer). The concentration of the remaining zinc(II) was determined by means of an atomic absorption spectrometer (AA-600 and AA-6700F, Shimadzu). The fluorescence spectra of the organic phases after the extraction of the zinc(II) complex were measured with an excitation wavelength of 390 nm. It was shown that the wavelengths at the emission maximum were 550 nm in the Hqn system and 570 nm in the Hocqn system, respectively. From the measurement of the atomic absorption intensity, it was confirmed that almost all zinc(II) ion was extracted into the organic phase as the complex in this condition. Although a self-adduct species, such as $Zn(qn)_2 \cdot Hqn$, was reported in the 8-quinolinol extraction system,[9] the extracted species were assumed to be $Zn(qn)_2$ and $Zn(ocqn)_2$ in the present study, since 1-butanol is a Lewis base solvent. These solutions were used as reference solutions for the conversion of the fluorescence intensity measured in the sheath flow system to the complex concentration.

Two-Phase Sheath Flow/Laser-Induced Fluorescence Microscopy. The detail of the two-phase sheath flow method has been described previously.[7] Briefly, the zinc(II) aqueous solution (saturated with 1-butanol) was introduced as a sheath flow in the micro-channel of the cell at a constant flow rate of 1.00 cm^3 min^{-1} with a syringe pump (Model 11 Single Syringe, Harvard Apparatus, Inc.). The organic phase (not saturated with water) was introduced into the aqueous phase flow from a fused silica capillary at a flow rate of 0.300 cm^3 h^{-1} with another syringe pump, and it formed a micrometer-sized cylindrical flow. In the case of the organic phase flow diameter equaling 20 μm, the linear flow velocities of the two phases were matched to 0.27 m s^{-1}.

The apparatus for laser-induced fluorescence microscopy was the same as that used in the previous study, and shown in Fig. 1. The second-harmonic light (390 nm) of a Ti:sapphire laser (Tsunami, Spectra-Physics) was reflected to the objective lens by a dichroic mirror (DM455, Nikon) and irradiated through a 20× microscope objective lens (S Fluor, NA 0.75, Nikon) to the cylindrical organic phase flow for the excitation of the zinc(II) complex. Prior to the introduction of the laser beam into the inverted microscope (TE300, Nikon), the beam was expanded by the combination of a concave and a convex lens, whose focal

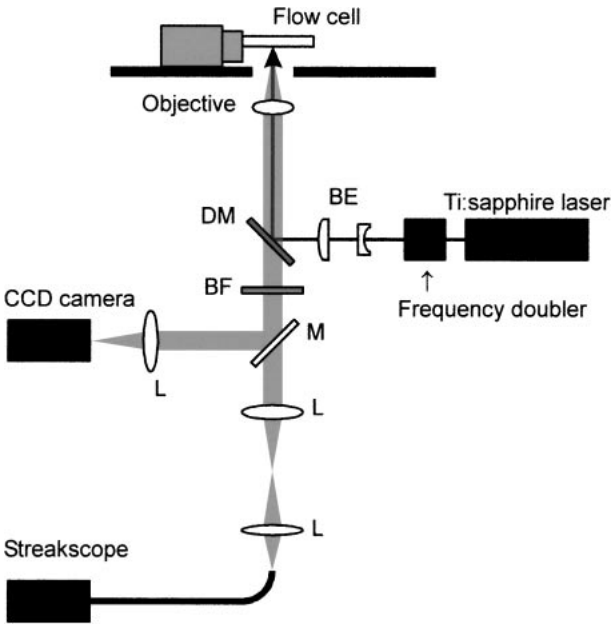

Fig. 1. The apparatus for laser-induced fluorescence microscopy. BE: beam expander, DM: dichroic mirror, BF: barrier filter, M: mirror for changing light path, L: lens for bearing images.

lengths were 15 mm and 100 mm, respectively. Emitted fluorescence was corrected with the same objective lens, and detected by an intensified CCD camera (PentaMAX, Princeton Instruments) placed at the focal position. Also, a streakscope (C4334, Hamamatsu Photonics) was used for the measurements of fluorescence spectra and lifetimes. For the reduction of undesirable signals, such as Rayleigh scattering of the excitation beam and Raman scattering from solvent molecules, a band-pass filter (535AF45, Omega Optical) was set in front of the detectors.

Measurement of Radial Fluorescence Intensity Distribution. In the present study, the fluorescence intensity in a single pixel on the image of a CCD camera was measured and used for the analysis of the complexation rate. Since the size of one pixel was 15 μm × 15 μm, and the area of 1 pixel was regarded as a pinhole, a quasi-confocal microscopy configuration was established. In the confocal fluorescence microscopy, the half depth of the observation region, z, at which the initial intensity is reduced to $1/e$, is calculated from the following equation,[10]

$$z = \frac{er}{\tan \alpha} \tag{1}$$

where r is the radius of a pinhole projected on to the sample plane and α is the aperture half-angle of the microscope objective lens. The numerical aperture, NA, of the objective lens is related to α through the equation,

$$\mathrm{NA} = n \sin \alpha \tag{2}$$

where n is the index of refraction of a medium. The magnification and NA of the objective lens used in the present study were 20 and 0.75, respectively, and thus the depth of the observation region was calculated as 2.0 μm. Figure 2 shows a schematic drawing of the observation region of the laser-induced fluorescence microscope. When the objective lens was moved along the z-axis, the fluorescence intensity distribution was measured along the z-axis. In the measurement of the complexation kinetics, the fluo-

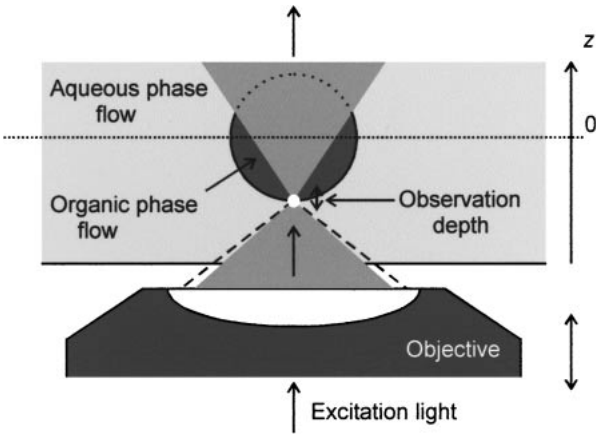

Fig. 2. Schematic drawing of observation area in the section of the two-phase sheath flow. The observation depth was 2 μm calculated with the Eq. 1 in the text. By moving the microscope objective, the fluorescence intensity profile was measured along z-axis.

rescence intensity in the interfacial region on the objective side was measured by moving the focal point downward from the center of the organic phase flow.

Results and Discussion

Formation of Zinc(Ⅱ)–Hocqn Complex. When a 1.1×10^{-2} mol dm^{-3} zinc(Ⅱ) aqueous solution and 1.0×10^{-3} mol dm^{-3} Hocqn 1-butanol solution were flowed at the linear flow velocity of 0.2 m s^{-1}, an increase in the fluorescence intensity was observed along the flow direction. For the identification of the species emitting fluorescence, the fluorescence spectrum and fluorescence lifetime were measured with the streakscope at different positions from the capillary outlet, that is, at different reaction times. Figure 3 shows the fluorescence spectra at the positions of 15 μm, 233 μm, 455 μm, and 1270 μm, which corresponds to the reaction times of 65 μs, 1.0 ms, 2.0 ms, and 5.7 ms, respectively. All of the fluorescence spectra showed an identical shape, with the maximum wavelength at 560 nm and the peak intensities increasing with the increase in the reaction time. Those spectra were similar to that of $Zn(ocqn)_2$ extracted in the organic phase. The fluorescence lifetime was also measured in the flow system. The observed fluorescence lifetime was 1.4 ns, which agreed well with 1.5 ns of $Zn(ocqn)_2$ in 1-butanol. These facts indicated that the increase in the fluorescence intensity was attributable to the formation of the zinc(Ⅱ) complex in the microflow system.

Interfacial Complexation Rate and Its Dependence on Hocqn Concentration. The fluorescence intensity profile in the organic phase flow was measured by moving the focal point of the microscope objective upward or downward. Figure 4 shows the fluorescence intensity profile measured at different distances from the capillary outlet (the reaction times, 0.28 ms, 0.88 ms, 1.4 ms, and 2.0 ms), in which the concentrations of zinc(Ⅱ) and Hocqn were 1.1×10^{-2} mol dm^{-3} and 1.0×10^{-3} mol dm^{-3}, respectively. In Fig. 4, z denotes the position of the focal point, with the origin of the z-axis corresponding to the center of the cylindrical organic flow. The diameter of the organic phase cylinder was 17 μm in this case,

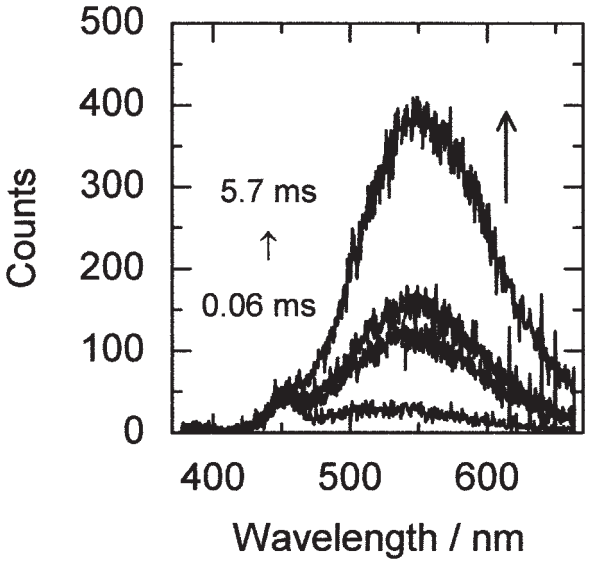

Fig. 3. Change in fluorescence spectrum measured with streakscope at the different reaction time. From bottom to top, the reaction times were 0.06 ms, 1.0 ms, 2.0 ms, and 5.7 ms. The counts (fluorescence intensity) at 560 nm increased with reaction time during 5 ms, proving that the complexation of zinc(Ⅱ) with Hocqn proceeded in the two-phase micro-sheath flow system.

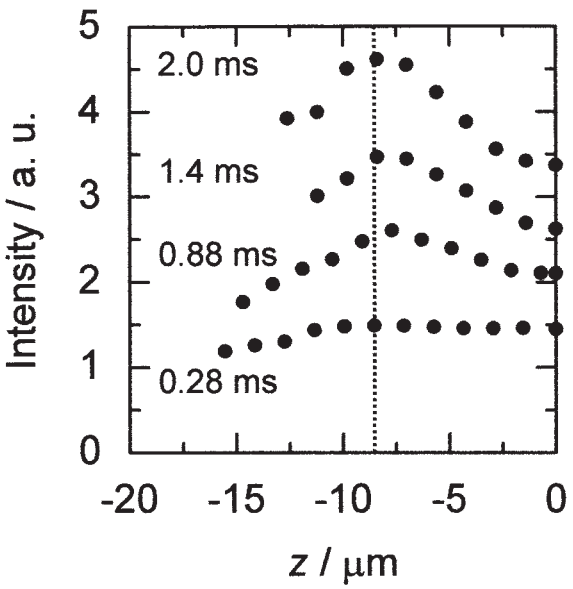

Fig. 4. The fluorescence intensity profiles measured at different reaction times in zinc(Ⅱ)–Hocqn extraction system. The concentrations of zinc(Ⅱ) and Hocqn were 1.1×10^{-2} mol dm^{-3} and 1.0×10^{-3} mol dm^{-3}, respectively. The diameter of the organic phase flow was 17 μm. Therefore, the 1-butanol/water interface was positioned at $z = -8.5$ μm, indicated by the dotted line.

and thus the position at $z = -8.5$ μm was the 1-butanol/water interface. The fluorescence intensity reached a maximum at the interface, and it increased with the reaction time. This result proved that the complexation between zinc(Ⅱ) and Hocqn proceeded in the 1-butanol/water interfacial region. It might

be noted that the measured fluorescence intensity profiles plotted against the position z in Fig. 4 were rather wide. Since the fluorescence intensity included those arising from the free HL in the organic phase and the scattered light which could not be blocked by the barrier filter, the shapes of the fluorescence intensity profiles were made broader, although the intrinsic observation depth was 2 μm in the quasi-confocal microscope used in this study. These wide intensity profiles do not correspond to the concentration profile of the formed complex.

The fluorescence intensities measured by the CCD camera in the two-phase flow system were converted to the concentrations of the zinc(Ⅱ) complex using the calibration curve which was made by the fluorescence intensity of the flowing 1-butanol phase with a known concentration of Zn(Ⅱ) complex. Figure 5(a) shows a plot of the complex concentration against the reaction time at the different initial Hocqn concentrations. The concentration of the complex in the interfacial region increased with the reaction time at all initial Hocqn concentrations. It is also clear that the increase of the concentration is proportional to the reaction time at higher initial Hocqn concentrations. The increase at the lower initial Hocqn concentration, however, was delayed a little and the increase at the initial stage of the complexation was small. This delay time in the initial stage of the complexation may be attributed to the lowering of the diffusion of Hocqn, which is caused by the contact of the 1-butanol and aqueous phases. Therefore, the complexation rates were obtained from the slope in the region excluding the initial stage. Similar experiments were conducted in the zinc(Ⅱ)–Hqn extraction system, and the results were shown in Fig. 5(b). In the case of Hqn, the increase in the complex concentration was proportional to the reaction time without delay. From the slopes in Fig. 5(b), the initial rates for the complexation were obtained. The dependences of the initial rate on the initial HL concentration were shown in Fig. 6. In both the Hocqn and Hqn cases, the initial rate increased with the initial concentration of HL, showing a saturation curve. Moreover, the rates in the Hqn system were about three times larger than those in the Hocqn system under an equivalent condition. One major difference between the properties of Hqn and Hocqn is the value of the distribution coefficient. The distribution coefficients in the heptane/water system were reported as $10^{1.35}$ for Hqn and $10^{4.46}$ for Hocqn.[6] In the 1-butanol/water system, it can be expected that the distribution constant of Hocqn is 10^4 times as large as that of Hqn. If the complexation of zinc(Ⅱ) with Hocqn and Hqn mainly proceeds in the aqueous phase, the complexation rate with Hqn will become 10^4 times faster than that of Hocqn. From the experimental results and this estimation, it was thought that the complexation between zinc(Ⅱ) and Hocqn or Hqn just after contact of the two phases proceeded at the 1-butanol/water interface.

The dependences of the formation rate of Zn(Ⅱ)–Hocqn complex on the zinc(Ⅱ) concentration and pH in the aqueous phase have been observed in the previous report.[8] The complexation rate was proportional to the zinc(Ⅱ) concentration from 0.5×10^{-2} mol dm^{-3} to 2×10^{-2} mol dm^{-3}. The experiments conducted under various pH from 2 to 6 showed that the protonation of Hocqn at the lower pH reduced the complexation rate, proving that the neutral form, HL, reacted with zinc(Ⅱ) ion.

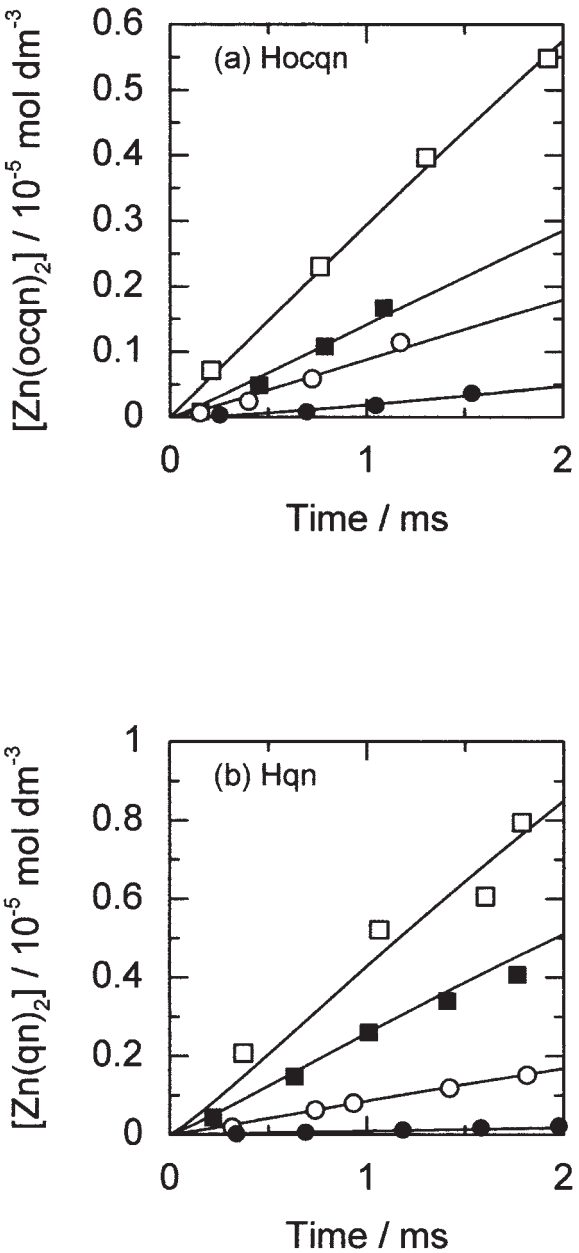

Fig. 5. Increase in the complex concentration in the interfacial region in Hocqn system (a) and in Hqn system (b). $[Zn^{2+}]$ was 1.1×10^{-2} mol dm^{-3} (pH 6.0). The initial HL concentrations were (a) ● 1.1×10^{-4} mol dm^{-3}, ○ 3.4×10^{-4} mol dm^{-3}, ■ 6.8×10^{-4} mol dm^{-3}, □ 4.5×10^{-3} mol dm^{-3}, and (b) ● 1.0×10^{-5} mol dm^{-3}, ○ 1.0×10^{-4} mol dm^{-3}, ■ 3.2×10^{-4} mol dm^{-3}, □ 5.6×10^{-4} mol dm^{-3}. Solid lines represent the initial rate, and the effect of the retarded diffusion on the initial complexation rate was appeared only in the Hocqn system.

Complexation Kinetics in 1-Butanol/Water System. The kinetic studies on the complexation rate of Ni(Ⅱ) with 8-quinolinol derivatives in some two-phase systems have been reported,[11] and it was pointed out that the complexation proceeded both in the aqueous phase and at the liquid/liquid interface. It is crucial to measure the interfacial concentration

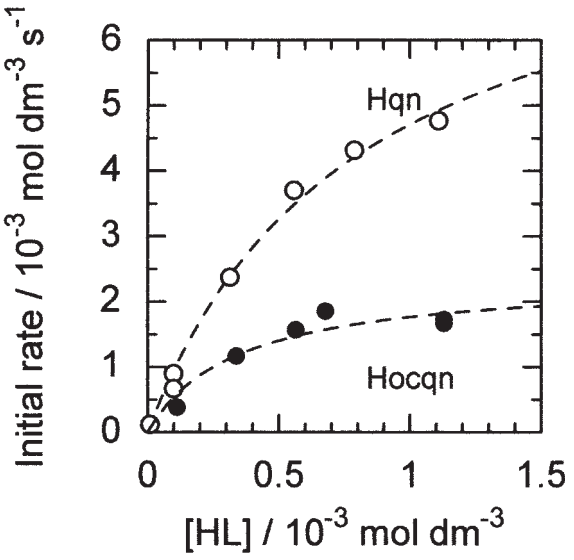

Fig. 6. The dependences of the zinc(Ⅱ)–HL complexation rate on the initial HL concentration in the organic phase. [HL] indicated the initial concentration of Hocqn or Hqn in the organic phase. The slopes obtained from Fig. 6 were plotted against the initial concentrations of HL. In both cases, the rate increased with [HL] and reached to saturation.

of HL and its time course in order to establish the rate law for the interfacial complexation. Under extremely short reaction times, below 2 ms, the transport distances of Hocqn, Hqn, and their zinc(Ⅱ) complexes by diffusion are less than several micrometers. Therefore, distribution equilibrium in the whole two-phase system is not attained. This means that the complexation of zinc(Ⅱ) with HL occurs in the liquid/liquid interfacial region. In this study, the rate law for the complexation of zinc(Ⅱ) with HL could be written from the experimental results in the form of,

$$\frac{\mathrm{d}[\mathrm{ZnL_2}]}{\mathrm{d}t} = k_\mathrm{r}[\mathrm{Zn^{2+}}]_\mathrm{a}\frac{a[\mathrm{HL}]_\mathrm{ini}}{b+[\mathrm{HL}]_\mathrm{ini}} \tag{3}$$

where $[\mathrm{ZnL_2}]$, $[\mathrm{Zn^{2+}}]$, and $[\mathrm{HL}]_\mathrm{ini}$ are the concentrations of $\mathrm{ZnL_2}$ and Zn(Ⅱ) ion, and the initial concentration of the extractants HL, respectively. The subscript a denotes the aqueous phase, k_r the apparent rate constant of the complexation, and a and b the constants.

Digital Simulation of Complexation Rate. The fast complexation rate, within several milliseconds just after contact of the two phases, was observed in this study. It is affected by the diffusion coefficients of the reactants in the bulk liquids, the adsorption and desorption rate constants of HL at the interface, and the complexation rate constants in the bulk aqueous phase and at the interface. Since the observation depth of the fluorescence microscope used in this study (2 μm) is much thicker than the thickness of the liquid/liquid interface (about 1 nm),[12] the observed concentration change includes both the change at the 1-butanol/water interface and that in the bulk phases within the observation depth. Therefore, a digital simulation was adopted in order to discuss the contributions of the diffusion rate and the reaction rate, individually.

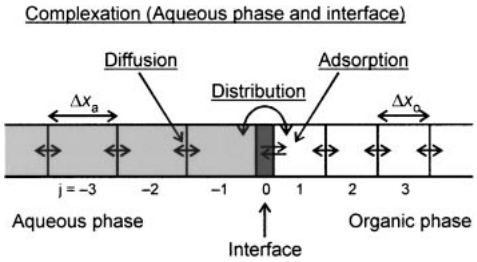

Fig. 7. Digital simulation model for the interfacial complexation. The numbers denote the variable j of each box.

Digital simulation techniques have been used a great deal in electrochemistry, in which an electrode reaction is accompanied by the diffusion of the oxidant and reductant. A conventional model was modified in the present study, as shown in Fig. 7.[13] The boxes at $j < 0$ denote the bulk aqueous phase and those at $j > 0$ are the bulk organic phase. The element at $j = 0$ is the interface, and the thickness of the interface is assumed to be zero. Except for the element at the interface ($j = 0$), each array box is a small cube, in which the cross section is a unit area. The thickness of an element box is Δx_a in the aqueous phase and Δx_o in the organic phase. The model diffusion coefficient, D_M, is defined by the following equation,[13]

$$D_\mathrm{M} = \frac{D_\mathrm{a}\Delta t}{\Delta x_\mathrm{a}^2} = \frac{D_\mathrm{o}\Delta t}{\Delta x_\mathrm{o}^2} \tag{4}$$

where D_a and D_o denote diffusion coefficients of a reactant in the aqueous phase and in the organic phase, respectively, and Δt is the time interval in the digital simulation defined by,

$$\Delta t = \frac{t}{l} \tag{5}$$

where l is the iteration number in the simulation. The value of D_M was set as 0.45 throughout the present simulation,[13] and the iteration parameter l was set as 10^4. Since the observation depth of the fluorescence microscope used in this study was 2.0 μm, and the measured complex concentration was averaged over the observation depth, the simulated complex concentrations were averaged over the boxes corresponding to the observation depth. Since the reaction time measured in this study was typically 2 ms, Δt was calculated as 200 ns in both extraction systems. The diffusion coefficient of HL was estimated from the volume of the molecule, which was approximated as a sphere. The volume of the ligand was calculated by the procedure already reported.[14] For example, in the case of Hocqn in the 1-butanol phase, D_o was estimated as 1.6×10^{-6} cm^2 s^{-1}. Strictly speaking, the value of Δx has to be defined with respect to every species having a different diffusion coefficient. In this simulation, however, the diffusion coefficients of all species were approximated by that of HL in order to fix the form of the box array. Although this approximation is oversimplified, it is acceptable in the case where the diffusion of HL has the predominant role in the rate-determining process. The diffusion length of Hocqn in the organic phase for 2 ms is calculated as 0.8 μm by using the value of the estimated diffusion coefficient, D_o. This length is smaller

than that of the observation region, 2 μm. The values of Δx_o were calculated as 8.4 nm in the Hocqn system and 11 nm in the Hqn system, respectively, from Eq. 4, when Δt was set as 200 ns. The number of boxes in the array was set at 600 in this simulation to cover the observation depth. The change in the concentration in each box was simulated through the following four steps.

[1] In the boxes corresponding to the aqueous phase and the interface, ZnL_2 is formed according to the complexation rate equation. The rate-determining step of complexation of 8-quinolinol derivatives with transition metal ions in aqueous solutions has been reported to be the formation of a 1:1 complex.[11] Therefore, in this digital simulation, it is assumed that the rate-determining step is the formation of the 1:1 complex. The increment in the complex concentration at the interface after the time Δt is represented as follows:

$$\Delta \Gamma_{ML_2} = k_i [Zn^{2+}]_{j=-1} \Gamma_{HL} \Delta t \tag{6}$$

where k_i is the rate constant for the interfacial complexation, and Γ_{HL} and Γ_{ML_2} are the interfacial concentrations of HL and ZnL_2, respectively. To consider the complexation in the bulk aqueous phase, Eq. 7 is used,

$$\Delta [ZnL_2]_j = k [Zn^{2+}]_j [HL]_j \Delta t \quad (j < 0) \tag{7}$$

where k is the rate constant for the complexation in the aqueous phase. In addition, it was assumed that the formation of the ZnL_2 from the 1:1 complex in both systems was sufficiently fast, and the concentration changes of the zinc(II) ion and HL in each box were calculated for the formation of ZnL_2 during Δt.

[2] It is assumed that the adsorption equilibrium of HL and the complex between the interface and the organic phase box adjacent to the interface ($j = 1$) is attained much faster than Δt. The local equilibrium concentrations of the complex and HL are correlated by the Langmuir adsorption equation,

$$k_1 C_{j=1} \left(1 - \frac{\Gamma}{\Gamma_{sat}}\right) - k_2 \frac{\Gamma}{\Gamma_{sat}} = 0 \tag{8}$$

where k_1 and k_2 are the rate constants for adsorption and desorption, respectively. The saturated interfacial concentration, Γ_{sat}, is correlated with the concentration of empty sites at the interface, $[S]_i$, which is actually occupied by 1-butanol molecules, by the following:

$$\Gamma_{sat} = [S]_i + \Gamma_{HL} + \Gamma_{ML_2} \tag{9}$$

It is expected that the ratio of k_1 to k_2 is different for HL and its zinc(II) complex. However, the values of this ratio were set to an identical value in this simulation, since the adsorptivities of both species were expected to be very low and not so different.

[3] The distribution equilibrium between the boxes at $j = 1$ and $j = -1$ is immediately attained with the distribution constant in the 1-butanol/water system defined by,

$$K_D = \frac{C_{j=1}}{C_{j=-1}} \tag{10}$$

where C denotes the concentration of the complex or HL in an element box corresponding to the bulk phases. The value of K_D for Hqn in the 1-butanol/water system was reported as $10^{1.66}$.[15] For Hocqn, K_D was estimated as $10^{4.5}$.[7] A change in the K_D value had no significant effect on the results of the simulation, provided that the value of K_D was larger than 10^2. Hence, the K_D values for HL were provisionally used for the zinc(II) complex in this simulation.

[4] The concentrations in bulk boxes are also changed by diffusion. For the boxes $j = \pm 2, \pm 3, \ldots$, the change in the concentration during Δt is represented as follows:

$$\begin{aligned} \Delta C_j &= \frac{D \Delta t}{\Delta x^2} (C_{j+1} - 2C_j + C_{j-1}) \\ &= D_M (C_{j+1} - 2C_j + C_{j-1}) \end{aligned} \tag{11}$$

where D denotes the diffusion coefficient of a substance.

The initial concentrations of HL and zinc(II) were set as $[HL]_0$ and zero in the organic phase boxes ($j > 0$), and zero and $[Zn^{2+}]_0$ in the aqueous phase boxes ($j < 0$), respectively. The initial concentrations of the zinc(II) complex were set as zero in all boxes, and the initial concentrations at the interface Γ were also set as zero for all species. The concentrations in the terminal boxes in both phases were always equal to the initial bulk concentrations. Appropriate simulation parameters were obtained so as to minimize the square of deviations from the experimental data.

The digital simulation using these initial conditions, however, could not reconstruct the experimental results, which showed a delay in the initial stage of the complexation at lower Hocqn concentrations, such as 1.1×10^{-4} mol dm^{-3}. A possible reason for this is that the 1-butanol solutions used in the measurement were not saturated with water. Therefore, the water molecules near the interface diffused into the organic phase. The flux of the water into the organic phase could reduce the diffusion rate of Hocqn to the interface just after the contact of the two phases, since the hydrophobicity of Hocqn is high. In order to consider the effect of the water flux on the diffusion of Hocqn just after the contact of the two phases, it was assumed that the initial concentration $[HL]_0$ in the boxes close to the interface was zero. The number of the boxes in which the initial HL concentration was set as zero was specified with the value n. The physical meaning of the empty boxes might be that the diffusion of Hocqn is retarded in the interfacial region at the initial stage of complexation because of the diffusion of water into the 1-butanol phase. A recent MD simulation study on the instability of the liquid/liquid interface suggested the formation of nano-droprets in the interfacial nanometer region by the mutual dissolution of the two phases within several microseconds.[16] An example of the change in $Zn(ocqn)_2$ concentration obtained by the digital simulation is shown in Fig. 8, together with experimental results and the time course of the interfacial concentration of Hocqn, simulated under $n = 43$. The best values of n were $n = 10$, 9, and 0 for the initial Hocqn concentration $[Hocqn]_{ini} = 3.4 \times 10^{-4}$ mol dm^{-3}, 6.8×10^{-4} mol dm^{-3}, and 4.5×10^{-3} mol dm^{-3}, respectively. This means that the retardation of the diffusion of Hocqn by water dissolution is larger in the smaller concentration gradient for Hocqn in the interfacial region. The digital simulation including the retardation boxes could accurately reconstruct the experimental results.

The simulation was conducted on other systems, and the parameters were obtained, as summarized in Table 1. In the Hqn system, the value of n was almost zero, and this was attributed

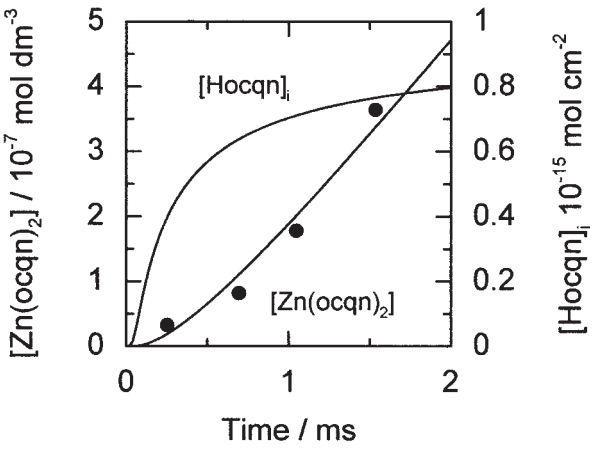

Fig. 8. A result of the digital simulation of the Zn(II)–Hocqn complexation. The initial concentration of Hocqn was 1.1×10^{-4} mol dm^{-3} and $n = 43$.

Table 1. Simulation Parameters that Reproduced Experimental Results of Interfacial Complexation of Zinc(II) in 1-Butanol/Water System

Parameters	Ligand	
	Hocqn	Hqn
$k_i \times \Gamma_{sat}$/m s^{-1}	6.2×10^{-7}	1.8×10^{-6}
k_1/k_2/mol^{-1} dm^3	1.3×10^3	5.5×10^2
K'/cm	1.1×10^{-8}	1.4×10^{-8}

k_i: rate constant of interfacial complexation, Γ_{sat}: saturated interfacial concentration, k_1 and k_2: rate constants for adsorption and desorption in Langmuir model, K': adsorption constant.

to the higher affinity of Hqn to water. From the fitting of the model, the value of k_1/k_2 and the product of k_i and Γ_{sat} were determined, but the individual values could not be obtained. In addition, it was revealed that the complexation in the aqueous phase was negligible in the present conditions.

The rate constant for the complexation of zinc(II) with dithizone in the aqueous phase was reported as 6.9×10^6 mol^{-1} dm^3 s^{-1},[17] suggesting Eigen mechanism. If the complexation in the present system proceeds by Eigen mechanism, the rate constant of the interfacial complexation of zinc(II) is assumed to be of equal order to this value. Hence, the values of Γ_{sat} for Hocqn and Hqn were obtained as 0.90×10^{-14} mol cm^{-2} and 2.5×10^{-14} mol cm^{-2}, respectively. The saturated interfacial concentration, Γ_{sat}, estimated kinetically for the 1-butanol/water system was much smaller than the usual value of 10^{-10} mol cm^{-2}. In the case of the adsorption of protonated Hocqn to the heptane/water interface, the value of Γ_{sat} was reported as 1.6×10^{-10} mol cm^{-2}.[18] The adsorptivity of HL is represented by the adsorption constant K', which is represented by the following equation.

$$K' = \frac{\Gamma}{C_{j=1}} = \Gamma_{sat}\frac{k_1}{k_2} \tag{12}$$

The calculated values of K' are also listed in Table 1. The values of K' are very small in both ligand systems, and it means that the adsorptivity from the 1-butanol phase to the interface is very low. The meanings of the parameters $k_i \times \Gamma_{sat}$, k_1/k_2, and K' refer to $k_r \times a$, $1/b$, and a/b in Eq. 3, provided that $b \gg [HL]_{ini}$.

The extremely low Γ_{sat} in the 1-butanol/water system is attributable to the high affinity of 1-butanol to water, since the interfacial tension in the 1-butanol/water system is 1.8 mN m^{-1} and quite low compared with other hydrocarbon/water systems. Since the affinity of 1-butanol and water molecules is high, 1-butanol molecules may occupy the interface more preferentially than HL. Therefore, the value of Γ_{sat} in this system can be regarded as a hypothetical one that is extrapolated from the kinetic data obtained in diluted conditions, including the occupation of 1-butanol at the interface.

Conclusions

In conclusion, the fast interfacial complexation between zinc(II) and Hocqn or Hqn at the 1-butanol/water interface was successfully measured by two-phase sheath flow/laser-induced fluorescence microscopy. The microscopic fluorescence intensity profiles normal to the flow direction were measured by moving the focal point of an objective lens, and it was proved that the complexation proceeded in the interfacial region. The initial complexation rate increased with the increase in the initial Hocqn concentration, showing a saturation curve. In the case of Hqn, the initial complexation rates were three times faster than those of Hocqn. A digital simulation revealed that the initial stage of the interfacial complexation was governed by the diffusion of the ligand, and suggested that the strong interaction between water and 1-butanol reduced the diffusion of Hocqn in the interfacial region. The detailed diffusion mechanism of the hydrophobic ligand at the interfacial nano-region is the subject of our continuing research. The present study demonstrated the utility of two-phase sheath flow/laser-induced fluorescence microscopy in the study of fast interfacial reactions.

This study was financially supported by a Grant-in-Aid for Scientific Research (A) (No. 12304045) and a Scientific Research of Priority Area (No. 13129204) from the Ministry of Education, Culture, Sports, Science and Technology, and by a fellowship of Osaka University, 21st Century COE Program, Creation of Integrated Ecochemistry.

References

1 A. G. Volkov, "Liquid Interfaces in Chemical, Biological, and Pharmaceutical Applications," Marcel Dekker, Inc., New York (2001).

2 H. Watarai, *Trends Anal. Chem.*, **12**, 313 (1993).

3 W. J. Albery, R. A. Choudhery, and P. R. Fisk, *Faraday Discuss. Chem. Soc.*, **77**, 53 (1984).

4 H. Nagatani and H. Watarai, *Anal. Chem.*, **68**, 1250 (1996).

5 H. Nagatani and H. Watarai, *Anal. Chem.*, **70**, 2860 (1998).

6 T. Shioya, S. Nishizawa, and N. Teramae, *Langmuir*, **14**, 4552 (1998).

7 T. Tokimoto, S. Tsukahara, and H. Watarai, *Chem. Lett.*, **2001**, 204.

8 T. Tokimoto, S. Tsukahara, and H. Watarai, *Anal. Sci.*, **17**, i1245 (2001).

9 F. C. Chou, Q. Fernando, and H. Freiser, *Anal. Chem.*, **37**, 361 (1965).

10 R. Rigler, Ü. Mets, J. Widengren, and P. Kask, *Eur. Biophys. J.*, **22**, 169 (1993).
11 M. L. Dietz and H. Freiser, *Langmuir*, **7**, 284 (1991).
12 H. Watarai, M. Gotoh, and N. Gotoh, *Bull. Chem. Soc. Jpn.*, **70**, 957 (1997).
13 A. J. Bard and L. R. Faulkner, "Electrochemical Methods, Fundamentals and Applications," John Wiley & Sons, Inc., New York (2001), p. 785.
14 D. R. Lide and H. P. R. Frederikse, "CRC Handbook of Chemistry and Physics, 76th ed," CRC Press, Boca Raton (1995), p. 245.
15 H. A. Mottola and H. Freiser, *Talanta*, **13**, 55 (1966).
16 A. Pfennig, *Chem. Eng. Sci.*, **55**, 5333 (2000).
17 J. S. Oh and H. Freiser, *Anal. Chem.*, **39**, 295 (1967).
18 T. Shioya, S. Tsukahara, and N. Teramae, *Chem. Lett.*, **1997**, 695.