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A Study on Liquid−Liquid Distribution Based on Single Picoliter Droplets and in Situ Electrochemical Measurements

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A microelectrode technique combined with the microcapillary injection/manipulation of a single organic droplet in water was developed. The technique was applied to the study of the distribution of a ferrocene derivative across a single-picoliter-nitrobenzene-droplet/water interface and to the simultaneous in situ electrochemical determination of the distributed solute in the picomole−femtomole range. The liquid−liquid distribution processes were discussed in terms of droplet size and solute concentration (in water) dependencies of the interfacial mass transfer rate.

Liquid−liquid extraction, used conventionally as a pretreatment technique for chemical analyses, is based on the separation of hydrophobic or hydrophilic solutes across oil/water interfaces. Recently, distribution of a solute in water into a single organic droplet with a microliter volume and subsequent gas chromatographic quantitation of the solute in the droplet have been reported by Jeannot et al.[1,2] The technique is certainly very useful for microanalysis of various species. It is noteworthy that the specific interfacial area of a droplet increases with decreasing droplet size, so that a solute in a solution phase should transfer more efficiently to smaller-sized droplets. Therefore, we were interested in investigating the distribution of a solute to a single picoliter−nanoliter droplet and simultaneous microanalysis of the solute in the droplet. To explore such a study, we applied following methodologies.

It has been known that a microelectrode can probe a local concentration of an analyte confined in small domains such as microdroplets and biological cells, when a minute sample is positioned *on* or *in* the vicinity of the microelectrode.[3−9] Previously, we reported electrochemical analyses of individual micrometer-sized droplets in oil/water emulsions and discussed microscopic mechanisms of the mass transfer processes of a solute across droplet/water interfaces.[9−12] Electrochemical responses of a microdroplet in a small electrolytic cell have also been reported by several research groups.[4,5,8] Therefore, microelectrode techniques have high potential for in situ analysis of a solute dissolved in a single microdroplet and can be used for detection of a solute in the picomole−femtomole range. For the present purpose, on the other hand, one must manipulate "single" microdroplets in solution. So far, we have reported that a laser trapping technique is very advantageous for manipulating single microparticles in solution.[9−12] Although the laser trapping technique can select and manipulate single microparticles among others, it is very difficult to position or trap a "*single microparticle in a cell*". When a temporal profile of solute distribution to a single microdroplet is followed, nontrapped microdroplets in a cell disturb quantitative analysis of the distribution processes, and therefore, the laser trapping technique cannot be applied directly to the present study. As a possible approach, we think that a microcapillary injection/manipulation technique is a powerful means to manipulate and position a "single" microdroplet in a solution phase, and actually, the technique has been used for studying chemical processes occurring in micrometer-sized biological cells. To study the distribution of a solute from a solution phase to a single droplet with a nanoliter−picoliter volume, we applied the microcapillary technique to injection/manipulation of a single droplet in solution. We expected that the microelectrode and capillary injection/manipulation techniques could provide a potential means to study characteristics of the liquid−liquid distribution processes across a single-microdroplet/solution interface.

In this article, we demonstrate the distribution of 1-ferrocenyl-2-butanol (FCB) from water to single picoliter nitrobenzene droplets and their in situ electrochemical analysis. Ferrocene derivatives are important redox-active species, and FCB possesses an appropriate coefficient of distribution between water and nitrobenzene for the present experiments. Thus, we use FCB as a probe for studying single-droplet-based, water-to-nitrobenzene

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distribution processes. The characteristic features of the present technique are also discussed in detail.

EXPERIMENTAL SECTION

Chemicals and Sample Preparations. FCB was synthesized and purified according to Rebiere et al.[13] Tetrabutylammonium tetraphenylborate (TBA^+TPB^-) was prepared from sodium tetraphenylborate (Kanto Chemical Co., Inc., GR grade) and tetrabutylammonium bromide (Tokyo Kasei Kogyo Co., Ltd., EP grade) and then purified by repeated recrystallizations from acetone/*n*-hexane. $MgSO_4$ (Kanto Chemical Co., Inc., GR grade) and tetrabutylammonium chloride (TBA^+Cl^-; Tokyo Kasei Kogyo Co., Ltd., GR grade) were used after recrystallizations from water and acetone/diethyl ether, respectively. Nitrobenzene (NB; Wako Pure Chemical Industries, Ltd., S grade) was purified by vacuum distillation after passing a column of activated basic alumina. Water was used after distillation and deionization (GSR-200, Advantec Toyo Co., Ltd.).

A water-saturated NB solution of TBA^+TPB^- (0.1 M) and NB-saturated water containing FCB, TBA^+Cl^- (1 mM), and $MgSO_4$ (0.1 M) were used as oil and water phases, respectively. The liquid-junction potential across the NB/water interface was determined by TBA^+ distributed in both phases as calculated by the equation $\Delta_0^w\phi = \Delta_0^w\phi^\circ + (RT/F)\ln([TBA^+(o)]/[TBA^+(w)])$,[14] where $\Delta_0^w\phi^\circ$, R, T, and F are the standard Galvani potential for the NB/water system (−248 mV), the gas constant, the absolute temperature (295 K), and the Faraday constant, respectively. $[TBA^+(o)]$ and $[TBA^+(w)]$ are the concentrations of TBA^+ in the NB and water phases, respectively. Under the present experimental conditions, the NB/water interface was nonpolarized and $\Delta_0^w\phi$ was controlled to be −131 mV.

Apparatus. A gold disk electrode (Bioanalytical Systems, Inc., 25 μm (diameter)), an Ag/AgCl/NaCl(satd) electrode (Bioanalytical Systems, Inc.), and a Pt wire (50 μm (diameter)) were used as working, reference, and counter electrodes, respectively. These electrodes were set in an electrolytic cell. Prior to experiments, the working electrode was polished by alumina (mesh size: 5, 0.5, and 0.05 μm). An aqueous solution (2 mL) containing FCB and the electrolytes was poured into the electrolytic cell set on a stage of an optical microscope (Nikon Co., Ltd., SMZ-U). A glass microcapillary (outer radius of the capillary ∼5 μm) was prepared by a puller (Narishige Co., Ltd., PC-10). The capillary connected with an injection/manipulation system (Narishige Co., Ltd., MN-151, MMW-200/IM-16) was set on the microscope, and a single NB droplet was pushed out to the water phase through the capillary and contacted on the working electrode, as illustrated in Figure 1. Electrochemical responses of FCB distributed from water to the single droplet were measured by using an electrochemical analyzer (Bioanalytical Systems, Inc., BS-1). Injection/manipulation behaviors of a single droplet in the vicinity of the microelectrode were monitored by a CCD camera (Hitachi Denshi, Ltd., KP-C550) attached to the microscope. All measurements were performed at ambient temperature (21−23 °C).

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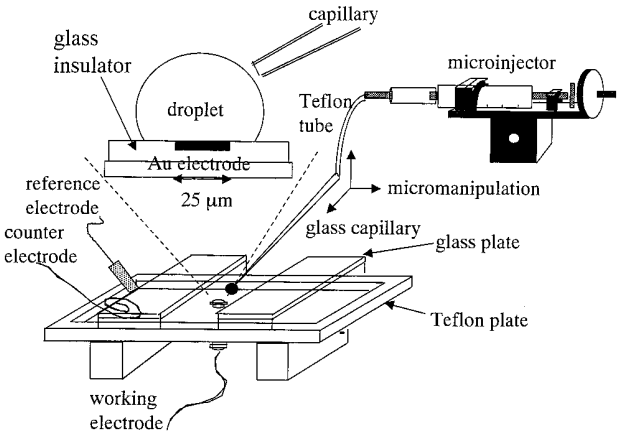

Figure 1. Schematic illustration of a microcapillary/manipulation−microelectrode system.

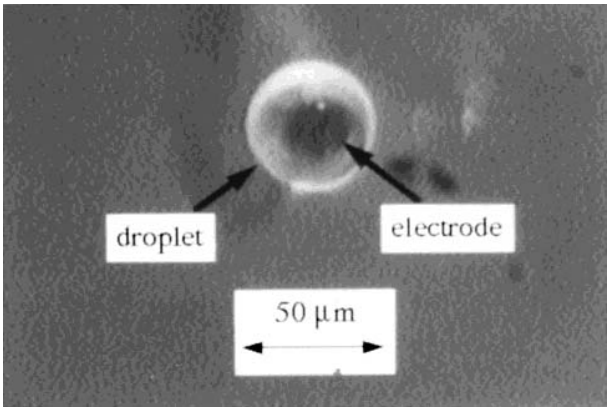

Figure 2. Photograph of a single NB droplet contacted on an Au microelectrode.

RESULTS AND DISCUSSION

Size Dependence of the Water-to-NB Droplet Distribution Rate. A single NB droplet was contacted on the microelectrode through a microcapillary using the injection/manipulation system. Figure 2 shows a typical example of a photograph of a single NB droplet contacted on the microelectrode. Single droplets with a radius (r) of >∼15 μm were easily injected and manipulated by the present system. The contact angle between an NB droplet and the glass insulator around the disk microelectrode was determined to be ∼105° by a separate experiment.[15] The droplet volume (V) can be thus approximated to $0.92\pi r^3$.

Upon injection of a "single" NB droplet into an aqueous FCB solution, FCB in water (concentration C_W) should distribute into the droplet on the microelectrode. The solute concentration in the NB droplet (C_{NB}) at an appropriate time after injection (t) was determined by cyclic voltammetry. Figure 3 shows cyclic voltammograms of FCB distributed from the water phase (C_W = 4.90 μM) to single NB droplets at t ∼ 30 min. The voltammogram of a droplet with r = 92 μm (V = 2.3 nL) showed a diffusion-limited sigmoidal curve, analogous to that observed in a solution or macrodroplet by a microelectrode (Figure 3a).[4,5,8] C_{NB} was then calculated to be 1.0 mM by the equation $I_d = 4nFC_{NB}D_{NB}r_E$, where I_d, n, D_{NB}, and r_E are the diffusion-limited current, the number of

(15) An NB droplet on the glass edge of the electrode in water was observed under an optical microscope to determine the contact angle of the droplet.

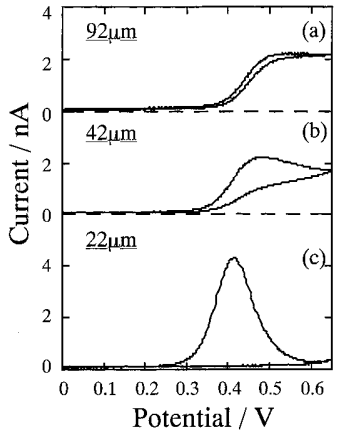

Figure 3. Cyclic voltammograms of FCB (scan rate 20 mV/s) in single NB droplets (C_W = 4.90 μM) with r of 92 (a), 42 (b), and 22 μm (c) observed at t = 33, 33, and 25 min, respectively.

electrons transferred (n = 1 for FCB), the diffusion coefficient of FCB in NB (4.2×10^{-6} cm^2 s^{-1}, determined by cyclic voltammetry), and the radius of the Au microelectrode (12.5 μm), respectively. The distribution coefficient ($K = C_{NB}/C_W$) was determined to be 1.9×10^3 by an ordinary extraction experiment. Therefore, C_{NB} in the droplet at t = 33 min (1.0 mM) was only 10% of that at the distribution equilibrium (9.5 mM).

On the other hand, the cyclic voltammogram for a droplet with r = 22 μm (V = 31 pL) exhibited a symmetrical peak at t = 25 min (Figure 3c). A cathodic current corresponding to reduction of the cation of FCB was not observed. Since $\Delta_0^w\phi$ (−131 mV) is more negative than the cation transfer potential (−75 mV for the ferrocenium cation), the cation exits quickly from the droplet to the water phase, as reported previously.[10,16] FCB in the droplet is electrolyzed completely during the forward potential sweep at 20 mV/s, owing to the small droplet volume and the relatively large electrode area (491 μm^2).[10,17] This is the primary reason for the characteristic voltammogram in Figure 3c. Thus, the current−voltage (I−E) curve in the figure affords information about the number of moles of FCB oxidized in the single NB droplet. The total electric charge (Q) of the FCB oxidation was determined to be 26 nC, which corresponded to the amount of FCB distributed to the droplet as 270 fmol. Therefore, C_{NB} in the droplet was calculated to be 8.7 mM on the basis of the equation $Q = nFVC_{NB}$.[17] The decrease in C_W upon distribution of FCB to a single microdroplet was negligibly small compared to the total amount of FCB in water, so C_{NB}/C_W was calculated to be 1.8×10^3, which is in good agreement with the K value ($=1.9 \times 10^3$).[18]

The I−E curve of FCB in the droplet with r = 42 μm (V = 210 pL) was an intermediate case between those for the droplets with r = 92 and 22 μm (Figure 3b). Since FCB distributed to the droplet is partly oxidized and transferred to the water phase during the forward potential scan at 20 mV/s, the voltammogram exhibits neiter a sigmoidal nor a symmetrical peak curve. The peak current (~2 nA) demonstrates that C_{NB} at t = 33 min is much lower than the value at the distribution equilibrium. Furthermore, the cyclic voltammogram of FCB was highly dependent on r at 30 μm < r < 50 μm (i.e., intermediate case), so cyclic voltammetry is not a direct means for determining the FCB concentration in the droplet, as reported recently.[5,8] In the present study, therefore, we focus our discussion on the results of the droplets with $r \sim 20$ μm.

According to a diffusion-limited distribution model, the rate of FCB distribution from water to a microdroplet is given as in eqs 1 and 2, as demonstrated theoretically and experimentally,[17]

$$Q(t) = Q^{eq}[1 - \exp(-t/\tau_{diff})] \quad (1)$$

$$1/\tau_{diff} = 3D_W/(r^2K) \quad (2)$$

where Q^{eq}, $Q(t)$, D_W, and τ_{diff} are the Q value at the distribution equilibrium, the time dependence of Q, the diffusion coefficient of FCB in water, and the diffusion-limited mass transfer time constant of FCB across the droplet/water interface, respectively. The model implies that the mass transfer rate across the droplet/water interface is equal to the diffusion-limited rate of FCB in water. In the initial stage of water-to-droplet distribution of FCB ($t \rightarrow 0$), the relevant rate is very fast and dependent on the solute concentration in water. A contribution of the reverse (droplet-to-water) mass transfer rate to the forward distribution rate increases with increasing solute concentration in the droplet, so the distribution rate decreases gradually with t ($t \gg 0$). For a given system (D_W and K are fixed constant), on the other hand, τ_{diff} is essentially independent of the solute concentration in water and is determined by r^2 alone. From the D_W ($=6 \times 10^{-6}$ cm^2 s^{-1}) and K ($=1.9 \times 10^3$) values, therefore, τ_{diff} for the present system is predicted to be 31 or 8.4 min for the droplet with r = 42 or 22 μm, respectively.[19] These values demonstrate clearly that distribution of FCB proceeds more quickly for smaller-sized droplets. Equation 1 also indicates that 95% of the FCB molecules to be distributed to a single NB droplet at the distribution equilibrium are transferred to the droplet at a time $t = 3\tau_{diff}$ (i.e., $Q(t) = 0.95Q^{eq}$). Indeed, the observed $Q(t)$ value for the droplet with r = 22 μm at ~30 min agreed very well with Q^{eq} expected for the droplet with r = 22 μm, as discussed above.

Figure 4 shows a time dependence of Q for the droplet with r = 22 μm. The observed time dependence was fitted by Eq 1 (solid curve in Figure 4), and the distribution time constant (τ_{obs}) was determined to be 9.9 ± 0.7 min. τ_{obs} was slightly larger than τ_{diff} (8.4 min, r = 22 μm) predicted by eq 2. Since FCB possesses both hydrophobic and hydrophilic parts, the mass transfer rate across the droplet/water interface is expected to be governed by both diffusion in water and adsorption/desorption of FCB at the droplet interface, similar to that of 1-(hydroxyethyl)ferrocene or 2-ferrocenyl-2-propanol.[11,12] It is noteworthy that $t = 3\tau_{diff}$ is a crude measure for the distribution time constant of FCB and, more precisely, the Q value calculated at $t \geq 4\tau_{diff}$ in eq 2 should be used as that at the distribution equilibrium in the present system.

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(18) Although the curvature of the microdroplet is relatively steep, the chemical potential difference between the droplet and water is calculated to be 0.3 J/mol at r = 20 μm using the Young−Laplace equation.[12] A change in K due to the Young−Laplace effect is thus negligibly small.

(19) Equation 1 is derived under the assumption of spherical diffusion of a solute from water to a droplet. The τ_{diff} value at r = 92 μm cannot be estimated by eq 1, since diffusion of the solute from water to the droplet surface is assumed to be one-dimensional.

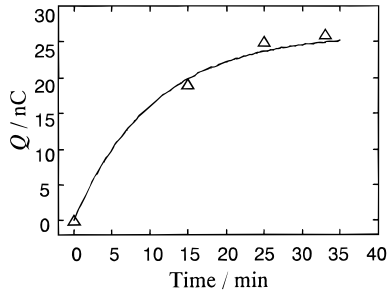

Figure 4. Time dependence of observed Q (△) in a single NB droplet ($r = 22$ μm, $C_W = 4.90$ μM). The solid curve represents the values calculated by eq 1.

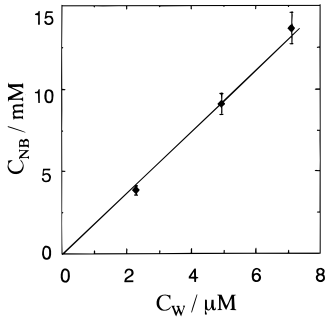

Figure 5. C_W dependence of C_{NB} determined for single droplets ($r = 20-22$ μm) at $t = 4\tau_{diff}$.

These results indicate that the water-to-droplet distribution rate of an electroactive solute increases with decreasing r so that solute distribution proceeds more efficiently for smaller-sized droplets at a given t. As a very important result, furthermore, an electroactive solute at concentrations as low as femtomole−picomole can be analyzed by the present single-picoliter-droplet-based technique.

Determination of the Distribution Coefficient. On the basis of the present technique, the distribution coefficient of a solute can be determined very easily by single-droplet measurements. To demonstrate this, C_{NB} distributed to single droplets was determined at various C_W values. Figure 5 shows the C_W dependence of C_{NB} determined at $t = 4\tau_{diff}$ (28−34 min) for single NB droplets with $r = 20-22$ μm. An error in determining C_{NB} was caused mainly by that in measuring the droplet size under an optical microscope. Thus, a calculation of C_{NB} was performed with an error in r being ± 0.5 μm. This corresponds to an error in C_{NB} of $\pm 8\%$ at $r = 20$ μm. As seen in Figure 5, we obtained a good linear relationship between C_{NB} and C_W. The slope of the $C_{NB}-C_W$ plot gave the distribution coefficient of FCB as $(1.9 \pm 0.1) \times 10^3$, which agreed very well with the K value ($=C_{NB}/C_W$ at the distribution equilibrium, 1.9×10^3). The results indicate that FCB is 1900 times more concentrated in a single droplet than it is in water. Quantitative analysis of FCB can also be done by the present method.

Jeannot et al. reported the distribution of a solute to a single microliter droplet under stirring of the surrounding solution phase and successive quantitation of the solute by injecting the droplet to a gas chromatographic system.[1,2] In the present study, since we used single micrometer-sized picoliter droplets, the distribution time could be reduced to as short as ~30 min without stirring or forced convection of the water phase. The distribution time decreases with decreasing r, which is one of the important advantages of the present method. In general, furthermore, kinetic analysis of liquid−liquid distribution processes is complicated in the presence of forced convection. In view of a kinetic study on liquid−liquid distribution, therefore, single-droplet measurements for a picoliter volume are highly preferable. On the other hand, the solute distributed to a droplet can be analyzed simultaneously by the electrochemical technique. It has been reported that an electroactive solute dissolved in a single droplet can be analyzed by cyclic voltammetry as low as several femtomoles.[10] These discussions and results indicate that the present approach is very simple and potential enough for microanalysis and is worth applying to various samples. At the present stage of the investigation, the technique is applicable to only electroactive solutes. Nevertheless, since fluorescence and absorption spectroscopies have been reported to be applied to single-droplet measurements, the present capillary injection/manipulation technique can be combined with these spectroscopic techniques.[20] Further studies on microanalysis of other samples on the basis of a microcapillary injection/manipulation−electrochemistry−spectroscopy technique are now in progress.

CONCLUSION

A microcapillary injection/manipulation−microelectrode technique was developed and applied to an electrochemical study of the liquid−liquid distribution of a ferrocene derivative. The volume of a micrometer-size droplet contacted on a microelectrode was in the picoliter range, so that the distribution equilibrium of the solute between the single microdroplet and the surrounding water phase proceeded very quickly. The present technique has great potential for mechanistic and kinetic studies of liquid−liquid distribution and interfacial processes at a liquid/liquid boundary. Furthermore, the technique of single-droplet-based distribution and simultaneous in situ analysis are highly promising for further advances in microanalysis.

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