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Integration of a Microextraction System on a Glass Chip: Ion-Pair Solvent Extraction of Fe(II) with 4,7-Diphenyl-1,10-phenanthrolinedisulfonic Acid and Tri-*n*-octylmethylammonium Chloride

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An ion-pair solvent extraction was performed in a microchannel fabricated in a quartz glass chip. The aqueous solution of Fe−bathophenanthrolinedisulfonic acid complex and the chloroform solution of tri-*n*-octylmethylammonium chloride were introduced into the microchannel, and a parallel two-phase laminar flow was formed. The ion-pair product extracted in chloroform was monitored by the thermal lens microscope. The ion-pair product was gradually extracted from aqueous solution into chloroform when the flow was very slow or stopped, while nothing was extracted into chloroform when the flow was fast. The time for extraction in the present 250 μm microchannel, 45 s, roughly coincided with the molecular diffusion time, and the extraction time was at least 1 order shorter compared with the ordinary extraction time using a separatory funnel and mechanical shaking. The microspace in the microchannel was characterized by the large specific interface area and short diffusion distance, and these characteristics may contribute to highly efficient extraction without mechanical shaking. The success of this molecular transport may lead to the integration of more complicated separation and chemical operations on a microchip and more applications.

The rapid development of systems for chemical analysis has been greatly promoted by the progress made within microfabrication technology. These are well-known as micro-total-analysis systems, μ-TAS,[1] or labs-on-a-chip[2] and DNA chips.[3] Most frequently reported have been chip-based capillary electrophoresis systems. Combination of capillary electrophoresis and fluorescence detection leads to an ideal system because of high performance for fluid control, separation, and detection sensitivity. On the other hand, molecular transport methods other than electrophoresis and electroosmosis have seldom been studied.[4−7] Recently, the H-filter, which is a microfabricated fluidic device, has been developed by Yager's group.[4] This is based on the interdiffusion between two fluids. Although this is not molecular transport between two different phases, the H-filter is very useful for the application of liquid−liquid partition on a microscale. Solvent extraction, for example, may also widen the applications of microfabrication technology to analysis. However, no solvent extraction has been performed on a microchip, because electrophoresis and electroosmosis are difficult to induce in typical organic solvents for solvent extraction such as chloroform and ethyl acetate. Extraction is one of the fundamental operations for chemical experiments and, thus, if molecular transport between two different phases, i.e., solvent extraction, can be carried out as chip-based technology, such areas as organic synthesis,[8] combinatorial chemistry,[9] and various analytical systems would be advanced.

The advantage of the microspace for solvent extraction on a microchip, in our opinion, is the scale merit of the small dimension itself, i.e., the large specific interface area, the interface-to-volume ratio, and the short diffusion distance which results in a short diffusion time. A solvent extraction system with high speed and high performance seems possible on a microchip without any mechanical stirring, mixing, or shaking.

We have developed a new method using a combination of capillary action and thermal lens microscopy (TLM).[10] Fluidic transport by capillary action is applicable to aqueous solutions and organic solvents. Photothermal methods are widely applicable because of their ability of detect fluorescent and nonfluorescent substances.[11−13] Recently, we have reported preliminary results

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that a Ni complex in aqueous solution could be extracted into chloroform in a microchannel using this method.[14] However, because the method used capillary action, it was difficult to control the flow rate and flow velocity of the liquid samples. In addition, the behavior of the extraction was still obscure because the initial time course of the extraction could not be observed.

In the present work, we applied the microsyringe pump to fluidic control of the liquid samples and investigated the extraction behavior in the microchannel. This is the full report on solvent extraction using a microchip.

EXPERIMENTAL SECTION

Glass Chip. The details of our glass chip have been previously published.[10,15] The chip was composed of three pieces of quartz glass plate, i.e., the cover, middle, and bottom plates having thicknesses of 170, 100, and 500 μm, respectively. Each plate was a 46 × 66 mm rectangle. A highly focused and intensified CO_2 laser beam was shone on the middle plate to pierce the channel part, and then the beam was scanned to inscribe the channel pattern. The microchannels were made inside the glass chip by sandwiching the middle plate between the top and bottom plates. Four small holes 1 mm in diameter were mechanically bored, ultrasonically, on the top glass for two inlets and two outlets (a drain). These three plates were laminated using optical contact, that is, the plates were polished to an optically smooth and flat ($\lambda/10$) finish, and then laminated together in an oven at 1150 °C without any adhesive.

Figure 1 shows the layout and dimensions of the glass chip. The microchannels were 250 μm wide and 100 μm deep. The glass chip had a solvent extraction region of 10 mm length.

Chemicals. Ammonium iron(II) sulfate, concentrated hydrochloric acid, 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt (bathophenanthrolinedisulfonic acid, disodium salt), sodium acetate, potassium dihydrogen phosphate, sodium hydroxide, tri-*n*-octylmethylammonium chloride (capriquat), and chloroform were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used as received. Chloroform was of analytical grade. The other chemicals were of guaranteed grade. Ultrapure water was obtained using an ultrapure water purification apparatus (Nomura Micro Science, TW-600RU).

The sample solutions were prepared using the protocol of Ishibashi's group.[16,17] Briefly, an 8.9×10^{-4} M Fe(II) stock solution was prepared by dissolving 8.71 mg of ammonium Fe(II) sulfate in 1 mL of concentrated hydrochloric acid and diluting to 25 mL with water. Next, 19.3 mg of 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt was shaken in 25 mL of 2.2 M sodium acetate, and the supernatant solution was used as the stock solution (1.25×10^{-3} M). A buffer solution of pH 6.5 was prepared from 25 mL of 0.2 M potassium dihydrogen phosphate solution, 15 mL of 0.1 M sodium hydroxide solution, and 60 mL of water. A 4 mL portion of 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt stock solution, 10 mL of buffer solution, and 0.05−3.6 mL of the Fe(II) stock solution were mixed and diluted to 50 mL with water and used as the sample solutions (8.9×10^{-7} to 2.1×10^{-5} M). Capriquat was dissolved in 250 mL of chloroform and used as extracting reagent (7.0×10^{-3} M).

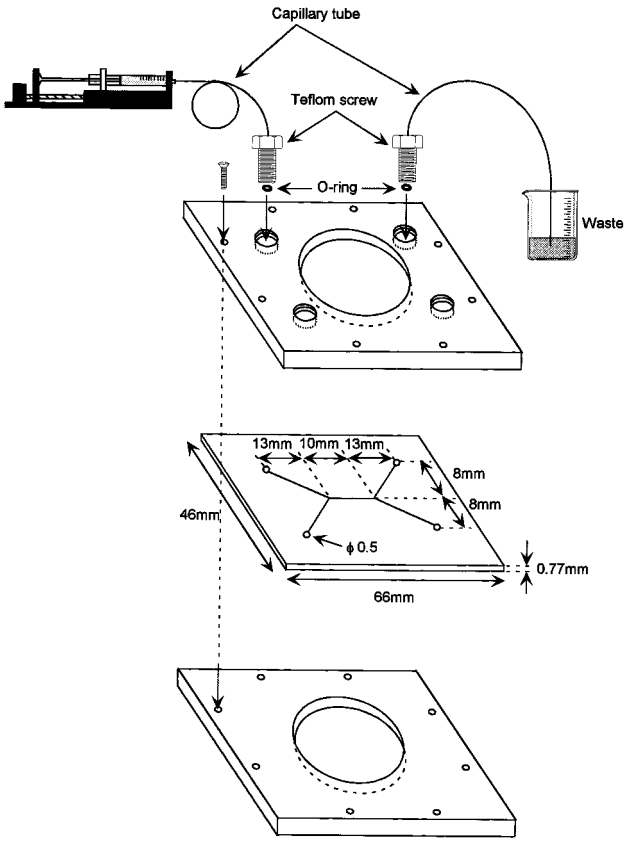

Figure 1. Layout and dimensions of the glass chip. The channel is 100 μm deep and 250 μm wide.

Operating Procedures. The flow rates of the liquid samples used as extracting reagent and aqueous phase were controlled by two syringes (Hamilton, 1710TLL) and microsyringe pump (KD Scientific, model-200). Each syringe needle (Hamilton, KF726) was connected to a custom-built Teflon screw with an O-ring (NOK; i.d., 0.74 mm; o.d., 2.78 mm) through a fused silica capillary tube (GL Sciences; i.d., 0.320 mm; o.d., 0.450 mm) using epoxy-based glue (Ciba-Geigy, Alraldite). The outlets were also connected to a custom-built Teflon screw with an O-ring and a fused silica capillary tube using the same method. They screwed down a custom-built PVC holder as shown in Figure 1. In all experiments, the detection point of the TLM signal was located at the center of the organic phase, just halfway between the interface and sidewall, 5 mm downstream from the intersection point in the Y-shaped microchannel.

Instrumentation. A schematic diagram of the TLM system is shown in Figure 2. An Ar^+ laser (Lexel, model-95, 514.5 nm, 200 mW), which was mechanically chopped by a light chopper (NF Electronic Instruments, 5584A) at 1.0 kHz, was used as an excitation laser and was introduced into the optical microscope (Nikon, specially made) after passing through some prisms and a beam expander. A He−Ne laser (Melles Griot, 632.8 nm, 15 mW) was used as a probe laser. Its beam was introduced from the opposite direction of the excitation laser into the microscope after passing through some prisms and a beam expander. Both laser beams were coaxially aligned by a dichroic mirror and a mirror in the bodytube of the microscope and then introduced into an objective lens (Nikon, CF IC EPI Plan ×20, NA 0.46).

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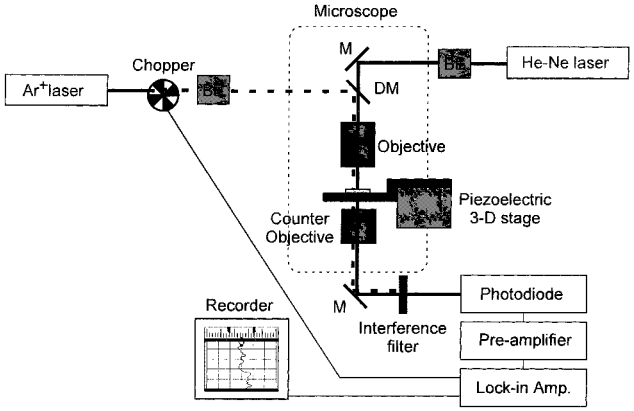

Figure 2. Schematic diagram of the TLM system: BE, beam expander; DM, dichroic mirror; M, mirror.

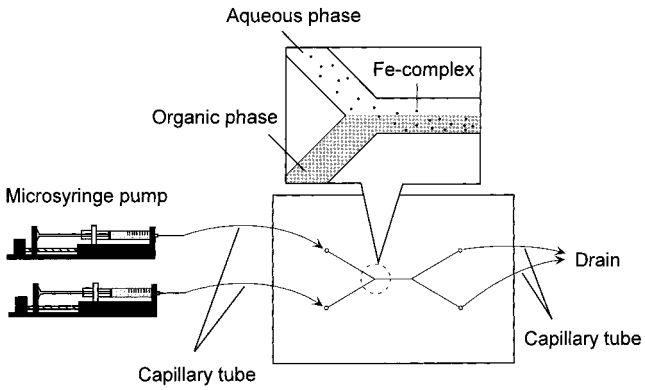

Figure 3. Schematic diagram of the integrated microextraction system.

Transient divergence of the probe beam, induced by the periodically chopped thermal lens effect, was detected as a change in the light intensity. The probe laser intensity passed through a condenser lens, a glass filter (Melles Griot, 03FCG089), and an interference filter (Melles Griot, 03FIL024) before being monitored with a photodiode (Electrooptics Technology, ET-2030). The intensity signal went to a low-noise preamplifier (NF Electronic Instruments, LI-75A) and a lock-in amplifier (NF Electronic Instruments, LI-575) before being recorded on a chart recorder (Rikadenki Electronics, R-62A). The glass chip was mounted on a 3D stage, which could be controlled in 0.5 μm and 0.1 μm steps in $X-Y$ (Sigma Koki, P-AES-60X (IS)-40) and Z (Melles Griot, Nonomover control System II) directions, and three steps were precise enough for positioning the foci of the laser beams. A CCD camera (Victor, KY−F55B), which was mounted on the microscope, displayed a picture image from inside the microchannel.

RESULTS AND DISCUSSION

A schematic illustration of the integrated microextraction system is shown in Figure 3. The aqueous solution of the Fe complex and the chloroform solution of capriquat were introduced into the microchannel by the microsyringe pump at a constant flow rate (2, 4, and 6.7 cm/s), and a parallel two-phase laminar flow was formed in the microchannel. The two solutions did not mix with each other under flow; there was a liquid−liquid (aqueous/organic) interface produced. In the case of ordinary solvent extraction using a separatory funnel, the two solutions in the separatory funnel are separated horizontally by the difference

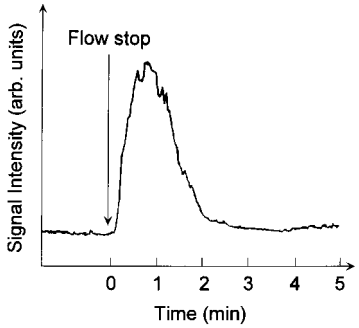

Figure 4. A typical time course of the TLM signal. The concentration of Fe(II) solution was 2.1 μM.

in their specific gravities. However, in a microchannel, the liquid−liquid interface does not have this upper/lower arrangement, rather it is paralleled side-by-side to the sidewalls of the microchannel because surface tension and friction force are much stronger than specific gravity in a microspace such as the microchannel.

The two introduced solutions formed a parallel laminar flow in the microchannel, and no ion-pair product could be extracted into chloroform during flow. This may be because hardly any shear flow diffusion occurs in the laminar flow, which is well-known from hydrodynamic theory. However, ion-pair product was rapidly extracted from aqueous solution into chloroform when the flow was very slow or stopped. In that case, the molecular diffusion can occur across the flow vector when the diffusion velocity component parallel to the flow vector becomes the same or larger than the flow velocity.

The liquid−liquid interface produced in the microchannel was kept for about 10 min after the flow was stopped. After that, the liquid−liquid interface was broken and became droplets. The time course of the ion-pair extraction was detected using TLM. The ion-pair product of the Fe complex and capriquat in chloroform was detected at the center of chloroform phase, just halfway between interface and sidewall, 5 mm downstream from the intersection point in the Y-shaped microchannel. A typical time course of the TLM signal is shown in Figure 4. The intensity of the TLM signal rapidly increased with lapse of time and gradually decreased after about 45 s. Finally, the intensity of the TLM signal was equal to that of the background level, i.e., there was an absence of the ion-pair product in the detection region. The behavior of the TLM signal from rise to peak may correspond to the ion-pair extraction from aqueous solution into chloroform. On the other hand, the behavior of the TLM signal after it reached the peak may correspond to back-migration of the product to the interface. The micelle formation of metal complexes and a quaternary ammonium salt such as trioctylmethylammonium chloride at an interface is a well-known example.[18] Therefore, the ion-pair product extracted may form reversed micelles in chloroform. The concentration of reversed micelles in chloroform would increase with time and the reversed micelles would be in high concentration. Then, the reversed micelles in chloroform would probably be thermodynamically unstable. In a bulk experiment using a separatory funnel and absorptiometer, we found that the absorbance of the ion-pair product in chloroform decreased with lapse of time (several hours). Then, the interface turned red locally; the Fe complex is red. Therefore, the ion-pair product extracted into chloroform was considered to migrate to the interface again. This phenomenon is probably responsible for the

thermodynamical instability of the reversed micelles in chloroform. In the present experiment, the decrease of the TLM signal in the microchannel was also caused by the same reason. Moreover, the aggregate was formed at the interface parallel to the microchannel walls after a long time experiment. This result also supports the assumption that the ion-pair product extracted into chloroform migrated to the interface again. Although further investigations are necessary on this point, the present work indicates that solvent extraction in the microchannel is possible.

In our previous study of Fe(II) chelating reaction, the reaction rate in a microchannel was governed by molecular diffusion.[10] In the present study of solvent extraction, we think that extraction time may be dominated by the molecular diffusion. The time required for molecular diffusion in the microchannel is roughly estimated on the basis of the following relation between diffusion time and diffusion length

$$l = \sqrt{Dt} \quad (1)$$

where l, t, and D are diffusion length, diffusion time, and diffusion coefficient, respectively. When the diffusion length is assumed to be the channel size, 250 μm, for sufficient extraction, and D is ca. 10^{-5} cm^2/s,[19] then the transportation time of the Fe complex from aqueous solution into chloroform, i.e., extraction time, is estimated to be about 60 s from eq 1. The observed value, 45 s, is roughly in accord with the estimated value. On the other hand, in the ordinary extraction method using a separatory funnel (aqueous solution, 10 mL; chloroform, 10 mL) and mechanical shaking (300 times/min), the shaking time for extraction equilibrium required about 20 min. Therefore, the extraction time in the microchannel was times shorter than that of the ordinary method.

One of the dominating factors for the extraction rate is the size of the specific interface area because the interface is the pathway of molecular transport. The specific interface area of our microchannel was 80 cm^{-1}, corresponding to a droplet with a diameter of 375 μm. Using an ordinary shaking machine with a separatory funnel, the specific interface area is about 10^0−10^1 cm^{-1}.[20] Thus, the specific interface area formed in our microchannel corresponds to that formed by a rather vigorous mechanical shaking. Then, the subject substance can be efficiently extracted using only contact with the aqueous solution and organic solvent, without any mechanical shaking. The success of solvent extraction on a microchip without mechanical shaking has invaluable merits such as simplified operation and easy miniaturization. Moreover, the microextraction system on a microchip with higher speed and higher performance may be realized by fabricating the microchannel with a large specific interface area, since the aspect ratio of a microchannel can be easily modified.

The dependence of the TLM signal on the concentration of Fe(II) in aqueous phase is shown in Figure 5. For comparison, the Fe complex was extracted from aqueous solution into chloroform by a separatory funnel (aqueous solution, 20 mL; chloroform, 20 mL) and introduced and then detected in the same microchannel for the reference experiment. This result is also shown in Figure 5. Both results showed good linear calibration curves. However, the TLM signal intensity in the microchannel

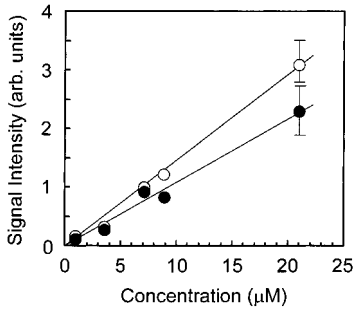

Figure 5. Dependence of the TLM signal intensity on the concentration of Fe(II) solution introduced: microchannel (●), separatory funnel (○).

was slightly smaller than that in the separatory funnel. This result may be caused by adsorption on the glass surface of tri-*n*-octylmethylammonium ions. Since the microchannel was not given any surface treatment, its inside walls would be negatively charged. Thus, a portion of the tri-*n*-octylmethylammonium ions in chloroform might be adsorbed on the walls of the microchannel. The ratio of the glass wall to sample volume in the microchannel is much larger than that in the separatory funnel. Consequently, the effect of adsorption on the inside walls would be greater in the microchannel than in the separatory funnel. Therefore, the extraction efficiency in the microchannel was poorer than that in the separatory funnel.

The volume of the extraction zone in the microchannel was 250 nL (assuming the introduction of same quantity; Fe complex aqueous solution, 125 nL; chloroform, 125 nL), and therefore, the absolute amounts of Fe complex taking part in the solvent extraction were calculated to be 0.1−2.6 pmol. Considering these values and that the detection volume was 8.6 fL,[13] we calculated that 7.7−180 zmol of Fe complex was detected in this integrated microextraction system.

Although further investigations are necessary, we have successfully integrated solvent extraction onto a microchip. The scale merit of the microspace makes the molecular transport easy, without using any mechanical operations. The present work is also the first to successfully perform a separation analysis on a microchip, excluding electrophoresis and electroosmosis methods. This technique is a good laboratory tool, providing small experimental devices which can be used in a wide range of research fields including chemical and biochemical analysis and chemical engineering.

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