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Continuous-Flow Chemical Processing on a Microchip by Combining Microunit Operations and a Multiphase Flow Network

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A new design and construction methodology for integration of complicated chemical processing on a microchip was proposed. This methodology, continuous-flow chemical processing (CFCP), is based on a combination of microunit operations (MUOs) and a multiphase flow network. Chemical operations in microchannels, such as mixing, reaction, and extraction, were classified into several MUOs. The complete procedure for Co(II) wet analysis, including a chelating reaction, solvent extraction, and purification was decomposed into MUOs and reconstructed as CFCP on a microchip. Chemical reaction and molecular transport were realized in and between continuous liquid flows in a multiphase flow network, such as aqueous/aqueous, aqueous/organic, and aqueous/organic/aqueous flows. When the determination of Co(II) in an admixture of Cu(II) was carried out using this methodology, the determination limit (2σ) was obtained as 18 nM, and the absolute amount of Co chelates detected was 0.13 zmol, that is, 78 chelates. The sample analysis time was faster than that of a conventional processing system. Moreover, troublesome operations such as phase separation and acid and alkali washing, all necessary for the conventional system, were simplified. The CFCP methodology proposed here can be applied to various on-chip applications.

The rapid development of microfluidic devices for chemical analyses has been pushed forward by great progress in microfabrication technology.[1] Such devices are known as micrototal analysis systems (μ-TAS)[2] or lab-on-a-chip.[3] The concept behind them is integration of different steps in an analytical process into a miniaturized flow system. The first realization of a μ-TAS was the integration of capillary electrophoretic (CE) separations in fabricated microchannels on microchips,[4] which demonstrated the enormous analytical potential for miniaturized separation devices. Since this realization of microchip-based CE separations, many studies have been made on this subject.[5]

On the other hand, to date, on-chip integration of analytical processes other than CE separations have received less attention.[6−15] All of these applications make use of laminarity of fluids in microchannels. The mixing in laminar flow occurs only by molecular diffusion, in contrast to turbulent flow. Using this characteristic of laminar flow, Weigl and Yager[8] demonstrated the analysis of blood by making use of the fast interdiffusion of human serum albumin from the sample stream into an adjacent, detection stream containing a fluorescent indicator. Whitesides and coworkers[12,14] generated concentration gradients using networks of microchannels designed to control diffusive mixing of substances.

Although a few components for chemical systems have been successfully integrated on a microchip, as described above, the integration of multicomponents has not been achieved. When we construct a chemical plant based on chemical engineering, we design chemical processing required for the chemical plant by combining unit operations. From the analogy of unit operations, such as mixers, reactors, etc., for chemical engineering, we introduced the concept of a microunit operation (MUO) which has led to integration of complicated chemical systems on a microchip. In previous studies, we demonstrated the integration

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of fundamental MUOs, such as mixing and reaction,[16−18] two- and three-phase formations,[19−21] solvent extraction,[19−25] solid-phase extraction,[26,27] heating,[28,29] and cell culture.[30] Moreover, we demonstrated that a stable multiphase flow network can be formed in microchannels.[19,20,31] By using a combination of MUOs and a multiphase flow network, we thought that a complicated chemical system including chemical processing could be integrated on a microchip. We call this methodology continuous-flow chemical processing (CFCP).

In this study, we present the concept of the CFCP and demonstrate, as an example, integration of Co(II) wet analysis in a continuous-flow system. To perform a Co(II) wet analysis, acid and alkali washing are necessary after the chemical reaction to remove the coexisting metal ions; specifically, washing by hydrochloric acid, water, and sodium hydroxide solution are repeated several times.[32] The complete process for traditional Co(II) wet analysis consists of about 40 unit operations. In our integrated system, it is not necessary to carry out as many unit operations as required for the conventional system, because simultaneous washing with acid and alkali is made possible by using multiphase flow in microchannels. The integrated system omits tedious operations and shortens analysis time.

EXPERIMENTAL SECTION

Microchips. Microchips used in this work were fabricated in Pyrex glass substrates using standard photolithographic and wet chemical etching techniques.[19] A schematic illustration and a photograph are given in Figure 1. Each microchip had five inlets and two outlets. The solid lines in Figure 1a corresponding to the microchannels were 50 μm wide and 20 μm deep, the dotted lines were 140 μm wide and 20 μm deep, and the chain line was 90 μm wide and 20 μm deep. The dotted-line parts were fabricated with guide structures at the bottom. A specially designed photomask pattern was used in order to fabricate the microchannels with guide structures to form the stable liquid−liquid interface. Since the photomask had three independent channel patterns for one microchannel, the three channels were etched independently on the glass substrate initially. The depth of the microchannels grew as they remained with etching HF solution as time passed. The three independent microchannels become one microchannel with guide structures at the bottom after a few minutes.

A schematic cross-sectional view of the guide structures is depicted in Figure 2a. Figure 2b shows a 3-D image of the etched

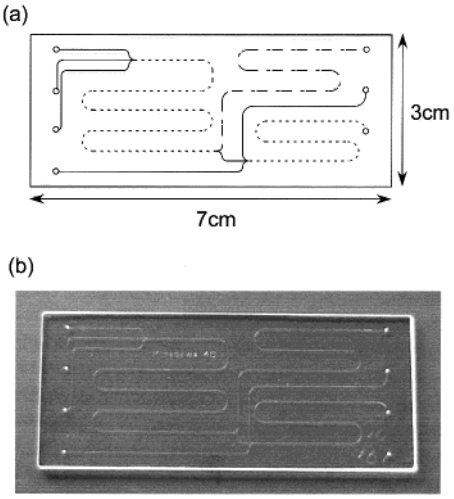

Figure 1. Microchip. (a) Schematic illustration. The solid (—), dotted (•••), and chain lines (-•-) are 50 μm wide and 20 μm deep, 140 μm wide and 20 μm deep, and 90 μm wide and 20 μm deep, respectively. (b) Photograph.

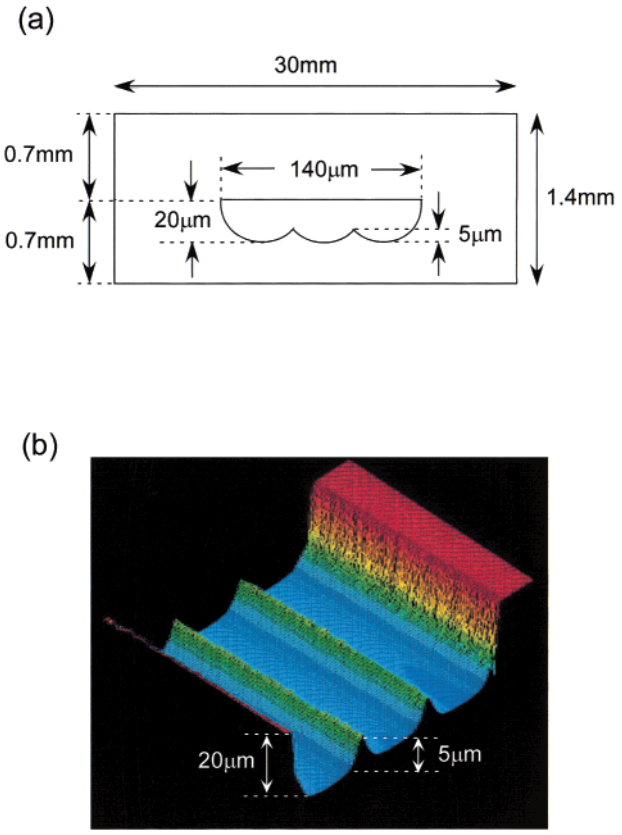

Figure 2. (a) Schematic cross-sectional view of the guide structures. (b) 3-D image of the guide structures.

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microchannel structures as observed with a color laser confocal microscope (Keyence Co., Osaka, Japan, VK-8500). The three-lobed, guide structures at the bottom of the microchannel were clearly seen. The guide height above the bottom of the microchannel was 5 μm. The guide shape and height were controlled arbitrarily using etching time and the gap between lines of the mask pattern.

Chemicals. The Co(II) standard solution (1000 ppm), Cu(II) sulfate pentahydrate, 2-nitroso-1-naphthol (NN), sodium hydroxide solution (1 M), concentrated hydrochloric acid (12 M), and *m*-xylene were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were analytical grade and used as received, except for NN. NN was purified by the literature procedure.[32] Ultrapure water was obtained using an ultrapure water purification apparatus (Nomura Micro Science, Kanagawa, Japan, TW-600RU).

The Co(II) stock solutions ($2-5 \times 10^{-7}$ M) were prepared by stepwise dilution of the standard solution with ultrapure water. The Cu(II) stock solution (1×10^{-5} M) was prepared by dissolving Cu(II) sulfate pentahydrate in ultrapure water. The sample solutions (including 1×10^{-6} M Cu(II) and $0.2-1.5 \times 10^{-7}$ M Co(II)) were prepared by changing the mixing ratio of Cu(II) and Co(II) stock solutions. A NN solution (ca. 3.4×10^{-4} M) including sodium hydroxide was also prepared by the literature procedure.[32]

Apparatus. The thermal lens microscope (TLM) used for detection has been described previously.[17,22,33] In brief, the TLM system consisted of an optical microscope (Nikon, Tokyo, Japan, special design); two lasers, one an Ar^+ laser (Lexel Laser, Fremont, CA, model 95, 488 nm, 200 mW) for the excitation beam and the other a He−Ne laser (Melles Griot, Carlsbad, CA, 05LHP171, 632.8 nm, 15 mW) for the probe beam; and optoelectronic detection systems. The excitation beam, which was modulated at 1060 Hz by a light chopper (NF Electronic Instruments, Yokohama, Japan, 5584A), and the probe beam were coaxially aligned by a dichroic mirror and a mirror in the bodytube of the microscope and then introduced into an objective lens with a numerical aperture of 0.46 and ×20 magnification (Nikon, CF IC EPI Plan). The transmitted beams were collected by a condenser lens. The beams were filtered by a glass filter (Melles Griot, Rochester, NY, 03FCG089) and an interference filter (Melles Griot, 03FIL024), and only the probe beam was monitored with a photodiode (Electrooptics Technology, Traverse City, MI, ET-2030). The signal was synchronously amplified with a lock-in amplifier (NF Electronic Instruments, LI-575) before being recorded on a chart recorder (Rikadenki Electronics, Tokyo, Japan, R-62A). The microchip was mounted on a 3-D stage, which could be controlled in 0.025-μm and 0.1-μm steps in $X-Y$ (Sigma Koki, Saitama, Japan, SGSP20-35) and Z (Sigma Koki, MINI-60X) directions by the stage controllers (Sigma Koki, Mark-102). These steps were precise enough for positioning the foci of the laser beams. A CCD camera (Victor, Kanagawa, Japan, KY-F55B), which was mounted on the microscope, displayed images from inside the microchannel.

Operating Procedures. The flow rates of the liquid samples were controlled through five syringes (Hamilton, Reno, NV, 1710TLL) and three syringe pumps (KD Scientific, Boston, MA, model 100, model 200 and Bioanalytical Systems, Lafayette, IN, MD-1020). A fused-silica capillary tube was used for connection of the syringes and microchip, as described previously.[17,22]

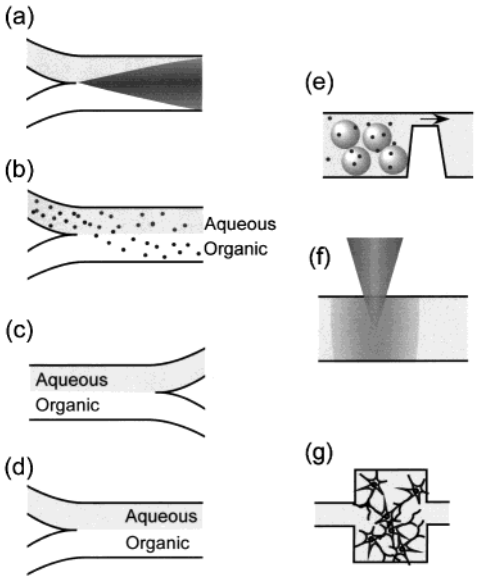

Figure 3. Schematic illustrations of microunit operations: (a) mixing and reaction, (b) solvent extraction, (c) phase separation, (d) two-phase formation, (e) solid-phase extraction, (f) heating, and (g) cell culture.

To measure the position-dependence of the TLM signal along the microchannel, the measurements were carried out by scanning the TLM focal point along the microchannel. In all experiments, the detection point of the TLM signal was located at the center of the *m*-xylene phase. The width of the *m*-xylene phase was about 50 μm.

Safety Considerations. All acids and bases should be handled with great care. *m*-Xylene is combustible, volatile, and toxic; exposure to the eyes or skin should be avoided.

RESULTS AND DISCUSSION

Concept of the CFCP. As mentioned above, we have demonstrated the integration of fundamental MUOs, such as mixing and reaction,[16−18] two- and three-phase formations,[19−21] solvent extraction,[19−25] solid-phase extraction,[26,27] heating,[28,29] and cell culture.[30] These MUOs are shown in Figure 3. By combining these MUOs, we integrated a chemical system on a microchip.

Very recently, we reported the chelating reaction of Co(II) ion with 2-nitroso-1-naphthol and solvent extraction of the Co chelates on a microchip.[33] This result corresponds to the integration of three MUOs (mixing and reaction, two-phase formation, extraction). However, it is difficult to integrate more complicated systems, since the necessary microfluidic components, such as microvalves, are not available at the present time.[34] Then we introduced the concept of continuous-flow chemical processing (CFCP) in order to integrate complicated chemical processing on a microchip. This concept is a combination of MUOs and a multiphase network under the continuous-flow mode.

Figure 4 shows example experimental procedures for conventional Co(II) wet analysis. The conventional procedures are fairly troublesome. In particular, washing processes by hydrochloric

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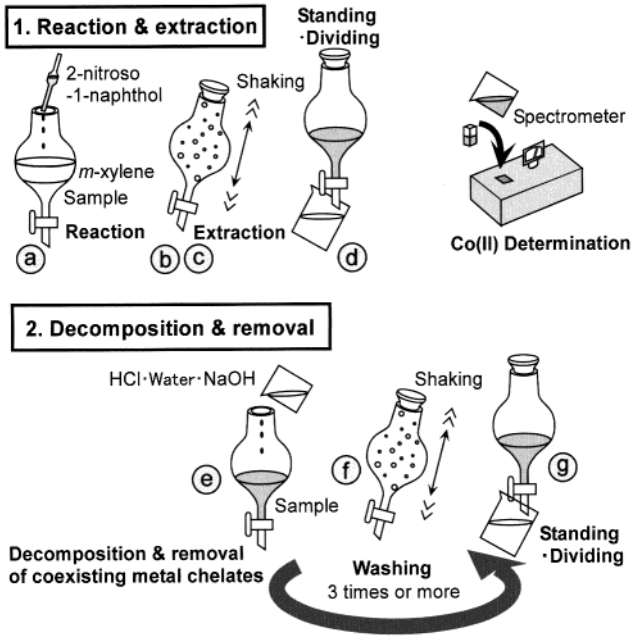

Figure 4. Schematic illustrations of unit operations for Co(II) wet analysis using conventional procedures. (1) Reaction and extraction: (a) confluence, (b) mixing and reaction, (c) extraction, (d) phase separation. (2) Decomposition and removal: (e) confluence (f) decomposition of coexisting metal chelates and removal of decomposed metal ions (HCl), removal of decomposed chelating reagent (NaOH), removal of HCl or NaOH (water), and (g) phase separation. (3) Measurement.

acid, water, and sodium hydroxide solution are repeated 3 or more times.[32] Thus, the number of unit operations for conventional procedures, including confluence, mixing and reaction, extraction, phase separation and so on, is about 40.

Moreover, conventional procedures need a long analysis time. If the Co(II) wet analysis is integrated on a microchip by using CFCP, these problems can be solved. A schematic illustration of Co(II) wet analysis by using CFCP is shown in Figure 5. The microchip consists of two different areas: the former is the reaction and extraction area and the latter is the washing, that is, decomposition and removal, area. In the former area, the sample solution containing Co(II) ions, the NN solution and *m*-xylene are introduced at a constant flow rate through three inlets using the microsyringe pumps. These three liquids meet at the intersection point, and a parallel two-phase flow, consisting of an organic/aqueous interface, forms in the microchannel. The chelating reaction of Co(II) and NN and extraction of the Co(II) chelates proceed as the reacting mixture flows along the microchannel. Since the NN reacts with coexisting metal ions, such as Cu(II), Ni(II) and Fe(II), these coexisting metal chelates are also extracted into the *m*-xylene. Therefore, washing is needed after extraction for the decomposition and removal of coexisting metal chelates.

The coexisting metal chelates decompose when they make contact with hydrochloric acid, and the metal ions are dissolved in the HCl solution. The decomposed chelating reagent, NN, is dissolved in the sodium hydroxide solution.[32] In contrast to the coexisting metal chelates, the Co chelate is stable in HCl and NaOH solutions and remains.[32]

In the latter (washing) area, the *m*-xylene phase containing the Co chelates and the coexisting metal chelates from the former (reaction and extraction) area is interposed between the HCl and NaOH solutions, which were introduced through the other two inlets at a constant flow rate. Then the three-phase flow, HCl/*m*-xylene/NaOH, forms in the microchannel. The decomposition and removal of the coexisting metal chelates proceed along the microchannel in a similar manner as described above. Finally, the target chelates in *m*-xylene are detected downstream by TLM.

The advantages of our approach compared with a conventional method are simplicity and omission of troublesome operations. The acid and alkali solutions cannot be used simultaneously in the conventional washing method, but this becomes possible by using three-phase flow in the microchannel. This chemical processing corresponds to the integration of eight MUOs on a microchip, two-phase formation, mixing and reaction, extraction, phase separation, three-phase formation, decomposition of coexisting metal chelates, removal of metal ions, and removal of reagents.

Stabilization of the Liquid/Liquid Interface Inside Microchannels. To integrate the sequential chemical processing as described above, it is necessary to form an aqueous/organic

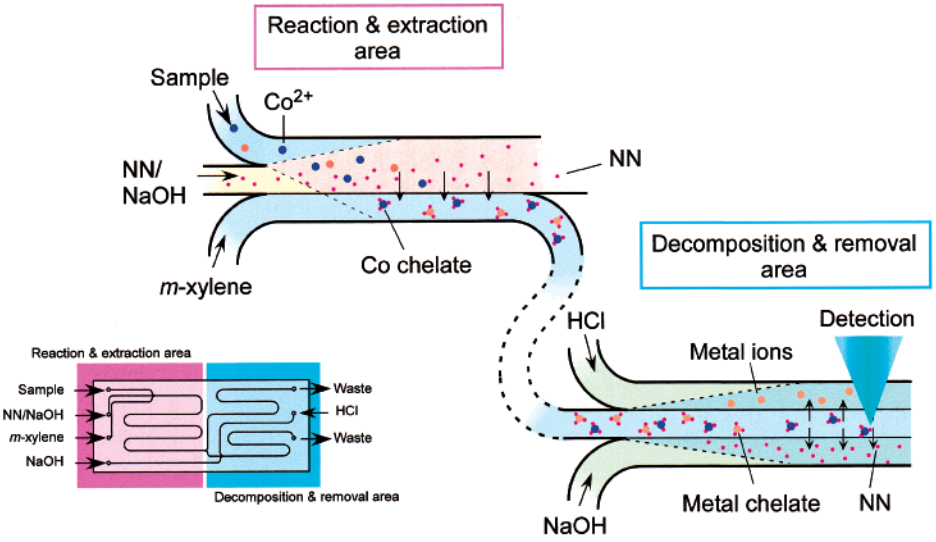

Figure 5. Schematic illustration of Co(II) determination by combining MUOs.

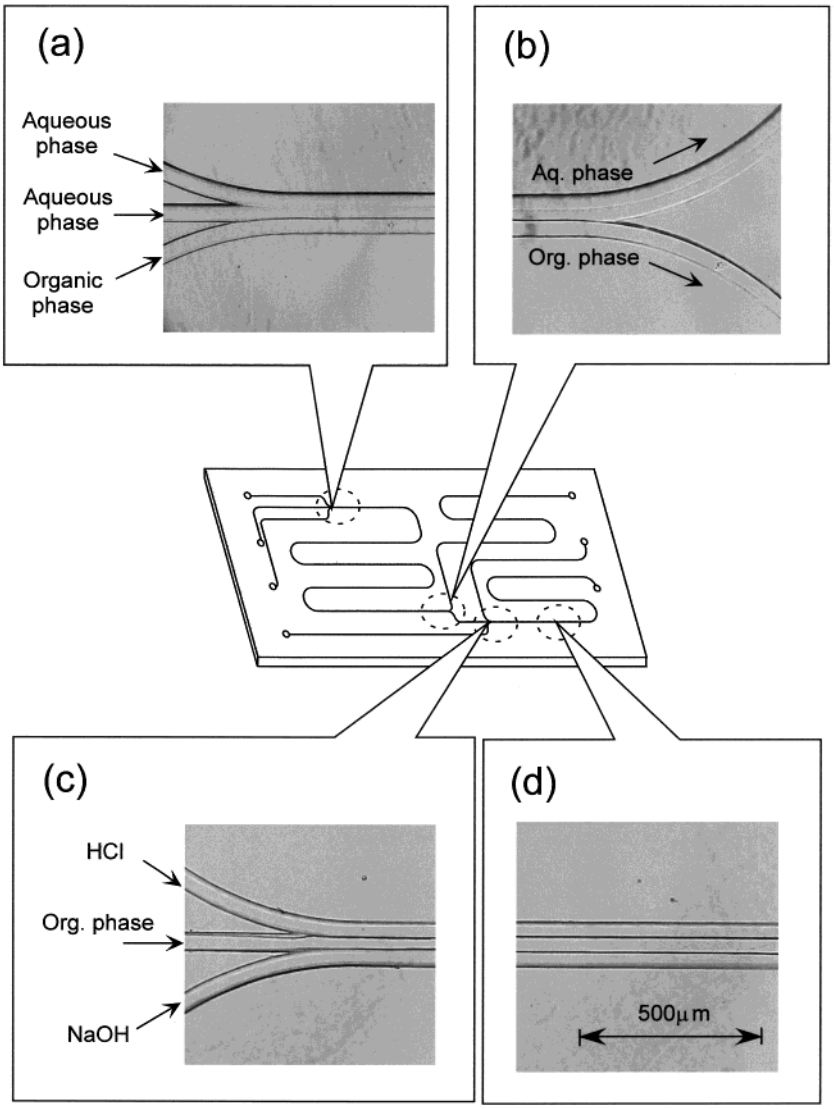

Figure 6. Photographs of the liquid−liquid interface formed in the microchannels: (a) confluence of water and *m*-xylene; (b) phase-separation part; (c) confluence of HCl, organic phase, and NaOH; and (d) three-phase flow.

interface inside the microchannels and to separate the two immiscible solutions. After that, only the separated organic solvent is introduced into the next area, where the formation of an HCl/organic/NaOH interface inside the microchannels is required. To form a stable liquid−liquid interface in the microchannels, we fabricated guide structures inside the microchannels as mentioned in the Experimental Section. These guide structures are a powerful tool for stabilization of the interface of immiscible liquids.[31]

Before carrying out the Co(II) determination, we examined whether stable interfaces are formed or not using the newly designed microchannel with the guide structures. The same experimental conditions as described in the next section were used, except that pure water served as the aqueous phase instead of an aqueous sample solution containing Co(II) and NN. Photographs of the liquid−liquid interface formed in the microchannels are shown in Figure 6.

The expected interfaces are formed throughout the microchannels. Although the reason for the interface stability was not analyzed using hydrodynamics, parameters influencing stability of interfaces, such as surface tension, contact angle, etc., may be favorably affected by the guide structures. Experimentally, it is impossible to form interfaces inside the microchannels over a long distance without the guide structures. Phase separation between aqueous and organic phases is also not possible without them.

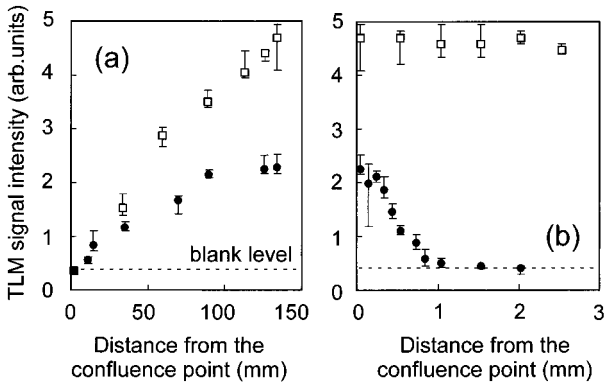

Figure 7. Dependence of the TLM signal of Co(II) (□, 1×10^{-7} M) and Cu(II) (●, 1×10^{-6} M) solutions on the distance from the confluence of (a) sample, reagent, and *m*-xylene and (b) HCl, *m*-xylene, and NaOH.

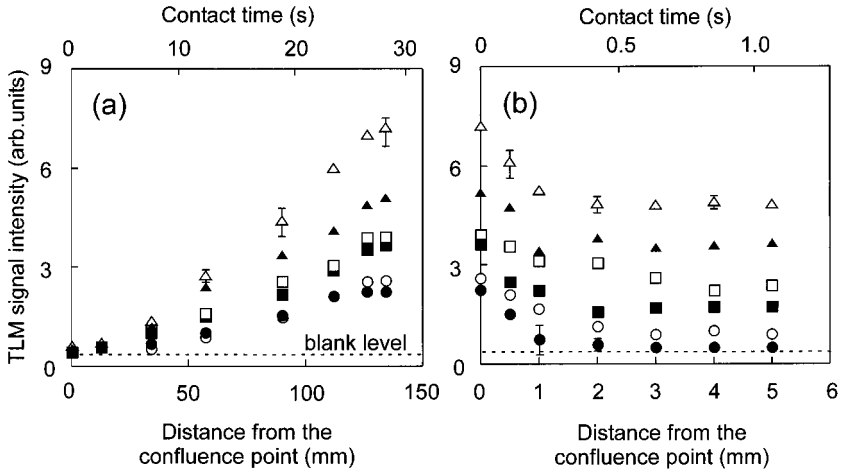

Figure 8. Dependence of the TLM signal of the admixture sample on the distance from the confluence of (a) sample, reagent, and *m*-xylene and (b) HCl, *m*-xylene, and NaOH. The samples were (△) 1.5×10^{-7}, (▲) 1.0×10^{-7}, (□) 8.0×10^{-8}, (■) 5.0×10^{-8}, (○) 2.0×10^{-8}, and (●) 0 M of Co(II). All samples included 1×10^{-6} M of Cu(II).

From the viewpoint of extraction efficiency, a lower guide height and lower flow rate are better, because extraction efficiency depends on the size of the specific interface area and the contact time between aqueous and organic phases. Since the interfaces became unstable when we used a microchannel having a guide height less than 5 μm under our experimental conditions as described later, the guide height was fixed at 5 μm.

Co(II) Determination. To carry out a performance test on our methodology, that is, sequential chemical processing including the chelating reaction, solvent extraction, and the decomposition and removal of coexisting metal chelates, we investigated the dependence of the TLM signal for *m*-xylene on the distance from the confluence point of the liquids. Test samples were Co(II) (1×10^{-7} M) and Cu(II) solutions (1×10^{-6} M). The flow rate of *m*-xylene was 0.4 μL/min; flow rates of the solutions, sample, reagent, HCl, and NaOH, were each 0.2 μL/min. For these flow conditions, extraction equilibrium was almost reached by the end of the microchannel in the reaction and solvent extraction area, that is, the position in front of the phase separation point. The length of the microchannel from the confluence point to the phase separation point was about 130 mm. The results are shown in Figure 7. The intensity of the TLM signal for both sample solutions gradually increased with the microchannel length, as shown in Figure 7a. The intensity of the TLM signal for Cu(II) solution was lower than that for Co(II) solution, because the absorbance of the Cu chelate at 488 nm is lower than that of Co chelate. As shown in Figure 7b, for the sample containing only Co(II), the intensity of the TLM signal remained constant. Thus, the Co chelates were not decomposed by HCl, and they were present in *m*-xylene. On the other hand, for the sample containing only Cu(II), the intensity of the TLM signal rapidly decreased with the microchannel length. Finally, the intensity of the TLM signal about 1 mm downstream from the confluence point of HCl, *m*-xylene and NaOH was equal to that of the blank level, indicating that the Cu chelates had been decomposed and completely removed.

Since satisfactory results were obtained, the admixture sample of Co(II) and Cu(II) was analyzed in our system. The results are shown in Figure 8. In the reaction and extraction area, the intensity of the TLM signal gradually increased with the microchannel length, since both Co(II) and Cu(II) ions reacted with NN and were extracted into *m*-xylene. In the washing area, the Cu chelates were decomposed and removed. In contrast with the Cu chelates, the Co chelates still remained in the *m*-xylene phase. Therefore, the intensity of the TLM signal gradually decreased with the microchannel length and became constant about 2 mm downstream from the confluence point. From the TLM signal 3 mm downstream from the confluence point of HCl, *m*-xylene, and NaOH, we obtained the calibration curve as shown in Figure 9. The calibration curve had good linearity ($R^2 = 0.979$). The determination limit (2σ) of Co(II) in the admixture of Cu(II) was 1.8×10^{-8} M. Considering this value and the detection volume of 7.2 fL,[24,35] we calculated that 0.13 zmol (78 chelates) of Co chelates were detected in this integrated system.

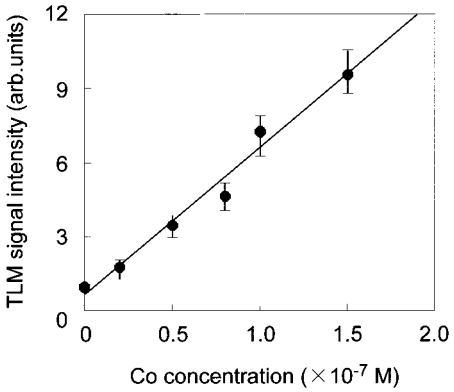

Figure 9. Calibration curve. The solid line is the least squares best fit.

CONCLUSIONS

The analyzing time for a sample in this integrated chemical system is faster than that of the conventional system. Moreover, troublesome operations, such as phase separation, and acid and alkali washing necessary for the conventional system are simplified. The methodology demonstrated here, CPCP, is expected to be applicable to development of more complicated processing

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systems. Although the results satisfied our current purpose, we saw an unexplained phenomenon. A fairly long time (ca. 30 s) was needed for the chelating reaction of metal ions with 2-nitroso-1-naphthol and solvent extraction of the metal chelates. On the other hand, the decomposition and removal of the coexisting metal chelates was completed in a short time (less than 1 s). This cannot be entirely explained by molecular diffusion theory in liquids. It has also been observed for different reaction systems using microchips.[36,37] This is an interesting topic in microchip chemistry and current studies in our laboratory are investigating it further.

(36) Tanaka, Y.; Slyadnev, M. N.; Sato, K.; Tokeshi, M.; Kim, H.-B.; Kitamori, T. *Anal. Sci.* **2001**, *17*, 809−810.
(37) Hisamoto, H.; Kikutani, Y.; Uchiyama, K.; Tokeshi, M.; Hibara, A.; Kitamori, T. Manuscript in preparation.

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