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Integration of a microextraction system Solvent extraction of a Co–2-nitroso-5-dimethylaminophenol complex on a microchip

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Abstract

A newly designed microchannel for solvent extraction was fabricated in a quartz glass chip and applied to solvent extraction of a Co–2-nitroso-5-dimethylaminophenol complex. The aqueous solution of Co complex and toluene were introduced into the microchannel, and the Co complex extracted in toluene was detected by thermal lens microscopy (TLM). The Co complex was quickly extracted into toluene when the flow was stopped. The observed extraction time, ca. 50 s, was almost equivalent to the value calculated using the diffusion distance and diffusion coefficient. The dependence of the TLM signal on the concentration of the Co complex showed good linearity in the range of $1 \cdot 10^{-7}$–$1 \cdot 10^{-6}$ *M*.

Keywords: Extraction methods; Microchips; Instrumentation; Thermal lens microscopy; Metal complexes; Nitrosodimethylaminophenol; Cobalt

1. Introduction

In recent years miniaturized devices, such as microchips, with microfabricated structures for the analysis and synthesis of chemicals and biochemicals have been shown to offer many advantages, e.g., reduced necessary sample quantity, shorter analysis time, and so on [1]. With the realization of chip-based technologies such as DNA analyses [2], drug discovery and screening [3], water and food analyses, environmental sample analyses and organic synthesis [4], the methodology of scientific research can be changed dramatically. On the other hand, combinatorial chemistry has been developed and applied by the pharmaceutical industry in particular [5]. This technology, which involves the microscale synthesis and screening of vast libraries of organic compounds, has resulted in great improvements in the efficiency of testing new molecules for drug discovery. Although combinatorial chemistry using microchips would be desirable, nothing has been published. This seems to be because fluid flows are driven using electroosmotic flow in most chip-based studies. Electrophoresis and electroosmosis are difficult to induce in organic solvents; their occurrence depends on the ratio of the solvent dielectric constant

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and viscosity [6]. Thus, methods other than electrophoresis and electroosmosis should be pursued in order to develop complicated systems such as for combinatorial chemistry.

We have developed a new method using a combination of pressure-driven flow and thermal lens microscopy (TLM) [7,8]. Fluidic transport by pressure-driven flow is applicable to aqueous solutions and organic solvents. TLM, based on the photothermal effect, is very useful because of its ability to detect fluorescent and non-fluorescent molecules [9]. This method seems to be more suitable for complex chemical systems.

Recently, we have succeeded in integrating an ion-pair solvent extraction of Fe(II) with 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid and tri-*n*-octylmethylammonium chloride on a microchip [7]. This is the first report of solvent extraction using a microchip. Solvent extraction is a fundamental operation in the chemical experiments which are necessary for organic synthesis.

In the present work, we have fabricated a newly designed microchip with reference to our previous study [7] in order to stabilize the liquid–liquid (aqueous–organic) interface in the microchannel and we have applied it to the solvent extraction of a Co–2-nitroso-5-dimethylaminophenol complex (Co–nitroso-DMAP).

2. Experimental

2.1. Glass chip

The details of our glass chip have been previously published [10,11]. The chip was composed of three quartz glass plates, i.e., cover, middle and bottom plates having thicknesses of 170, 100 and 500 μm, respectively. Each plate was a 52×32 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 (drains). 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.

Fig. 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 2 mm length.

2.2. Chemicals

All reagents were spectral- or analytical-grade and purchased from Wako (Osaka, Japan) and used as received. Ultrapure water was obtained using an ultrapure water purification apparatus (Nomura Micro Science, TW-600RU).

2-Nitroso-5-dimethylaminophenol (nitroso-DMAP) was prepared by nitrosation of *N*,*N*-dimethyl-*m*-aminophenol with sodium nitrate in hydrochloric acid solution. The crude product obtained was recrystallized from hydrochloric acid solution [12,13]. Briefly, 10 g of *N*,*N*-dimethyl-*m*-aminophenol was dissolved in 10 ml of concentrated hydrochloric acid and cooled below 5°C. Then 12 ml of 0.074 *M* sodium nitrate was added dropwise while mechanically stirring. The solution was filtered, and the precipitates were dissolved in hydrochloric acid solution by heating.

The Co–nitroso-DMAP complex was synthesized by the following procedure: 500 ml of 1.67 m*M* cobalt standard solution, 10 ml of 2 *M* citric acid and the hydrochloric acid solution of nitroso-DMAP were mixed in a separatory funnel, and the pH was adjusted to 5.3 by adding sodium hydroxide solution.

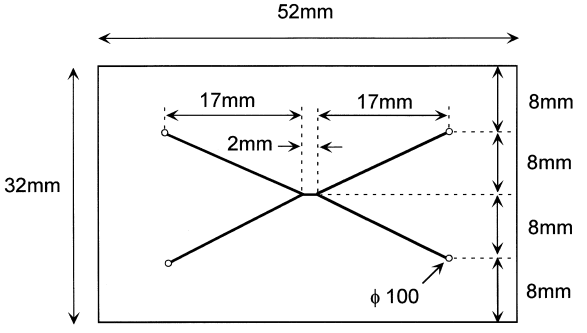

Fig. 1. Layout and dimensions of the microchip. The microchannels were 100 μm deep and 250 μm wide.

After adding 100 ml of 1,2-dichloroethane the funnel was shaken for 10 min with a mechanical shaker. Then, the aqueous phase was discarded. The obtained 1,2-dichloroethane phase was washed by shaking in the separatory funnel with 50 ml of 1 *M* potassium hydroxide (two times), 50 ml of hydrochloric acid, and 50 ml of water (several times). The 1,2-dichloroethane phase was collected by filtration after dehydration with anhydrous sodium sulfate. Then, the Co–nitroso-DMAP complex was obtained by concentrating and drying the 1,2-dichloroethane phase. The sample solutions ($1 \cdot 10^{-7}$–$1 \cdot 10^{-6}$ *M*) of Co–nitroso-DMAP were prepared by dissolving in water.

2.3. Operating procedures

The flow-rates of the liquid samples used as extracting reagent and aqueous phase were controlled through two syringes (Hamilton, 1710TLL) and a microsyringe pump (KD Scientific, Model-200). Each syringe needle (Hamilton, KF726) was connected to a custom-built PTFE screw with an O-ring (NOK, 0.74 mm I.D.×2.78 mm O.D.) through a fused-silica capillary tube (GL Sciences, 0.320 mm I.D.×0.450 mm O.D.) using epoxy-based glue (Ciba-Geigy, Alraldite). The outlets were also connected to a custom-built PTFE screw with an O-ring and a fused-silica capillary tube in the same way. They screwed down a custom-built poly(methyl methacrylate) holder [7]. In all experiments, the detection point of the TLM signal was located at the center of the organic phase (toluene phase), just halfway between the interface and microchannel sidewall, 1 mm downstream from the intersection point in the Y-shaped microchannel.

2.4. Apparatus

The TLM system has been described elsewhere [7]. Briefly, an Ar^+ laser (Lexel, Model-95, 488.0 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, custom-made for our purposes) 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 other prisms and another 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). 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 (Electro-Optics 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 three-dimensional 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. Three steps were precise enough for positioning the foci of the laser beams. A charge coupled devise (CCD) camera (Victor, KY-F55B) which was mounted on the microscope displayed picture images from inside the microchannel.

3. Results and discussion

The aqueous solution of the Co–nitroso-DMAP complex and toluene were introduced into the microchannel by the microsyringe pump at a constant flow-rate, e.g., 4 cm/s. The two introduced liquids formed a two-phase laminar flow in the microchannel, and no Co complex could be extracted in chloroform during flow, which could be explained using hydrodynamic theory. Under flow, the two liquids could never mix with each other and a liquid–liquid (aqueous–organic) interface was produced in the microchannel. This interface remained for about 15 min after the flow was stopped. After that, the liquid–liquid interface broke up, forming droplets. Stability of the liquid–liquid interface in the present microchannel was much better than that of our earlier one [7]. This was due to modifications

of the microchannel pattern, i.e., a shortened length of the extraction zone and a smaller angle of encounter of the two liquids. The time course of the solvent extraction was monitored using TLM. A typical time course of the TLM signal is shown in Fig. 2. The intensity of the TLM signal rapidly increased with time and became constant after about 50 s. In our previous study of the ion-pair solvent extraction, the extraction time was governed by molecular diffusion [7]. This is correct when the influences of interfacial adsorption and interfacial potential can be neglected [14]. Assuming the diffusion distance was the channel width, 250 μm, and D was ca. 10^{-5} cm^2/s [15], we estimated the diffusion time, i.e., extraction time, from the following equation as about 60 s:

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

where l, D and t are diffusion distance, diffusion time and diffusion coefficient, respectively. This agreed very closely with the observed value. We then made a comparison with the conventional solvent extraction method using a separatory funnel (aqueous solution, 10 ml; toluene, 10 ml) and mechanical shaker (300 times/min). The dependence of the absorbance on the shaking time was measured by a conventional spectrophotometer as shown in Fig. 3. The shaking time to reach extraction equilibrium was about 10 min. The extraction time using the microchip was one-order shorter than that of the conventional method. This result was due to the large specific interface area, the interface-to-volume ratio, of the microchannel [7].

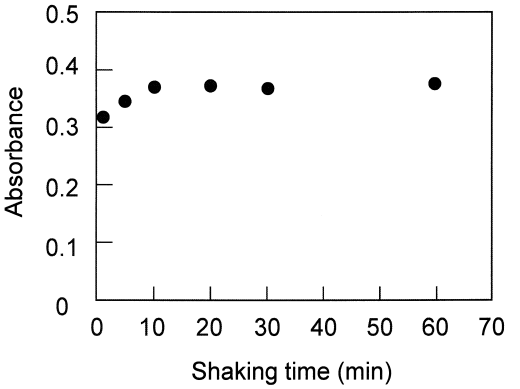

Fig. 3. Dependence of the absorbance on the shaking time as measured by a conventional spectrophotometer. The concentration of the Co complex solution was 20 μ*M*.

The dependence of the TLM signal on the concentration of Co–nitroso-DMAP in aqueous phase is shown in Fig. 4. We obtained good linearity in the range of $1 \cdot 10^{-7}$–$1 \cdot 10^{-6}$ *M*. The volume of the extraction zone in the microchannel was 50 nl (assuming introduction of the same quantities, Co complex aqueous solution, 25 nl and toluene, 25 nl). Therefore, the absolute amounts of the Co complex

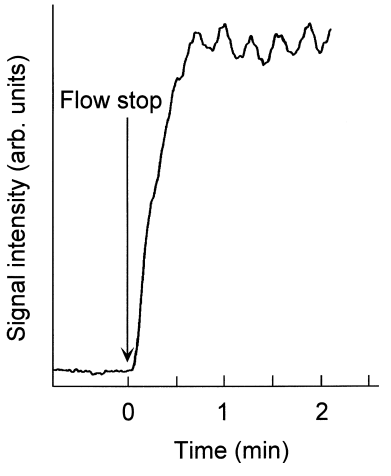

Fig. 2. A typical time course of the TLM signal. The concentration of the Co complex solution was 1 μ*M*.

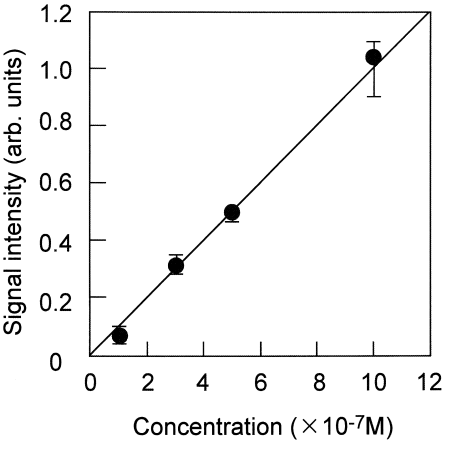

Fig. 4. Dependence of the TLM signal intensity on the concentration of the Co complex solution introduced in the microchannel.

participating in the solvent extraction were calculated to be 5–50 fmol. Considering that the absolute amounts of the Co complex in the microchannel were 5–50 fmol and the detection volume was 7.2 fl [16], we calculated 0.72–7.2 zmol of the Co complex were detected in this integrated microextraction system.

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