A Microfluidic Platform for Preconcentrating and Detecting Cu(II) with a Fluorescent Chemosensor and Cu(II)-Chelating Alginate Beads

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Long-term exposure to copper can cause headaches. stomachaches, dizziness. vomiting and diarrhea. Especially. children under 10 years of age have increased susceptibility to copper toxicity mainly due to their lack of a fully developed homeostatic mechanism of copper.[1,2] The Maximum Contaminant Level Goal (MCLG) was set at 1.3 mg/liter of Cu ions by the US Environmental Protection Agency (US EPA). Therefore. the determination of Cu(II) in natural waters remains an important task in environmental monitoring.

Existing methods to determine Cu(II) include stripping voltammetry. graphite furnace atomic absorption spectroscopy (AAS), atomic emission spectroscopy, inductively coupled plasma - atomic emission spectrometer (ICP-AES). inductively coupled plasma mass spectroscopy (ICP-MS). and flow injection analysis (FIA).[3,4] In spite of their superb sensitivity. the equipment used in these methods can only be operated by experts and require pre-treatment steps longer than 10 hours.[5,6] On the other hand, spectrophotometric methods have been often used for the determination of copper ions due to their advantages of simplicity and inexpensive instrumentation. However. spectrophotometric methods are. unfortunately, can not be practically used because of their lack of sensitivity and selectivity.[7] To increase the sensitivity of metal ion in solution. methods for preconcentrating metal ions using silica gel or agarose beads were reported.[8,9]

The lack of sensitivity and selectivity in conventional detection methods can be improved by the use of a fluorescent chemosensor. defined as a molecule comprising a ligand recognizing a metal ion and a fluorophore that is triggered upon metal ion binding.[10] We have recently reported a fluorescent chemosensor based on the fluorescein derivative which can effectively recognize Cu(II) in the nanomolar range at pH 7.4.[10] The response of the chemosensor is based on the fluorescence quenching of the chemosensor by binding with Cu(II). It showed an excellent selectivity for Cu(II) over other transition metal cations.[10]

Microfluidic devices possess many features such as high throughput. short analysis time. small volume and high sensitivity. which are appropriate for improving the performance of many bioanalysis techniques. For example. the diffusion distance between interacting molecules in the microwells of a microtiter plate is in the range of a few millimeters. compared to tens of microns in a microchannel, thereby reducing incubation and mixing times.[11] Also. the option to tailor-make an integrated system enables researchers to easily adopt microfluidic devices for the specific analysis of different biological analytes.[11] The most commonly used method to fabricate microfluidic devices is soft lithography. which offers easy fabrication and rapid prototyping.[11]

Herein. we report the highly sensitive fluorescent detection of Cu(II) using a microfluidic device packed with Cu(II)-chelating alginate beads.[12] A previously synthesized, fluorescent chemosensor[10] for detecting Cu ions in aqueous samples was used in the microfluidic device for the optical detection of Cu^{2+} in the ppm range.

Experimental Section

Materials. SU-8 2050 was purchased from MicroChem Corp. (Newton. MA). PDMS prepolymer and curing agent (Sylgard 184) were purchased from Dow Corning (Midland, MI). Cu(II)-chelating alginate beads (average diameter: 55-90 μm) from BioBead Science (Selma. AL). and $Cu(ClO_4)_2$ and other metal compounds Sigma Chemical Co.

Design of a microfluidic device. The device (10 mm × 5 mm) was composed of three different functional zones. as shown in Figure 1A: a sample treatment zone designated in microchannels 1-3, a mixing zone in microchannel 4, and a fluorescence detection zone in microchannel 5. As shown in Figure 1B. the width in the part of microchannel 2 where the microchannel meets the other microchannels was designed to be only 50 μm in order to entrap any microbeads larger than the reservoir ending. The dimensions (8 mm × 50 μm) of microchannels 1 and 2 were identical and the channels were used to load a solution containing a copper-sensing and a chemosensor neutralizer solution. respectively. In microchannel 4, herring-bone structures were installed in the channel wall to completely mix the three different laminar flows from microchannels 1-3 by generating chaotic mixing.[13] The fluorescence in the well mixed flow passing through microchannel 5 was either observed by a fluorescent stereomicroscope or was measured in microchannel 5 by a fluorescence scanner (Fluorolog 3. HORIBA Jobin Yvon Inc.. Edison. NJ).

(A)

(B)
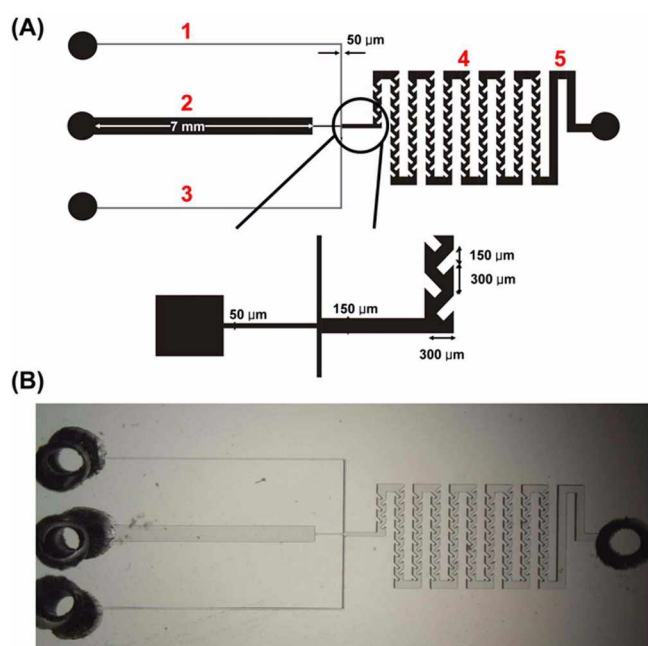
Figure 1. A microfluidic platform for fluorescent detection of Cu(II). (A) Design of a microfluidic device. (B) PDMS replica of a microfluidic device.

Fabrication of the microfluidic device. A PDMS microfluidic device was fabricated by the usual photolithographic method and replica molding, as described elsewhere.[11] First, SU-8 2050 was spun-coated on a silicon wafer to create a SU-8 replica master containing the above-mentioned design. Next, a 10:1 mixture of the polydimethylsiloxane (PDMS) prepolymer and curing agent was cast on the SU-8 replica master to generate negative patterns of the SU-8 patterns. The surfaces of the PDMS replica and slide glass were treated with oxygen plasma (25 W, 30 sec) in an O_2 plasma cleaner (Harrick Scientific, Ithaca, NY) and bound together. Holes were punched on the inlet and outlet ports and silicone tubes (ID = 1 mm, OD = 2 mm) were inserted into these ports to introduce the beads and sample solutions.

Operation of the microfluidic device for preconcentration and detection of Cu(II). Cu(II)-chelating alginate beads were first immobilized in the weir-shaped reservoir by flowing a slurry of the beads into microchannel 2. Then, solutions of varying concentration (0-1 ppm) and volume (0-1 mL) of $Cu(ClO_4)_2$ were supplied into the microchannel at the flow rate of 10 μL/min by a syringe pump (KD Scientific Co., New Hope, PA). Then, unbound or excessive Cu(II) were removed by flowing 5 mL of 20 mM HEPES buffer (pH 7.4) at 10 μL/min. After the preconcentration step, a suitable volume of elutant (pH 2.2) was introduced through microchannel 2 while the same volume of chemosensor and neutralizer solutions was simultaneously supplied to microchannels 1 and 3, respectively. Each laminar flow was then merged and chaotically mixed while passing through microchannel 4. Finally, the fluorescent intensity in microchannel 5 was either observed by a fluorescent stereomicroscope (SMZ 1500, Nikon Co., Kanagawa, Japan) with a Peltier-

cooled CCD camera (SPOT INSIGHT[TM] Diagnostic instruments. Sterling Heights. MI) or measured by a fluorescence scanner. Fluorolog 3 with a F-3000 fiber optic mount and a fiber optic cable bundle (1 mm) at excitation and emission of 505 nm and 522 nm, respectively.

Results

Fabrication of the microfluidic device. The microfluidic device was fabricated with PDMS and a glass slide using soft lithography.[11] The mold made of SU-8 on silicon substrate is shown in Figure 1A. Fabrication of the microfluidic device was completed by binding the PDMS replica to a glass slide, as shown in Figure 1B.

Mixing of laminar flows in herring-bone structures. Efficient mixing is a prerequisite for concentrating and detecting the metal ions in this microfluidic device. Thus, herring-bone structures were installed in microchannel 4 to prevent poor mixing and ensure chaotic mixing of laminar flows.[12] To test the mixing efficiency in microchannel 4, mixing among the chemosensor, elutant and neutralizer solutions was tested by flowing each solution in microchannels 1-3 without packing beads in microchannel 2. After loading a suitable amount of Cu(II) solution at different concentrations (0-100 ppm), the fluorescence intensities of the sample solutions were measured in microchannel 5. As shown in Figure 2, the fluorescence of the copper chemosensor started to decrease at 1 ppm without any preconcentration step. The minimum detectable concentration was 1 ppm in the microfluidic device.

Preconcentration and detection of Cu(II) in a sample solution. To lower the minimum detectable concentration, Cu(II)-chelating beads were incorporated into microchannel 2 and copper solution at varying concentrations (0-1 ppm) and volumes (0.01-1 mL) of copper solution were then
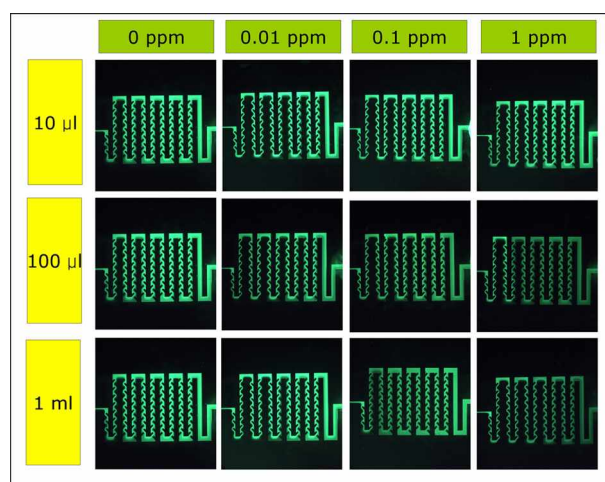
Figure 2. Representative fluorescent images of Cu(II) specific sensor depending on Cu(II) concentration. Preconcentration of copper ions depends on the volume and concentration of sample solutions containing the metal ions. The fluorescence intensity decreased by increasing either the volume or concentration of the sample solutions supplied to microchannel 2.
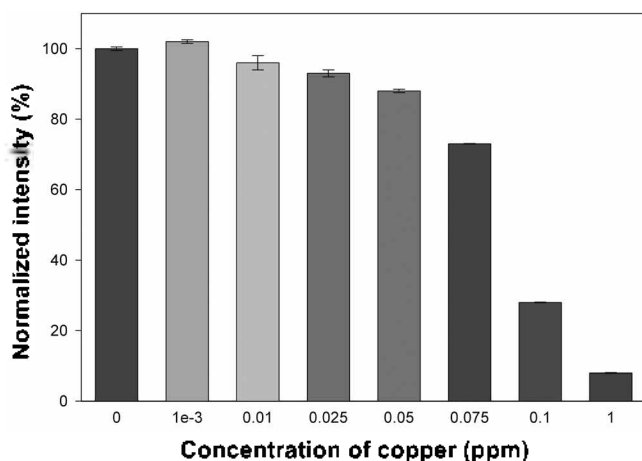
Figure 3. The standard curve of fluorescent titrations in the microfluidic device. The fluorescence data of compound 1 (100 μM) with $Cu(ClO_4)_2$ at pH 7.4 (10 mM HEPES) was obtained by a fluorescence scanner, Fluorolog 3 (HORIBA Jobin Yvon Inc., Edison, NJ) with a F-3000 fiber optic mount and a fiber optic cable bundle (1 mm) at excitation and emission of 505 nm and 522 nm, respectively.

supplied into microchannel 2 at the flow rate of 10 μL/min to preconcentrate the metal ion. After repeated washing of the bead-packed reservoir, the same volumes of copper chemosensor, elutant and neutralizer solutions were simultaneously introduced into their respective microchannels and the fluorescence intensity in microchannel 5 was then observed or measured. With increasing copper ion concentration or sample volumes, the fluorescence intensities in microchannel 5 were decreased, as shown in Figure 3. The fluorescent intensity of sensor was decreased as Cu(II)-concentration increased and thus a exponential decaying curve was obtained as a standard curve. The estimated equation using origin program (OriginLab Corp. Northampton, MA) was followed;

$$y = 93.11 / (1 + \exp((x - 6.63)/0.74)) - 6.55.$$

This indicated that an increasing number of copper ions accumulated in the Cu(II)-chelating beads with increasing concentration or volume of the sample solution. The limit of detectable concentration was 100 ppb by preconcentrating copper ions in 1 mL sample solution, as shown in Figure 3.

Discussion

Previously, we reported a fluorescein derivative which displayed large chelation-enhanced quenching effects with Cu(II) and Ni(II).[10] Although the selectivity of the derivative was more than 25-fold greater for Cu(II) than for Ni(II), this derivative may not be able to accurately detect Cu(II) in a sample containing a relatively higher concentration of Ni(II). This selectivity problem can be overcome by selectively pre-concentrating Cu(II) on metal ion-chelating beads before the fluorescent detection. However, this preconcentration step renders the overall detection procedure more cumbersome and laborious. Therefore, in the present study we integrated the bead-based preconcentration module with miniaturized detection in a microfluidic device to improve the sensitivity of chemosensor-based Cu(II) determination. We successfully developed a microfluidic platform for sensing copper ions by adding a chemosensor after preconcentrating Cu(II) on Cu(II)-chelating alginate beads. This device demonstrated a high specificity of 0.1 ppm on a chip and the potential for application to detect other heavy metals in water/environment samples using chemosensors.

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