Microfluidic Detection of Multiple Heavy Metal Ions Using Fluorescent Chemosensors

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Many heavy metals are taken into the body as ions or in certain compounds and tend to inhibit the function of particular enzymes. This causes serious health problems. Especially, lead is a potent poison causing serious diseases such as anemia, neurological disorders, *etc*.[1] Cadmium and its environmental compounds are extremely toxic, even in low concentrations, and will bioaccumulate in organisms and ecosystems.[2] Mercury and most of its compounds are extremely toxic. It can cause damage to the brain, kidney, and lungs.[3]

A great deal of effort has been made to develop devices and methods for detecting toxic heavy metals and their compounds. Traditionally, methods such as mass spectrometry and atomic absorption spectrometry have been used for the detection of these heavy metal ions. However, in spite of their superb sensitivity, the equipment used in these methods can be operated only by experts and requires pre-treatment steps taking more than 10 hours. On the other hand, spectrophotometric methods have often been used for the determination of metal ions, due to their advantages of simplicity and inexpensive instrumentation. However, they cannot be used in practice because of their lack of sensitivity and selectivity. Many improved techniques including electrochemical sensors,[4-6] optical sensors[7,8] and biosensors[9] have been developed, but they still need to be further improved to enable portable and real-time heavy metal monitoring systems to be developed.

In this regard, there is growing interest in the use of fluorescent chemosensors for detecting heavy metal ions, because of their sensitivity and selectivity.[10,11] A recent report demonstrated that a fluorescent chemosensor could detect mercuric ions in aqueous solution in the micromolar range.[12] Similarly, we recently reported two different chemosensors which can detect cadmium and mercury in aqueous solution in the micromolar range, respectively.[13] This kind of single fluorescence measurement is considered rather tedious when it comes to detecting either several heavy metals in a sample solution or a heavy metal in many sample solutions.

The chemical analysis of multiple samples can be easily performed in microfluidic devices having multiple channels, where each sample can be analyzed in a single channel. Recently, we showedthat single toxic compounds including heavy metals can be easily detected by the combined use of microfluidic devices and fluorescent chemosensors.[14,15] However, our previous works were also limited to the detection of a single metal ion at a time.

Herein, we introduce a microfluidic platform for the simultaneous detection of multiple heavy metals using three different fluorescent chemosensors. To demonstrate the feasibility of this platform, we chose cadmium(Cd), lead(Pb) and mercury(Hg) ions as the target analytes and their respective specific chemosensors[16-18] as the detection probes. The microfluidic device was designed to contain three parallel chaotic mixing channels, allowing each chemosensor to be independently mixed with a sample solution. Once the mixing between the chemosensors and the sample was completed, a characteristic fluorescencechange was observed from each microchannel, due to the binding between the chemosensor and its counterpart metal ion.

Three different chemosensors (Fig. 1)[16-18] were selected for the detection of Cd^{2+}, Pb^{2+} and Hg^{2+}. The Cd^{2+} sensor has boradiazaindacene (BODIPY) as the fluorophore and *N*,*N*-bis-

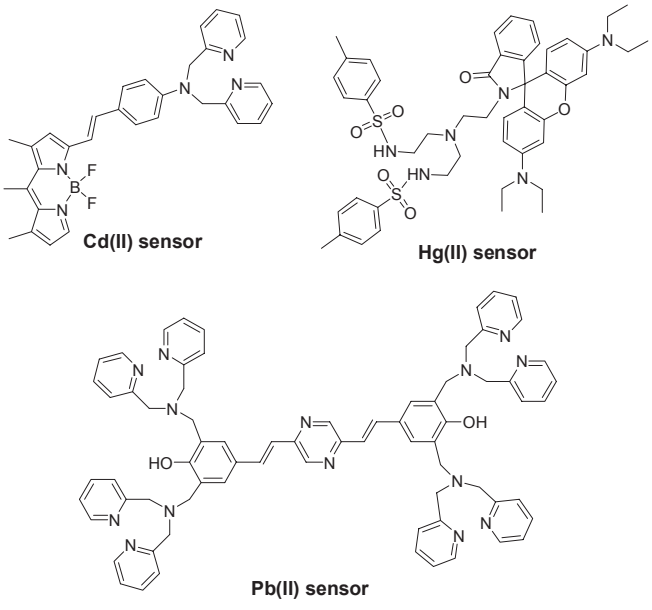

Figure 1. Structures of Cd sensor,[16] Pb sensor[17] and Hg sensor.[18]

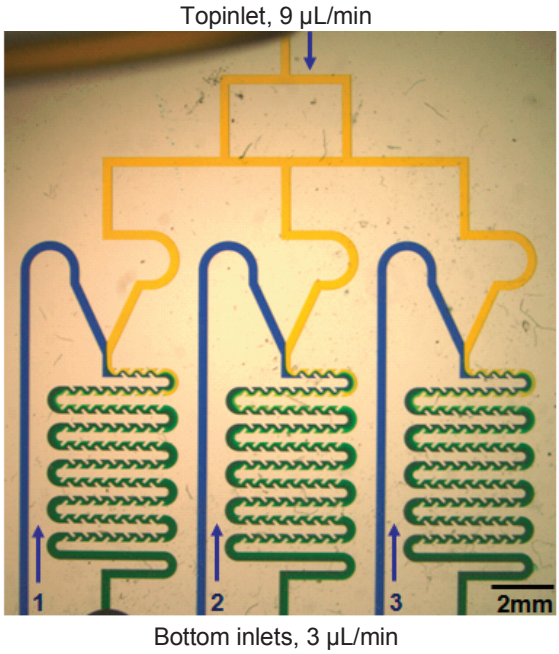

Figure 2. Optical images of three-parallel-mixer microfluidic chip tested with food dyes. The mixing test was carried out by introducing yellow dye from the top inlet at a flow rate of 9 μL/min and blue dye from the three bottom inlets at a flow rate of 3 μL/min separately.

(pyridin-2-ylmethyl)benzenamine as the Cd^{2+} receptor.[16] The emission intensity at 597 nm tends to increase upon the gradual addition of Cd^{2+}, which allows for the detection of Cd^{2+} by fluorescence methods. The Pb sensor is a single agent fluorescent sensor containing four bis(2-pyridylmethyl) amine (Dpa) groups which exhibits a highly selective and sensitive response to lead ions compared to other heavy metal ions at pH 7.0.[17] Its fluorescence intensity at 560 nm is increased by the formation of a complex between Pb^{2+} and the chemosensor, which blocks the photo-induced electron transfer.[17] The Hg sensor is a tren-based rhodamine derivative and a new emission band with a peak at 575 nm appears with increasing intensity, upon the addition of increasing concentrations of Hg^{2+} ions.[18]

As shown in Fig. 2, the device was composed of three major functional parts; the sample loading, mixing and chemosensor loading parts. In the mixing part, herring-bone structures were installed in the channel wall to completely mix the laminar flows from the top and bottom inlets by generating chaotic mixing.[15] To test the mixing capability in the chip, food dyes of different colors were loaded. Yellow and blue dyes were loaded from the top inlet at 9 μL/min and the bottom inlet at 3μL/min, respectively. This flow difference enables an equal volume of each dye to be loaded into the mixing part. When the solutions of the two dyes ran through the chaotic mixer, the thickness of the green color gradually increased along the mixing channel due to the chaotic advection and enhanced mixing, indicating that the mixing of the laminar flows with different colors was completed in the latter part of the mixing part.

Before investigating the possibility of the simultaneous detection of multiple heavy metal ions in a sample in the chip,

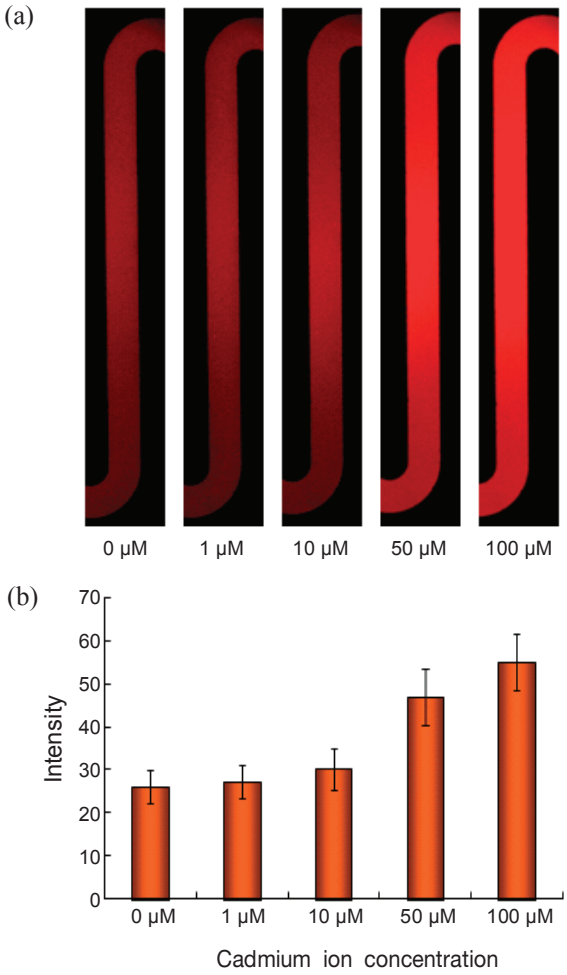

Figure 3. (a) Fluorescence images of Cd sensor (3 μM) mixed with different concentrations (0-100 μM) of Cd^{2+} in PBS in a single micromixer channel. (b) The fluorescent intensity of the Cd sensor only and the intensity enhancement with increasing Cd^{2+} concentration.

it is necessary to verify the fluorescence detection of a single heavy metal ion in the chip. For this purpose, various concentrations of cadmium ions (1-100 μM) and a 3 μM solution of the Cd sensor were loaded into the chip from the top inlet at 9 μL/minand the bottom inlet at 3 μL/min, respectively. Fig. 3a shows five images captured from the Cd sensor mixedwith different concentrations of cadmium ions and the solvent only. These images indicate that the fluorescence intensity of the Cd sensor increased as the concentration of the heavy metal ion increased. This was verified by Image J analysis demonstrating the correlation between the metal ion concentration and fluorescence intensity, as shown in Fig. 3b. In the microfluidic device, concentrations of Cd^{2+} as low as 50 μM were detectable. Based on these results, it was expected that a similar level of detection performance could be obtained for the other two metal ions.

The simultaneous detection capability of the microfluidic platform was evaluated using three different chemosensors. For this purpose, a 3 μM solution of the Cd chemosensor, 5 μM solution of the Pb chemosensor and 25 μM solution of the Hg chemosensor were injected into the device from bottom inlets 1, 2 and 3 at 3 μL/min, respectively. At the same time, a

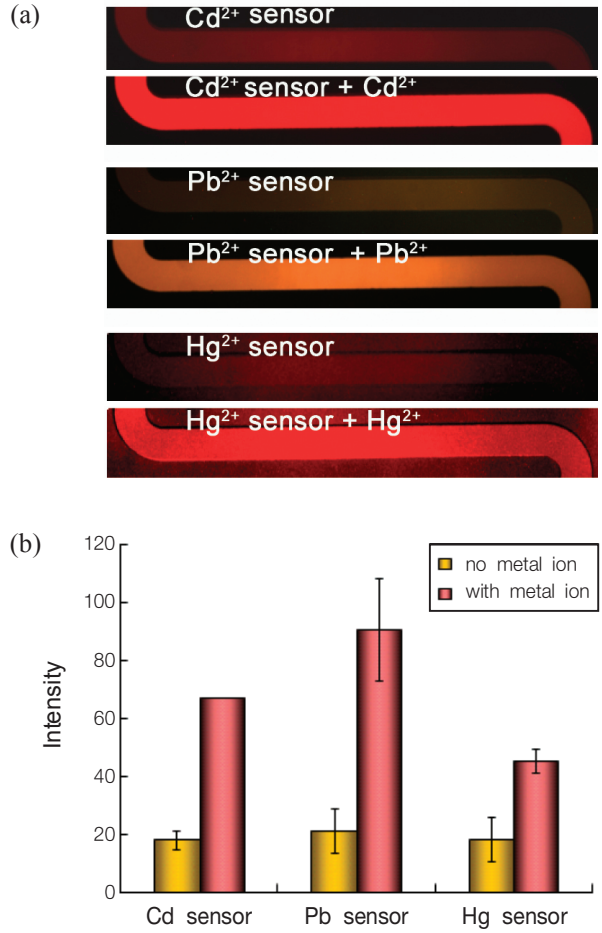

Figure 4. (a) Fluorescence images of Cd sensor (3 μM), Pb sensor (5 μM), and Hg sensor (25 μM) mixed with 20 mM PBS and corresponding detectable minimum concentrations of Cd^{2+} (50 μM), Pb^{2+} (50 μM) and Hg^{2+} (50 μM) in the three-parallel-mixer microfluidic chip. (b) Fluorescent intensity of Cd sensor, Pb sensor and Hg sensor only and the intensity enhancement after mixing with Cd^{2+}, Pb^{2+} and Hg^{2+}.

sample solution (pH 7.4) containing 20 mM HEPES and metal ions was injected into the device from the top inlet at 9 μL/min. When the mixing between the metal ions and the chemosensors were completed, a characteristic fluorescent response to each heavy ion was observed in the mixing part, as shown in Fig. 4a. Depending on the chemosensors, different optimum concentrations of the sensors and proper minimum detectable concentrations of the heavy metal ions were obtained. Based on these results, it is suggested that the present simple microfluidic platform is highly suitable for the simultaneous detection of heavy metal ions in industrial discharge water and metal mining wastewater containing heavy metals at concentrations higher than the safety limits. This method combines the advantages of selective fluorescent chemosensors and cost-effective, high throughput microanalytical devices in a microfluidic system for chemical sensing. More effort needs to be made to improve the sensitivity of the chemosensors, since this is the main factor determining the sensitivity of the microfluidic detection platform.

Experimental Section

Materials. SU-8 2100 was purchased from MicroChem Corp. (Newton, MA, USA), the PDMS (polydimethyl siloxane) prepolymers (Sylgard 184A and 184B) were purchased from Dow Corning (Midland, MI, USA).

Fabrication of Microfluidic Device. The mask designs were created in AutoCAD (San Rafael, CA, USA) and printed on high-resolution transparency films (Han & All Tech., Seoul, Korea).[19] The dimensions of the fluidic channels were 300 m 150 m (width height). A master mold was made by spinning a layer (150 m) of epoxy-based negative-tone photoresist (Nano SU-9 2010, MicroChem, Newton, MA, USA) on a polished silicon waferat two different speeds (500 rpm for 5 s and 1500 rpm for 30 s). The spin-coated silicon wafer was baked in two steps on a hotplate (65 °C, 30 min; 95 °C, 60 min). After cooling, the photoresist was exposed to UV light through a transparency mask for 15 s in a mask alignment system (MDA-400, Midas System Co., Daejon, Korea) with a 350 watt UV light and post-baked at two different temperatures (65 °C, 30 min; 95 °C, 30 min). After being developed for 10-15 min, the molds were treated with trimethylchlorosilane (United Chemical Technologies, Inc., Bristol, PA, USA) vapor for 15 min to facilitate the release of PDMS from them. The PDMS (Sylgard® 184 Silicone elastomer kit, Dow-Corning, Cortland, NY, USA) microfuidic device was fabricated from the molds using soft lithography.[19]

Detection. The device was mounted on an inverted microscope (Nikon Eclipse TE 2000-U). Fluorescence excitation was provided by a mercury lamp (100 Watt). Two filter sets were used. One consisted of an EX 510-560 excitation filter, DM 575 beam splitter and BA 590 barrier filter, while the other consisted of an EX 450-490 excitation filter, DM 505 beam splitter and BA 520 barrier filter. The image was recorded using a SPOT insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Fluorescent images in the microchannel were obtained using Peltier-cooled CCD camera (SPOT INSIGHT™, Diagnostic instruments, Sterling Heights, MI, USA). The fluorescence images were analyzed by Image J program (NIH, USA)

All the chemoensors were originally dissolved in CH_3CN : H_2O = 9:1 and later diluted in 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (pH 7.4) for its use in the detection. A 3 μM solution of the Cd sensor, 5 μM solution of the Pb sensor and 25 μM solution of the Hg sensor in 20 mM HEPES buffer (pH 7.4) were introduced separately into the microchannel from the three bottom inlets at a flow rate of 3 μL/min and a mixture of 50 μM Cd^{2+}, 50 μM Pb^{2+} and 250 μM Hg^{2+} in 20 mM buffer was introduced into the microchannel from the top inlet at a flow rate of 9 μL/min. The flow-rates of the liquid samples were controlled by two microsyringe pumps (KDS 220, KD Scientific, New Hope, PA, USA). Each syringe needle was connected to the inlet through a polyethylene tubing (TYGON®, Saint-Gobain PPL Co., Cleveland, OH, USA) with a stainless steel pin (New England Small Tube, Litchfield, NH, USA).

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