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Potential integration of cadmium lab chip with immunoassay using quantum dot/antibody probe for detection of microcystin-LR

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ABSTRACT

Quantum dots (QDs) were used to detect quickly pathogenic microorganisms in sea water. The size is an important parameter that determines the chemical and physical properties of nanoparticles. In order to measure the size of water soluble nanoparticles, QDs, field-flow fraction (FFF) was used. Different sized water-soluble nanoparticles were effectively separated. As an indirect measurement of target analyte, instead of conventional fluorescent optical methods, we measured amount of cadmium ions using anodic stripping voltammetry since major component of core-shell CdSe/ZnS QDs used for immunoassay of cyanobacterial hepatotoxin microcystin-LR was cadmium. In addition, we developed a lab chip (LC) to measure electrochemically cadmium in water. For our further research, it will be integrated with immunoassay method to develop portable lab chip monitoring system QDs for the detection of cyanobacterial hepatotoxin microcystin-LR. The quantum dots-antibody (QD/Ab) and QD-labeling methods were simple, sensitive, reproducible, and selective.

Keywords: Field-flow fraction (FFF); Lab-on-a-chip; Nanoparticle; Quantum dots (QDs); Quantum dot-antibody probe

1. Introduction

Drinking water supplies are considered to be most susceptible to contamination by pathogenic bacteria and their toxic compounds. The content of pathogenic microorganisms in water has been raising much concern as regards their impact on human health [1]. For example, microcystin is a prevalent cyanotoxin produced by the massive occurrence of blue-green algae in eutrophic freshwater and marine habitats. Cases of adverse health effects of microcystins have been reported in many countries, with some reports of liver cancer and even death. To date, approximately 80 variants of heptapeptide microcystins have been discovered, the most common and toxic peptide of them being microcystin-LR, in which leucine (L) and arginine (R) are combined with a constant ring composed of five amino acids [2]. Infection occurs after ingestion of contaminated water. The risk of human illness associated with pathogenic microorganisms can be prevented by regular continuous monitoring of pathogens and toxins in water.

There are many conventional techniques, such as RT-PCR, fluorescence in situ hybridization (FISH), antibody assay, and culture methods, have been used to study and

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quantify bacterial species in water. However, these classic methods are tedious, and time and labor-consuming, results are usually not available until the water has been consumed, thereby increasing the risk of uptake or transmission of pathogens [3]. Improved pathogenic health risk analysis techniques require a rapid and sensitive test which would reveal the presumptive presence of pathogenic microorganisms.

The application of nanomaterials for microbiological analysis can increase speed, specificity, and sensitivity. Quantum dots (QDs), semiconductor nanocrystal, have recently attracted strong interest from the biological environments as optical and/or electrochemical nanomaterials [4–6]. The advantages of QDs are that compared to the radioactive probes, fluorescent probes are safer; they offer better resolution and do not need additional detection steps. Moreover, fluorescent probes can be labeled with dyes of different emission wavelength thus enabling detection of several target sequences within a single hybridization step [7]. Due to their many benefits, obviously, QDs-based biosensor is a powerful alternative to conventional analytical techniques.

Recent developments in micro electro mechanical systems (MEMS) manufacturing techniques have offered exciting possibilities for the miniaturization of analytical instruments on chips the size of a few millimeters on edge by 1 mm in thickness, which provides a lab chip (LC). Since the LC allows us to manipulate very small volumes, there are several advantages; small sample and reagent volume (less than a few uL) can be used, which is suitable for determining the analytes which are present within the host without doing any damage to the host system; less sample wastage can be produced, which is cost effective and environmental friendly way; and rapid analysis times can be expected, due to high surface area to volume ratio. Integrated with immunoassay using quantum dot/antibody (QD/Ab) probe, thus, miniaturized on-chip electrochemical sensor would lead to numerous benefits, like a high throughput analytical system for environmental screening.

Accordingly, the objectives of the study were to apply the QDs/antibody to detect electrochemically microcystin-LR, and to study the LC for rapid measurement of cadmium using square wave anodic stripping voltammetry (SWASV).

2. Materials and methods

2.1. Bioconjugation of quantum dots with monoclonal antibodies

The monoclonal antibody against microcystin-LR (MC10E7) was obtained from Alexis, and QDs with a Qdot525. Antibody Conjugation Kit was purchased from Invitrogen. In this study, the semiconductor nanocrystal has a CdSe core encapsulated in the shell of ZnS and polymer. Then, the QD/Ab probe was obtained based on manufacturer's protocols. The detail descriptions were mentioned in our previous work [8].

2.2. Fabrication of microfluidic chip

As shown in Fig. 1, the SU-8 2075 photoresist (Microchem Corp., MA, USA) was spin-coated on the 3-inch nickel (Ni) disk to achieve a 100 μm thickness, followed by a pre-bake process. After the photoresist layer was exposed to a UV source (365 nm UV), it was baked again for SU-8 cross-linking for about five hours. A development process was followed by immersing the Ni disk into SU-8 patterns. After developing, Ni electroplating was performed in a Ni plating bath, using a two-electrode system with a Ni anode and the patterned Ni disk cathode. Finally, a Ni mold with a 100 μm-thick plating Ni microstructure was obtained after removal of the residual SU-8. The microfluidic chip was then replicated from this mold in a cyclic olefin copolymer (COC) substrate by a

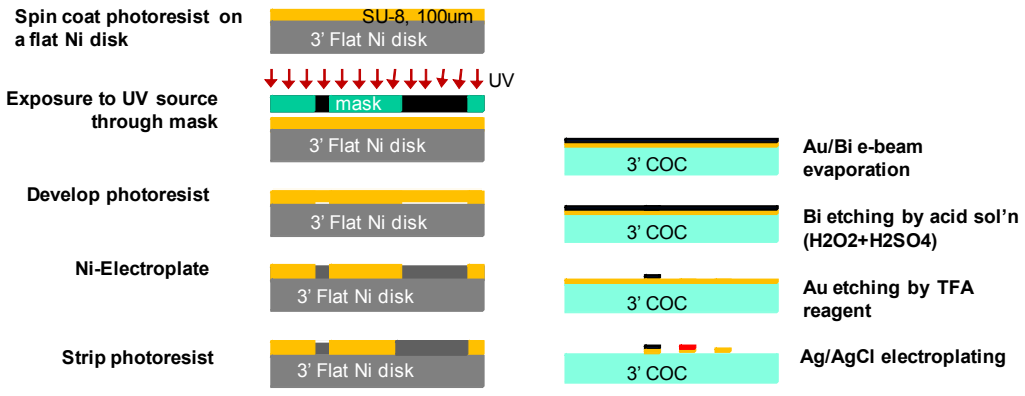

Fig. 1. Schematic procedure of microfabrication processes of the Cd(II) chip with microchannel (left) and microelectrodes (right).

high-throughput injection molding machine (BOY 22A, BOY Machines Inc., PA, USA).

2.3. Fabrication of microelectrodes

As shown in Fig. 1, first, an Au layer of 100 nm and a Bi layer of 300 nm was-beam evaporated on the 3-inch blank polymer COC substrate, respectively. Gold (Au) and bismuth (Bi) electrodes was then patterned by photoresist after photolithography, and etched by Bi (2% H_2O_2 and 2% H_2SO_4) and Au (TFA) etchant. The Ag/AgCl reference microelectrode was also used for voltammetric measurement. The microfluidic chip was bonded with the microelectrodes chip using UV adhesive bonding method at room temperature to make the final chip sensor.

2.4. Bonding and packing

Commercially available UV adhesive with a low viscosity was applied to one substrate by spin coating for 60 s at 4500 rpm. Once the adhesive spreads evenly on the glass surface, the plain substrate was gently lowered onto a clean surface. The substrate containing the channels was aligned and carefully placed on top of the substrate with the adhesive. Appropriate pressure was applied to the substrates to even the adhesive at the interface. A UV light source was used to cure the adhesive for 4 min at 7 mW/cm^2.

2.5. Condition optimization for electrochemical transduction

Electrochemical factors were optimized to improve the response to anodic current of QDs. For this optimization, 7.23×10^{12} QDs was dissolved to cadmium ions with 1 M HNO3, which was then transferred to a 0.2 M acetate buffer (pH 4.76) as an electrolyte in an electrochemical cell. SWASV was performed by Epsilon Potentiostat/Galvanostat (Bioanalytical Systems, Inc.) with three three-electrode system that included a gold-coated working electrode (3 mm diameter), an Ag/AgCl reference electrode saturated with 3 M KCl, and a platinum wire counter electrode. The SWASV was performed after cathodic deposition at −1.3 V for 210 s of electrodeposition time at a 500 rpm stirring condition. The stripping step was then carried out by scanning the potential toward more positive values from −1.3 to 0 V.

2.6. Characterization of nanoparticle by field-flow fractionation (FFF)

An asymmetric FFF system (Postnova) equipped with a membrane with 1000 kD MWCO (molecular weight cut-off), and a microchannel employing both laminar channel and crossflow was utilized to obtain chromatograms by UV detection at 254 nm. The eluent was composed of 0.01% FL-70 (anionic and neutral surfactants) (Fisher Scientific) and 0.1 mM NaN_3.

3. Results and discussion

3.1. Determination of the nanoparticle size by FFF

By altering the QD size and its chemical composition, fluorescence emission may be tuned from the near ultraviolet, throughout the visible, and into the near-infrared spectrum, spanning a broad wavelength range of 400–2000 nm. Since QD photoluminescence emission maximum can be manipulated by changing the particle size, their use as fluorescent labels for biological macromolecules has attracted considerable attention. Thus, for numerous such applications precise determinations of average particle size are highly essential. Notwithstanding many efficient tools, there has been a constant need for improved rapid analysis, characterization, and separation of nanoparticles in solid state and in solution. Particularly for biological-labeling studies the exact size of the nanoparticle in solution should be accurately know. In this study we observed that accurate characterization of nanoparticles dispersed in aqueous solutions is still more complex largely due to aggregation of the particles. The analysis of some typical water-soluble nanoparticles and quantum dots in solid state using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies and in solution using dynamic light scattering (DLS), the tools commonly used for the purpose, are not entirely reliable [9]. These conventional analytical tools have suffered from certain drawbacks due to aggregation and decomposition of organic ligand coating [10].

The field-flow fraction, on the other hand, is one of the most versatile families of separation techniques known. As shown in Fig. 2, there are two different types of flows inside the micro channel of FFF, the first is channel flow (direction to the right), and the second is crossflow (field flow; in downward direction). Under the crossflow conditions these smaller nanoparticles reach the exit faster than the larger ones. More interestingly, since aggregation of the water-soluble nanoparticles is prevented, particles are accurately characterized by UV absorbance measurements that relate diffusivity of particles with size. The FFF cross-flow condition is apparently noninvasive and hence the technique was very effective in characterizing the nanoparticles. Furthermore, using this simple technique it was possible to fractionate a sample of the AuNPs (Fig. 2). From the laminar and cross flow rates, as well as the void and sample peak positions, the average hydrodynamic radii of the AuNPs computed from the standard equations was found to be 6.64 ± 0.58 nm, which is a statistical average of eighteen runs. Fractionation of such particles was an added feature not achievable with SEM, TEM, and DLS. In spite of the efficacy of the FFF in determining the size of particles in such broad range, till date it has not been significantly explored as a routine tool for the characterization and separation of nanoparticles and QDs. Accordingly, we reported that the FFF can be a very reliable tool for determination of the diameter of the water-soluble nanoparticles.

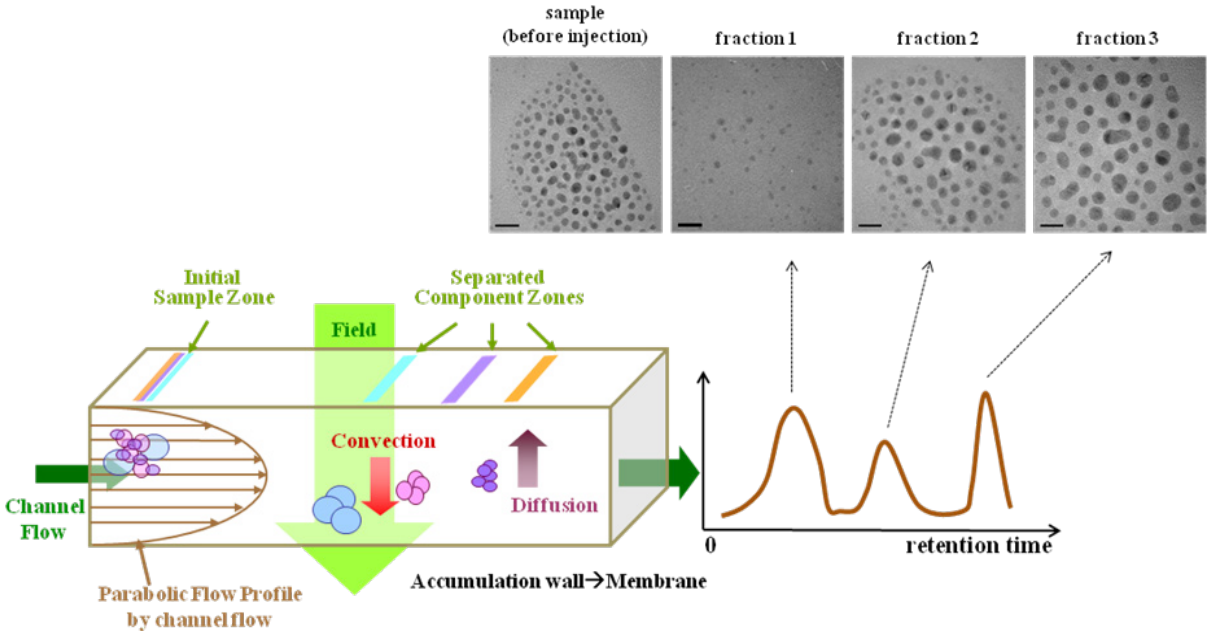

Fig. 2. Schematic representation illustrating the principle of field-flow fractionation technique for particle size analysis, and TEM micrograph of Au nanoparticle fractionation. Scale bar 10 nm.

3.2. *Electrochemical immunoassay using QD/Ab probe for identification of cyanobacterial hepatotoxin microcystin-LR*

Even though QDs have been previously used as fluorescent labels with unique optical properties, recent attention on this semiconductor nanocrystal has focused on its potential for use in electrochemical methods in a variety of point-of-care environments. Electrochemical detection of microcystin-LR was explored using a QD/Ab probe for nanoparticle based amplification and direct electrochemical transduction [8]. The immunological recognition of microcystin-LR using the QD/Ab probe was amplified and converted to an electrochemical signal by measuring the cadmium ions released from QD based

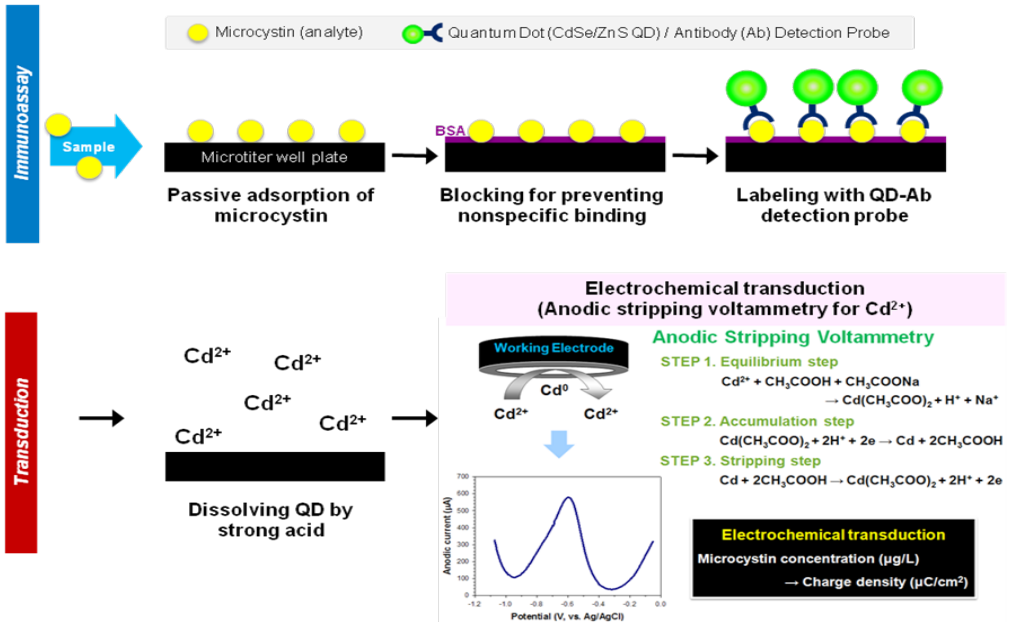

Fig. 3. Conceptual scheme for immunological recognition and electrochemical transduction of microcystin-LR.

on SWASV under optimized electrochemical factors. Fig. 3 shows the principal of immunological recognition and electrochemical transduction of microcystin-LR. The toxins were first passively adsorbed onto the wells of a microtiter plate and the unbound parts of wells were then blocked with bovine serum albumin (BSA) to prevent nonspecific adsorption. After adding QD/Ab probe, a monoclonal antibody against microcystin-LR, as a mouse IgG isotype, recognizes the positively charged arginine (R) at position 4 of the heptapeptide. In this way, the immunological binding event of microcystin-LR with the QD/Ab probe was amplified and converted to an electrochemical signal by measuring the cadmium ions acidically dissolved from QD using SWASV under the optimized electrochemical factors.

3.3. Cadmium measurement with SWASV sensing principle

The concept of polymer lab chip-based systems started from the integration of the various chemical operations involved in conventional analytical processes in a laboratory, such as sampling, preparation, mixing, reaction, and separation into a polymer lab chip system, requiring only a tiny volume of chemicals and sample and only a fraction of the time needed for the conventional approach. Inherent advantages of MEMS devices are high reliability, low power operation, small size, and low cost to manufacture. In this study, MEMS technology was considered to be integrated with the immunoassay using QD probe for the trace determination of cadmium ions in the samples since major component of cadmium in core-shell CdSe/ZnS QDs was cadmium.

A number of bonding techniques for glass substrate can be used to seal the microchannel, including fusion and adhesive bonding. However a few fabrication approaches have certain limitations for biochip packaging. Fusion bonding requires high temperatures, long hours, and a high quality, expensive polished substrate. Further, it has a poor efficiency requiring repeated attempts to achieve a leak-proof bond, and the process is irreversible. Some bonding techniques needing high temperature may not be suitable for bonding of substrates that have a temperature sensitive material, such as enzyme or polymer layers. Efforts to overcome the above-mentioned limitations have led us to the development of a reversible room-temperature UV adhesive bonding technique for the rapid fabrication of biochips presented herein. The fabrication process was a rapid room temperature process avoiding long fabrication hours and high temperatures. The fabricated chips are highly reliable, as the process has a nearly 100% yield. In addition, the process is reversible and does not require extremely flat and smooth surfaces, enabling the possibility of bonding two flexible non-uniform substrates. Glass substrates and the medical-grade adhesive enhance the biocompatibility of the chip, allowing its use in a biological environment in the presence of aqueous fluids. Due to the toxicity of mercury or mercury precursor, bismuth (Bi) was introduced as an alternative working electrode material for SWASV for detection of cadmium. Bismuth has much less toxicity while maintaining similar electrochemical performance to Hg including well defined, undistorted stripping response, wide linear dynamic range, excellent resolution of neighboring peaks. Bi is an environmentally friendly element, with a very low toxicity, and a widespread pharmaceutical use. Fig. 4 shows the photograph of the fabricated LC, which illustrate of three sensors with planar electrode arrays, electrical connections, and microchannels. The entire chip size was 3 cm × 2 cm, and the inlet and outlet channels have a width of 400 μm and a depth of 100 μm. The Bi working, Ag/AgCl reference, and gold counter electrodes have same size as follows: length of 3 mm, width of 500 μm, and a spacing of 500 μm.

One of the most critical things to achieve detection below the ppb level is the choice of electrochemical sensing method. It is widely accepted that SWASV is one of the most promising methods for detection and determination of heavy metals in water, whether from rivers, lakes, process streams or drinking sources [11]. SWASV sensing method was used to follow the Cd(II) stripping process at the Bismuth (Bi) bulk working electrode because in previous many studies it offered high sensitivity in the detection over the linear sweep and differential pulse voltammetry for rapid analysis of heavy metals [12,13]. As shown in Fig. 5, SWASV is a voltammetric method for quantitative determination of specific metal ionic species. The cadmium ions in the sample solution are electrochemically deposited onto a working electrode

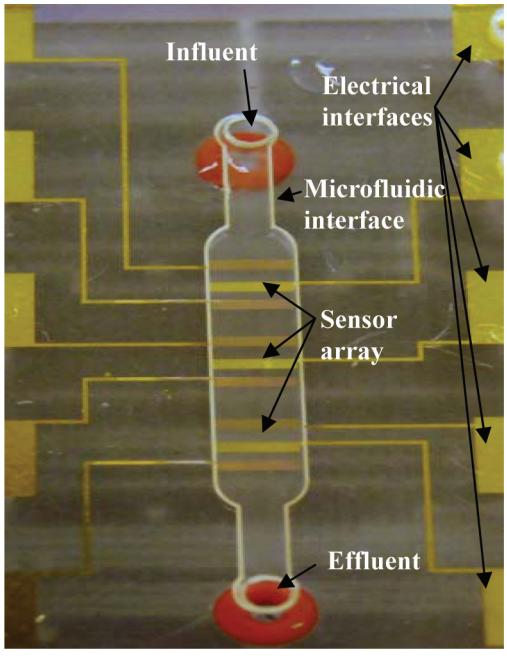

Fig. 4. Photograph of the fabricated cadmium LC.

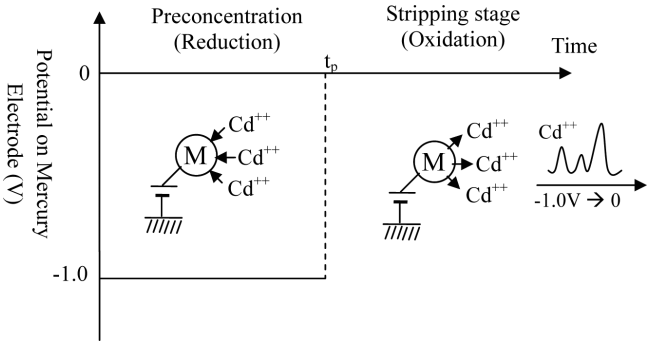

Fig. 5. Conceptual diagram illustrating anodic stripping voltammetry in the pre-concentration and stripping stage of the analysis.

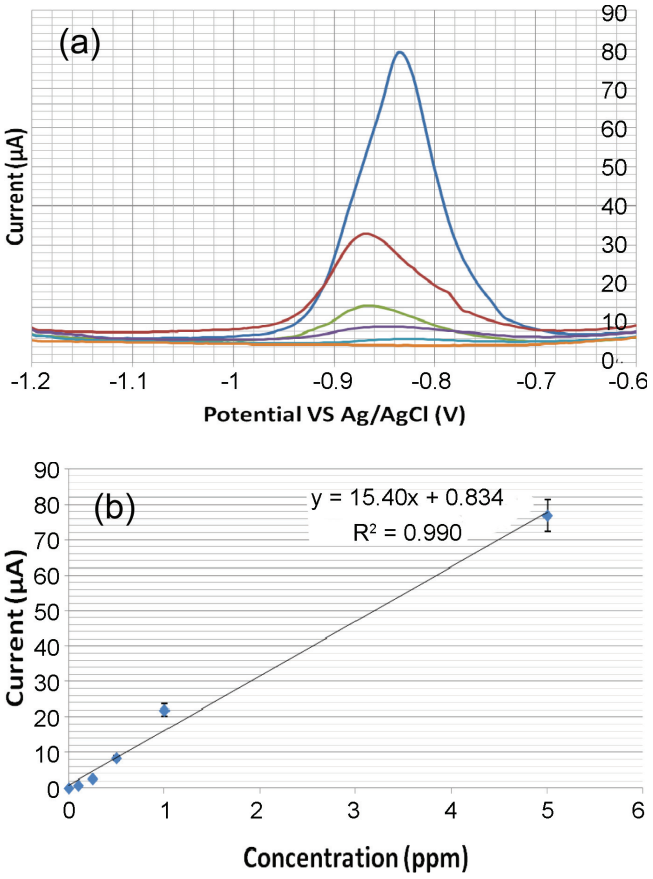

Fig. 6. (a) SWASV of Bi working electrode for measuring Cd(II) in acetate buffer (pH 4.65) from the concentration 100 ug/L to 5 mg/L and (b) calibration curve.

during a time period by applying a negative potential on the working electrode. The oxidation current is measured while these deposited cadmium ions are stripped and oxidized from the working electrode by scanning the electrode potential in a positive direction during the stripping step. The oxidation of species is registered as a peak in the current signal associated with the potential at which the species is known to be oxidized [14].

The –1.2 V (vs. integrated Ag/AgCl pseudo reference electrode) of deposition potential was found to be the most appropriate value since replicate additions gave reproducible peak current values and the symmetry of Cd(II) peaks were confirmed at each given condition. As shown in Fig. 6, the Bi bulk electrode shows a peak potential of –0.85 V and for increased concentrations of Cd(II), the peak stripping current increased linearly. These preliminary results show the potential development of miniaturized electrochemical cadmium lab chip sensor by SWASV sensing method.

Another issue to be addressed is that a lab chip and electrochemical sensing methods-based portable monitoring system could also offer the potential for highly efficient, simultaneous, rapid and on-site determination of pathogenic baterials in water. As a result, it appears well suited to complement standard analytical methods for a number of environmental monitoring applications. The lab chip integrated with electrochemical sensing systems can provide a way to save tremendous amounts of time and costs, with a possibility of making rapid decisions on local environmental problems. More importantly, in the long term, with more development of the instrument, including a smart sipping system for automated sampling which will obviate the need for operators to frequently change sensor chips, a more convenient and smart monitoring system for simultaneous determination of a couple of environmental contaminants (e.g., ammonium, nitrite, nitrate, phosphate), pH, ORP and heavy metals will also be possible in the future. These benefits will influence to a large extent the potential application of the sensor to a variety of environmental fields such as rivers and streams, even in remote and hard to reach locations. If current and emerging wireless communications technology could be integrated with our portable monitoring system, it would have a radical impact on future water monitoring because the wireless sensor networks (WSNs) will enable water researchers and decision makers to have quick access to the abruptly changing contaminant levels of water with less effort and cost.

4. Conclusions

Collectively, the aforementioned MEMS and polymer micromachining techniques are a proof-of-concept which is useful to develop a miniaturized, on-chip, and highly sensitive electrochemical sensor for detecting cadmium ions. SWASV is one of the most sensitive, convenient, and cost effective analytical methods. In the voltammetry, cadmium ions were electroplated on a working electrode during a deposition step, and oxidized from the working electrode during a subsequent stripping step. Accordingly, a qualitative analysis for microcystin-LR would

be possible to measure amount of cadmium ions based on the peak potential specific for the Cd(II)/Cd(0) redox couple of anodic voltammogram at −0.85 V, concentration of the toxin was quantified using the charge density of Cd(II) during the stripping step corresponding to the amount of QD.

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