IOP PUBLISHING MEASUREMENT SCIENCE AND TECHNOLOGY

Meas. Sci. Technol. **22** (2011) 032001 (18pp) doi:10.1088/0957-0233/22/3/032001

TOPICAL REVIEW

State-of-the-art lab chip sensors for environmental water monitoring

Am Jang[1], **Zhiwei Zou**[2], **Kang Kug Lee**[2], **Chong H Ahn**[2] **and Paul L Bishop**[1,3]

[1] Department of Civil and Environment Engineering, University of Cincinnati, OH, USA
[2] Microsystems and BioMEMS Laboratory, Department of Electrical and Computer Engineering, University of Cincinnati, OH, USA

E-mail: Paul.Bishop@UC.edu

Received 20 June 2010, in final form 7 October 2010
Published 24 January 2011
Online at stacks.iop.org/MST/22/032001

Abstract

As a result of increased water demand and water pollution, both surface water and groundwater quantity and quality are of major concern worldwide. In particular, the presence of nutrients and heavy metals in water is a serious threat to human health. The initial step for the effective management of surface waters and groundwater requires regular, continuous monitoring of water quality in terms of contaminant distribution and source identification. Because of this, there is a need for screening and monitoring measurements of these compounds at contaminated areas. However, traditional monitoring techniques are typically still based on laboratory analyses of representative field-collected samples; this necessitates considerable effort and expense, and the sample may change before analysis. Furthermore, currently available equipment is so large that it cannot usually be made portable. Alternatively, lab chip and electrochemical sensing-based portable monitoring systems appear well suited to complement standard analytical methods for a number of environmental monitoring applications. In addition, this type of portable system could save tremendous amounts of time, reagent, and sample if it is installed at contaminated sites such as Superfund sites (the USA's worst toxic waste sites) and Resource Conservation and Recovery Act (RCRA) facilities or in rivers and lakes. Accordingly, state-of-the-art monitoring equipment is necessary for accurate assessments of water quality. This article reviews details on our development of these lab-on-a-chip (LOC) sensors.

Keywords: environmental monitoring, heavy metals, monitoring of water quality, nutrients, portable monitoring system, state-of-the-art lab chip, MEMS

(Some figures in this article are in colour only in the electronic version)

1. Water pollution

Acute drinking water shortage and water quality degradation will remain important barriers to global sustainability for the foreseeable future. Water pollution in surface water and groundwater from point and non-point sources (NPS) is a widespread problem in the USA and worldwide. Due to strict enforcement of water quality regulations at major point sources, these sources in the USA are now largely under control. However, even in the most advanced countries, NPS pollutants such as nutrients, pesticides, and heavy metals are transported from many different sources by surface water and groundwater pathways (Jones 1999). Runoff from our farms and cities is a major source of nutrients (mainly phosphorus and nitrogen) entering surface waters such as lakes, rivers, and coastal waters. The most common impairment of surface

---

[3] Author to whom any correspondence should be addressed.

waters is eutrophication caused by excessive inputs of nutrient (Arhonditsis *et al* 2003, Huang *et al* 2003). Definition, causes, extent and severity of eutrophication are extensively studied elsewhere (Bizsel and Uslu 2000, De Marco *et al* 1998, Arhonditsis *et al* 2003, Huang *et al* 2003). In addition, with the heavy use of heavy metal salts, an alarming increase has been taking place in the concentrations of these ions in the biosphere. Generally, heavy metals are the most insidious pollutants because of their nonbiodegradable nature and ability to persist for long periods, thus giving rise to significant contamination of surface waters and groundwater resources. It is well known that the relative distributions and chemical species of toxic contaminants are highly influenced by environmental parameters such as pH and oxidation–reduction potential (ORP). Accordingly, continuous surveillance of contaminant concentrations and geochemical parameters in water bodies is of prime importance to better understand their long-term impact on aquatic systems.

1.1. Water contamination due to nutrients

There is currently a great public concern due to nutrient contamination of surface waters and groundwater resources (Liu *et al* 2005). The problem is complicated by the fact that the negatively charged nitrate ions (NO_3^-) and phosphorus ($H_{3+x}PO_4^x$) ions are not held by soil particles and can be leached down through the soil profile, leading to nutrient enrichment of downstream groundwater.

Although the nitrate ion is a chemical species of the basic nitrogen cycle (Alvarez-Romero *et al* 2007) and is commonly found in a diversity of natural processes, nitrate contamination is most frequently associated with anthropogenic activities at the ground surface, such as the fertilization of agricultural crops, animal feces and urine, chemical synthesis as precursors of a large variety of chemicals, and in the food industry as preservatives and disinfectants. Recent research indicates that elevated nitrate concentrations above the background levels are commonly found in shallow groundwater systems underlying regional sources such as agricultural lands on which fertilizer or pesticides have been applied or from local sources such as municipal landfills or waste spills (Liu and Lu 2008). In Germany, about 30% of the observations within the national groundwater-observation network showed elevated nitrate concentrations (more than 25 mg NO_3^- L^{-1}), mostly located in areas dominated by agricultural land use (Kersebaum *et al* 2006).

Due to their direct toxicity to humans and animals, it has been stressed by various authors that nitrogenous compounds in drinking or ground water are of particular concern. It is known that, although ammonium itself is relatively nonpoisonous to macroinvertebrates (Berenzen *et al* 2001), its products—ammonia and nitrite—can be extremely toxic. Water containing ammonia when treated with chlorine forms chloramines, which have recently been found to be carcinogenic (Singleton *et al* 2007). The elevated levels of nitrate in drinking water are related primarily to human health effects such as methemoglobinemia (infantile cyanosis), cancer, neural tube effects, or spontaneous abortions (Fewtrell 2004). It has been reported extensively that with, greater than 10 mg NO_3^-–N L^{-1}, nitrate in infants under six months is reduced to nitrite, which combines with hemoglobin in the blood to form methemoglobin, a process known as blue baby syndrome (Powlson *et al* 2008, Knobeloch *et al* 2000). The interaction of the nitrite with hemoglobin (oxyHb) has been suggested to explain the formation mechanism of the methemoglobin (metHb) and nitrate. As a consequence of the formation of metHb, oxygen delivery to tissue is impaired (Santamaria 2006). Recently, epidemiological studies have suggested that high nitrate ingestion may also be a contributing factor in gastric cancer (Singleton *et al* 2007). When nitrites are endogenously reduced from nitrate, they may react with secondary amines and amides in food products producing *N*-nitrosodimethylamine. Recent research showed that precursors of *N*-nitroso compounds are potentially carcinogenic (Santamaria 2006). These problems have been widely recognized, and as a consequence, the current stringent potable water standard (maximum contaminant level, MCL) for nitrate has been set by the US EPA (1994) at less than 10 mg NO_3^-–N L^{-1} which is equivalent to 45 mg NO_3^- L^{-1} (Alvarez-Romero *et al* 2007). In most European countries the maximum permissible nitrate concentration in drinking water is now 11.3 mg NO_3^-–N L^{-1} (50 mg NO_3^- L^{-1}) (Strebel *et al* 1989). Despite these regulatory efforts, nitrate is a significant contaminant to water in many areas and effort is required to minimize future contamination. It was reported that among 123 656 wells tested in the USA, 6.4% contained greater than 10 mg NO_3^-–N L^{-1} and 13.2% contained 3.1–10 mg NO_3^-–N L^{-1} (Huang *et al* 2003). Furthermore, it has been established that upstream areas and non-point sources contribute the majority of the nitrate discharged into 85 estuaries in the US coastal zone (Olguin *et al* 2004).

Phosphorus in water is not considered directly toxic to humans and animals, but is indirectly the first cause of oxygen deficit and toxic algal blooms of coastal and estuarine ecosystems (Arhonditsis *et al* 2003, Huang *et al* 2003), eventually resulting in death of several aquatic animals and fishes due to suffocation (Mak *et al* 2003).

1.2. Water contamination due to heavy metals

Environmental concentrations of heavy metals have increased significantly in the last 50 years because, while heavy metals occur naturally, they are also found as a result of a variety of anthropogenic activities. For example, it is reported that there are over 100 000 abandoned or inactive mining sites across the USA (Caruso *et al* 2008). These abandoned waste materials usually contain metal-rich sulfides which have adverse effects on the environment through the discharge of acid mine drainage (AMD) (Hezard *et al* 2009). Many abandoned mines are considered by the US EPA as extremely hazardous toxic waste sites, including about 60 waste sites designated by Superfund (Fortin *et al* 1995). The heavy metals most frequently encountered in these sites include arsenic, lead, mercury, cadmium, chromium, zinc, nickel and copper, all of which pose risks for human health and the environment and are included on the US EPA's list of priority pollutants (Mulligan *et al* 2001). In spite of intensive research efforts, spreading of AMD from the source through the integrated groundwater–surface water system has become a rapidly emerging concern in the USA. According to the reports from the US EPA, AMD has already contaminated the headwaters of more than 40% of the watersheds in the West (Fortin *et al* 1995).

The toxic effect of heavy metals usually involves an interaction between the heavy metal ion and the specific target protein, resulting in a change in protein structure and function. Cells involved in the transport of trace metals are particularly susceptible to toxicity (Erie *et al* 2005, Wagner *et al* 1998). Many heavy metals, such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), are known to be potentially mutagenic or carcinogenetic to humans and other soil organisms and plants (Ho *et al* 2002, Singleton *et al* 2007, Bagal-Kestwal *et al* 2008). Health-related studies have shown that excessive intake of toxic trace metals can directly influence the body's metabolic regulation in numerous ways, resulting in neurological and cardiovascular diseases, as well as renal dysfunction (Tariq *et al* 2008). More specifically, chronic Cu intake is related to hemochromatosis. Cadmium adversely affects several important enzymes and is known to accumulate in the human kidney for a relatively long time, from 20 to 30 years. At high dose, it is also known to produce health effects on the respiratory system and it has also been associated with bone disease (ATSDR 1999). Excessive zinc uptake may also cause or contribute to gastrointestinal problems, hair loss, anemia, loss of libido, impotence, prostatitis, ovarian cysts, menstrual problems, depressed immune functions, muscle spasms, sciatica, renal tubular necrosis/interstitial nephritis, dizziness and vomiting, among others (ATSDR 1999). Lead is of specific concern due to its relative abundance at contaminant sites and its known potential to cause adverse health effects (Szitanyi *et al* 1996, Martin and Ruby 2004), including damage to central and peripheral nervous systems (Gupta and Torres 1998). Long-term occupational exposure can cause adverse health effects on the lungs and kidneys.

2. Contaminant monitoring procedures

Traditional monitoring of contaminants is generally accomplished by invasively collecting samples in the field and transporting them to centralized laboratories for analyses. Unfortunately, since their speciation may be changed quickly as a result of chemical, biological and physical reactions, the long time delays associated with this procedure are frequently unacceptable. Thus, one of the best preventive measures is to rapidly determine the contaminants on-site, which is a crucial issue in environmental monitoring.

Electrochemical electrode operation may be based on potentiometry, amperometry, or voltammetry (Sawyer *et al* 1994). Many of these measurements are currently made using chemical electrodes of various types, including those for measuring pH, dissolved oxygen (DO), ORP, and various cations and anions. These devices have been shown to be valuable tools for direct detection of pollutants in streams or lakes, wastewater treatment reactors, and water distribution systems due to the speed of analysis, low maintenance cost and availability of necessary equipment. Unfortunately, most analytical sensing electrodes are relatively large in size, on the order of 1–3 cm in diameter. They can be used to monitor bulk liquid concentrations when there is sufficient volume to wet the electrode contacts, but they are often inappropriate for measurements in small volumes of liquids or in soils. Further, their size makes it impossible to make spatial measurements over small distances, as is needed for biofilm or soil pore water monitoring. Due to these limitations, the continuous surveillance of hazardous areas is not possible.

2.1. Microscale monitoring

The development of a fast, accurate and robust microscale *in situ* monitoring technique, which can cope with these problems, would be highly desirable. With this, microscale *in situ* measurements of target ions at a specific location where biodegradation of toxic organics occurs in soil, biofilm, sediment or other systems could be performed to explain many of the complex reactions which take place in the system being tested. They may also be able to provide continuous *in situ* monitoring of contaminants flowing in surface water and groundwater. The need for accurate, robust *in situ* microscale monitoring is not limited to just hazardous waste scenarios, but is more universal. Remediation of Superfund and other hazardous waste sites, particularly those using bioremediation techniques, requires significant use of monitoring procedures. Rapid information feedback during waste site remediation for real-time *in situ* monitoring capabilities is essential for the sustainable management of soils. This is further exacerbated if one needs to know what is happening at the soil biofilm or pore water level. Other environmental applications include the monitoring of stream or lake sediments, water and wastewater treatment reactors, and water distribution systems. Microscale monitoring may also find significant uses in industry for monitoring industrial reactors and processes, including the healthcare industry for medical diagnosis, pharmaceutical manufacturing, and biologics research, and the food industry for monitoring bioreactors and fermentation processes. Many *in situ* microscale monitoring methods have recently been proposed as an alternative for environmental measurements, such as DO, nitrate, pH, or ORP, over micrometer distances in extremely small volumes.

2.2. Microelectrodes for in situ biofilm monitoring

Biofilms can serve a very useful purpose by biodegrading organic contaminants in water or converting unwanted inorganic materials into harmless ones (Bishop 2007). Biofilms are a critical component of membrane bioreactors and of many biological reactors used for water treatment. They are also found in soils where they may impact water injection or removal systems. They are also the key component in contaminant bioremediation. Knowledge of the way biofilms form, how they grow and how to control them is critical for effective design and operation of many water reclamation facilities. But frequently, biofilms may result in problems for water treatment systems, due to biofouling or the growth

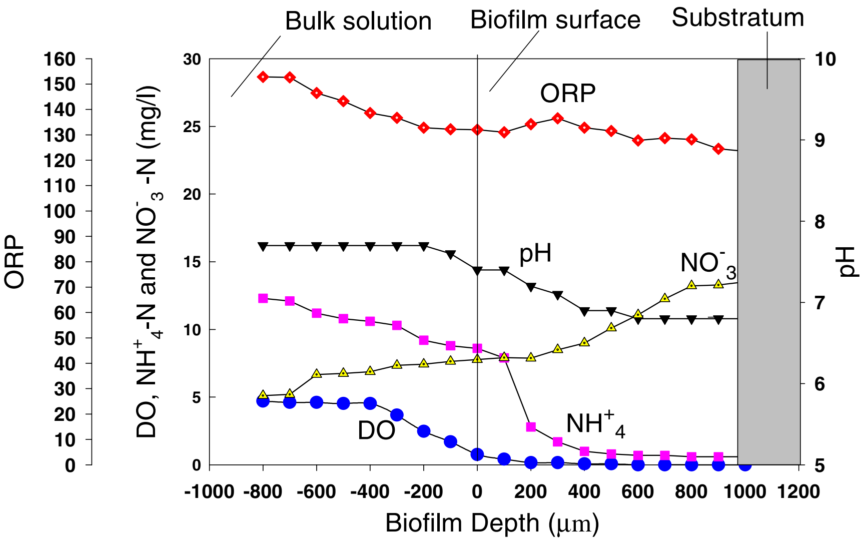


Figure 1. Typical profile of constituents in a biofilm using various microelectrodes.

of pathogenic or other unwanted microbes. So, the need for accurate, robust *in situ* microscale monitoring of contaminants in biofilm systems is essential for continuous water quality monitoring. However, necessary *in situ* monitoring systems do not currently exist.

Over the last two decades numerous papers have been published to illustrate the state-of-the-art of microelectrodes for physiological, biochemical and environmental applications (Bishop and Yu 1999, de Beer and Schramm 1999, Nelson *et al* 1986, Revsbech and Jorgensen 1986, Revsbech *et al* 1983). Microelectrodes are powerful and unique tools and the only method currently available for *in situ* measurement of concentration gradients in extremely small volumes with high resolution and no disturbance of biofilm structure (Yu and Bishop 1998, Zhang and Pang 1999, Zhang *et al* 2003, Horn 1994). Their use can be extended to describe growth of biofilms and substrate utilization by the bacteria in biofilms, to measure the effective diffusive boundary-layer thickness, and to explore metabolic activity gradients and local mass-transfer coefficient profiles in biofilm as a function of depth in the biofilm (Horn 1994, Fitch *et al* 1999). In addition, they are not greatly affected by stirring the solution since the concentration gradient exists only a few diameters from the electrode, which is very convenient in the case of *in situ* measurements where stirring is possible (Pizeta *et al* 2003). We have extensive experience with the development of microelectrode systems to monitor for such things as pH, ORP, DO, nitrogen, phosphorus and heavy metals. Microelectrodes with tip diameters of 1–10 μm have been utilized for environmental measurements over micrometer distances in biofilms, in activated sludge floc particles, in soil columns, and in gel beads containing immobilized bacteria for over 15 years (Bishop and Yu 1999, Li and Bishop 2004, Yu and Bishop 1998, Jang *et al* 2003, 2005, 2006). These biofilms were only 20–1000 μm in thickness. It is not possible to measure contaminant concentrations in these biofilms, or even in the pore water surrounding the soil biofilms, using conventional techniques. It is often essential to know such factors as DO concentration, pH and ORP at the actual location of biodegradation, rather than in the bulk liquid, because these control the biodegradation reactions. Also, rates of change of these and other constituents can be used to determine both the biodegradation rates and the rates of mass transfer into and within the biofilm. An example of the constituent profiles that can be obtained using microelectrodes is shown in figure 1.

Although these sophisticated microelectrodes have been used to determine or verify predicted gradients of environmental constituents in biofilms, their use has been limited to only a few researchers. The main reasons, as shown in figure 2, are due to the small electrical signal produced and the need to usually use them in specialized laboratories under highly controlled conditions such as inside a well-grounded Faraday cage to prevent external electrical interferences. They require trained personnel because they are difficult to manufacture and operate under micro-scale conditions. They require high concentration by personnel when the microelectrodes approach to the surface of the supporting media because they are very fragile. Although they are used inside a well-grounded Faraday cage, they may still be subject to electrical interferences because they require a length of cabling needed to connect both the microelectrode and a commercial external reference electrode to the signal processor. It is difficult to measure multi-ions at the same time or to ensure that replicate measurements are made in the same place. These have many benefits, but these pitfalls often negate their use in the natural environment. One way to overcome these problems is the development of a thin-film chip sensor which is able to measure simultaneously and rapidly multi-ions in waters when only small quantities are available, such as soil pore water. In this device, the integrated signal processing device is intimately coupled to the reference electrode and ion measuring sensors, allowing a reduction in the electrical interferences caused by the connection of long cabling.

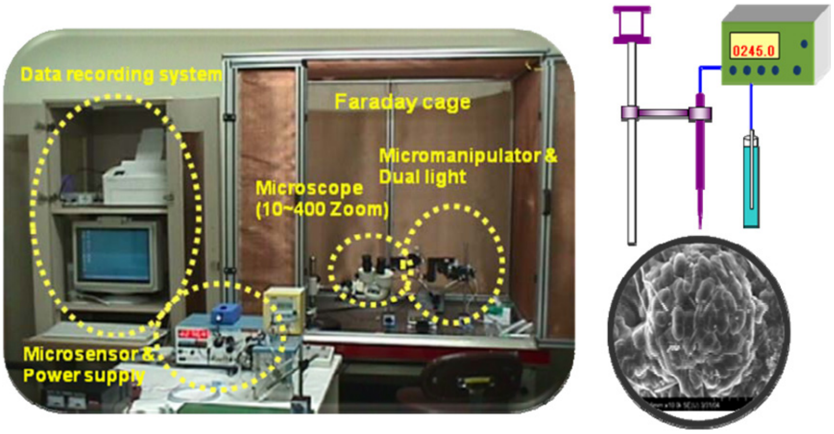


Figure 2. Picture of experimental set-up for microelectrode measurements in a Faraday cage (left); the working microelectrode is approaching the biofilm to measure the concentration profiles of interest in the biofilm (right).

Table 1. Some general requirements for environmental monitoring sensors (Rogers 2003).

Requirement	Specification range
Cost	\$1–15 per analysis
Portability	Can be carried by one person, no external power
Assay time	1–60 min
Personnel training	Can be operated after 1–2 h training period
Format	Reversible, contiguous, *in situ*
Matrix	Minimal preparation for groundwater, soil extract
Sensitivity	mg L^{-1} to μg L^{-1}
Dynamic range	At least two orders of magnitude

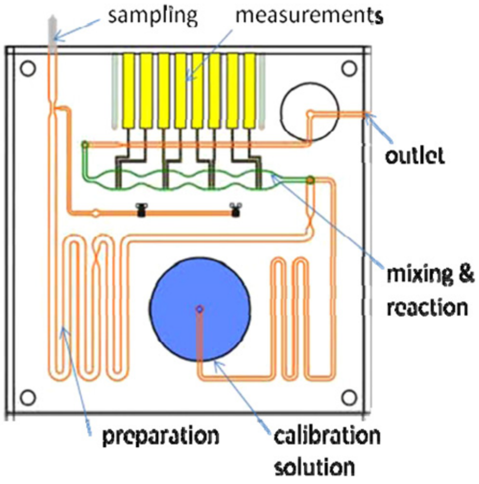


Figure 3. Schematic diagram of a state-of-the-art LOC chip.

3. Application of MEMS technology to environmental water quality monitoring

Since on-site measurement is essential to achieve effective monitoring strategies, there has been a large demand for the development of a new portable system to monitor the contaminated surface water or shallow groundwater in a cost-effective and sustainable manner. Although specific requirements must be met for each field monitoring scenario, some general requirements for environmental monitoring sensors are listed in table 1.

In order to achieve the required specifications shown in table 1, micro electro mechanical system (MEMS) and polymer micromachining techniques can be considered. Recently, there has been a growing interest in the application of MEMS technologies to monitor and measure contaminants in waters. Generally the smart lab-on-a-chip (LOC), a parallel term to micro total analysis systems (μTAS), consists of passive microfluidic components, a biochemical sensor array, on-chip pressure sources, and a sample injection interface (Ahn *et al* 2004a). As shown in figure 3, 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. A significant advantage of MEMS is the ability to make possible mechanical parts of micron size that can use very small volumes (a few μL). Miniaturized on-chip electrochemical sensors with planar microelectrodes would lead to numerous benefits such as greatly reducing the sensor cost due to mass production, precise control of dimensions, higher processing speed, excellent uniformity, reproducibility, making the entire sensing system portable and easy to use. In addition, the mass production ability of MEMS technology, due to its batch fabrication methods, permits use of low cost components with high accuracy and reliability, which are vital for effective sample mixing, separation, etc. Use of the LOC concept enables miniaturized instruments for detecting nitrate, pH, ORP and heavy metals in surface water and groundwater at shallow depths.

Since electrochemical detection provides a rather easy procedure for direct and selective detection, we have integrated MEMS technology with electrochemical sensing methods (potentiometric and voltammetric) for the simultaneous determination of toxic compounds and environmental parameters. The LOC integrated with electrochemical sensing systems could act as a high throughput analytical system for environmental screening. It can provide a way to save time and costs, with a possibility of making rapid decisions on local environmental problems. Moreover, combining the specificity of the electrochemical sensor and inherent biological sensitivity on a small lab chip, the bio-electrochemical lab chip can be expanded into an additional system for rapid and early warning detection of the presence of contaminants. Obviously, it is a powerful alternative to conventional analytical techniques. However, more accurate but time-consuming and expensive conventional methods are also needed for further validation and additional investigations of the environmental samples previously tested by electrochemical lab chip systems.

3.1. Electrochemical polymer LOC

The concepts of LOC fabricated by MEMS technology have gained tremendous popularity in the analytical chemistry community over the past decade because they allow down-scaling of processes traditionally carried out in a larger volume (Johnson *et al* 2008). However, in recent years, the range of environmental applications for lab chip systems has also increased (Ong *et al* 2001, Jaffrezic-Renault and Dzyadevych 2008) due to several promising aspects: (a) smaller sample and reagent volume (less than a few μL) is required, which is suitable for determining the analytes which are present within the host without doing any damage to the host system; (b) less sample wastage can be produced, which is cost-effective and environmental friendly way; (c) it is possible to place many microfluidic architectures in a relatively small area, thus allowing multiple studies to be performed in parallel; (d) rapid analysis times can be expected, due to the large increase in the surface area to volume ratio associated with reduction in dimensions; (e) thin-film microelectrodes are suitable for miniaturization and large-scale production using inexpensive technology; and (f) errors occurring due to manual steps can significantly be reduced because sample preparation processes are integrated and automated on a chip.

3.1.1. Plastic substrate. One of the most critical decisions for a lab chip platform is the choice of substrate material. Silicon (Si) and glass have been commonly used materials with miniaturization purposes because of their ability to support substantial electro-osmotic flow, their versatility and chemical resistivity, and their relatively straightforward fabrication (Johnson *et al* 2008, Ibanez-Garcia *et al* 2006). However, the development of polymer microstructures for the fabrication of disposable microfluidic devices and lab-on-a-chips is becoming increasingly popular because of their high volume production and, more importantly, lower fabrication cost, which reduces the overall device cost and allows the construction of disposable devices (Ibanez-Garcia *et al* 2006). Most commonly, polycarbonate (PC) and polydimethylsiloxane (PDMS) have been used as alternatives to Si and glass.

Although PDMS outshines other substrates in its high optical clarity, gas permeability, deformable mechanics, and ability to produce prototype devices quickly, many reports also suggest some drawbacks to PDMS including analyte absorption, evaporation, and low throughput production (Mukhopadhyay 2007, 2009). Compared with PDMS, cyclic olefin copolymer (COC) is particularly attractive because of its high throughput, minimal water evaporation, and solvent resistance making it suitable for integration of other electronic components, as compared in table 2 (Ahn *et al* 2004a). We have chosen COC as the favored substrate material for the environmental lab chip applications, as it has a relatively high solvent resistance.

Table 2. Comparison of COC and PDMS physical parameters relevant to microfluidic cell culture.

	COC	PDMS
Density (g cm^{-3})	1.02	0.97
Water adsorption (%)	0.01	0.2–0.8
Refractive index	1.53	1.43–1.47
Contact angle (degree)	~96	~113
Fabrication yield	High	Low
Gas permeability	Relatively low	Relatively high
Solvent resistance	High	Low
Integration of electronics	Easy	Difficult

3.1.2. Plastic micromachining using the replaceable mold disk technique. Several techniques used to fabricate the polymer lab chip involve: (1) a photolithography and metal patterning step for the microelectrode chip, (2) UV LIGA (a sequence of lithography, electroplating, and molding; the German acronym for lithografie, galvanoformung, abformung); and (3) micro polymer injection molding for the microfluidic chip. In particular, micro polymer injection molding technology is ideally suited for the mass fabrication of polymer lab-on-a-chips because of its rapid replication capability at low cost.

The micro injection molding technique is combined with a replaceable mold disk technique for rapid fabrication of the replaceable micromold inserts (Choi *et al* 2001). In this technique, the replaceable mold disk, which contains the high aspect ratio microstructures, is fabricated on a separate circular nickel disk of about 7.6 cm diameter and 1.6 mm thickness and is inserted into the molding block, as shown in figure 4. This reduces many complexities of mold fabrication such as fabrication cost, turnaround time, micromachining, etc. The mold disk can be removed from the molding block and subsequently replaced with other mold disks with different patterns without replacing the entire molding block. The injection molding can be performed using the existing injection molding system, so this replaceable disk molding technique can be considered as economically viable. The process steps for the fabrication of the micro mold insert are: (1) surface preparation of the nickel substrate, where the nickel substrate is lapped flat and parallel and polished using a lapping machine; (2) photolithography using SU-8 photoresist; (3) electroplating of nickel; and (4) photoresist stripping. An additional polishing step can be completed for the reduction of the channel surface roughness (Lee 2008).

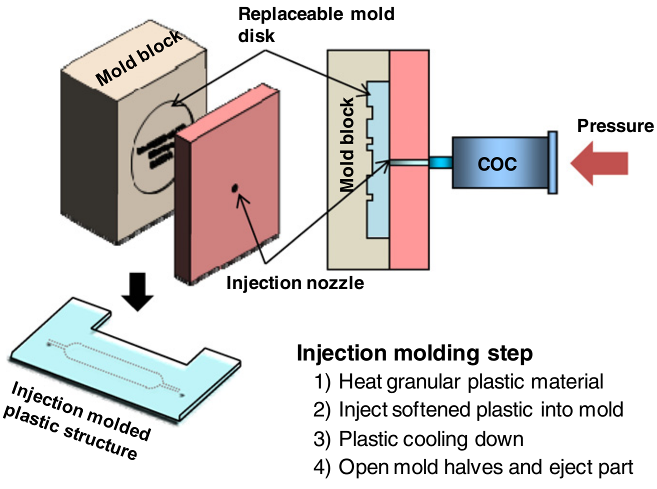


Figure 4. Schematic illustration of the plastic microinjection molding technique.

4. LOC chip sensors

4.1. Phosphate LOC sensor

Due to their instrumental simplicity, moderate cost and portability, most recent analytical method development has concentrated on either simple electrochemical sensors, such as ion-selective electrodes mainly based on the use of highly selectively ionophore materials in an immobilized PVC membrane (Choi *et al* 2005) or biosensors based on specific binding proteins (Verma and Singh 2005, Kuswandi 2003). Although in the last decade there has been growing interest in the use of these methods in environmental pollution monitoring and detection of certain types of analytes , their configuration with an internal filling solution is not well suited for miniaturization; and the relatively high cost and instability of enzyme materials limit the use of enzyme-based heavy metal sensors. Alternately, the replacement of the liquid internal solution with a solid-state membrane for heavy metals seems quite promising and has been the subject of a number of recent investigations (Zine *et al* 2006). The use of solid-state membranes has many advantages over other conventional methods, including simple structure, high sensing performance and ease of compatibility with other advanced technologies such as MEMS techniques.

As an example, we developed a miniaturized phosphate sensor with an on-chip planar cobalt (Co) microelectrode and integrated microfluidic channels (figure 5) using standard MEMS fabrication technology. The proposed sensor has been realized very cheaply and is suited for large-scale mass production and disposable usage without cross contamination. Further benefits of the proposed sensor include low volume of analyte consumption and waste generation, rapid sensing time, and elimination of the extensive polishing step used for bulk Co-wire, while maintaining comparable stability and sensitivity to traditional Co-wire electrodes. Eventually, this sensor can be used for large-scale field deployment for environment applications and disposable point-of-care testing (POCT) in clinical diagnostics. Moreover, it can be easily integrated into lab-on-a-chip devices, coupled with sample preparation and additional analyses.

4.1.1. Theoretical background for potentiometric measurement of phosphate. The interaction of the cobalt surface with oxygen has been suggested to explain the sensing mechanism of the cobalt toward the phosphate ions by several authors (De Marco *et al* 1998, Englblom 1999, Meruva 1996, Xiao 1995). They have realized that in both acidic and basic medium there is an oxidation reduction on the surface of the

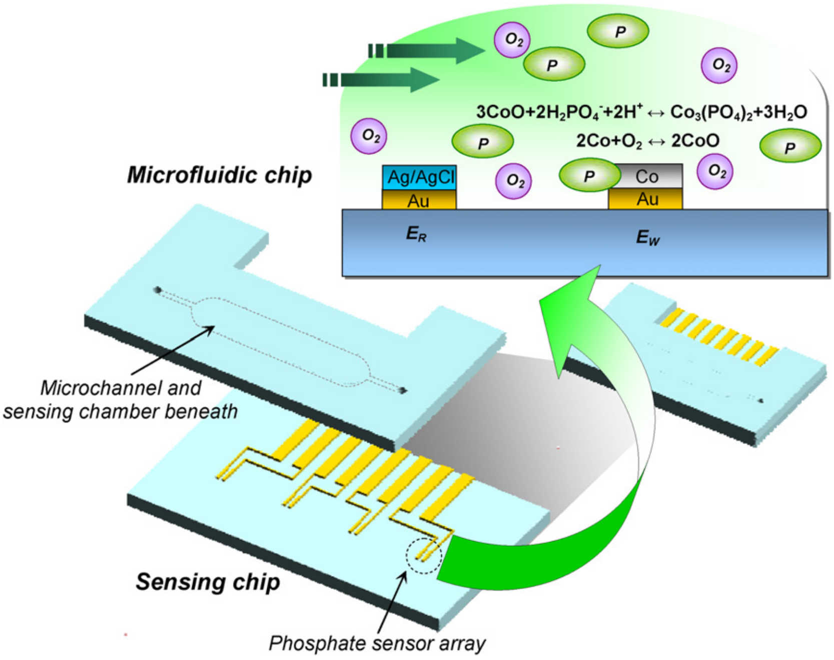


Figure 5. Schematic view and working principle of the on-chip phosphate sensor with planar Co electrodes on polymer substrates.

cobalt electrode by following a pretreatment procedure (De Marco *et al* 1998, Meruva 1996), schematically in both cases:

$$2Co_{(s)} + 2H_2O_{(l)} \leftrightarrow 2CoO + 4H^+ + 4e^-. \qquad (1)$$

In this way, it has been postulated that the CoO layer formed at the electrode surface serves as the sensitive membrane responding toward phosphate ions according to the host–guest mechanism (Xiao 1995). Specifically, in the presence of phosphate in the solution, cobalt phosphate is produced on the surface of cobalt by the following three reactions proposed (Engblom 1999, Meruva 1996), depending on the pH value of the solution:

at pH 4 in 25 mM potassium hydrogen phthalate (KHP, K_2HPO_4) buffer:

$$3CoO + 2H_2PO_4^- + 2H^+ \leftrightarrow Co_3(PO_4)_2 + 3H_2O \qquad (2)$$

at pH 8 in *N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid (HEPPS) buffer:

$$3CoO + 2HPO_4^{2-} + H_2O \leftrightarrow Co_3(PO_4)_2 + 4OH^- \qquad (3)$$

at pH 11 in 10 mM 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS) buffer:

$$3CoO + 2PO_4^{3-} + 3H_2O \leftrightarrow Co_3(PO_4)_2 + 6OH^-. \qquad (4)$$

The Nernst equation for the electron transfer and side reaction can be written as (De Marco *et al* 1998)

$$E = \text{const.} - 2\cdot\frac{303RT}{F}\text{pH} - 2\cdot\frac{303RT}{F}\log(\gamma_{H_{3+x}PO_4^x}) - 2\cdot\frac{303RT}{F}\log\left(H_{3+x}PO_4^x\right) \qquad (5)$$

where γ is activity coefficient and R, T and F have their traditional meanings as universal gas constant (8.314 J K^{-1} mol^{-1}), absolute temperature and Faraday's constant (96 485 C mol^{-1}). At constant pH and ionic strength in buffer, equation (5) is simplified as

$$E = \text{const.} - 2\cdot\frac{303RT}{F}\log\left[H_{3+x}PO_4^x\right]. \qquad (6)$$

In this way, the corresponding electrode potential response versus the logarithm of the phosphate concentration can be determined directly by the proposed Nernst equation (6).

4.1.2. Fabrication of a phosphate sensor and a microfluidic chip on a polymer substrate. Briefly, an Au layer of 100 nm and a Co layer of 300 nm were deposited on the 3 inch blank COC wafer using an e-beam metal evaporator. Au and Co electrodes were patterned by photolithography and etched by Co (0.5% HNO_3) and Au (TFA) etchants. The Ag/AgCl layer (~1 μm thick) was deposited on the reference electrode using electroplating on the Au seed layer (Zou *et al* 2007). The analyte consumption and sensing time of the proposed sensor can be significantly reduced by using the integrated polymer microfluidic chip. The plastic injection molding and UV adhesive bonding technique have been developed in our group for high throughput polymer biochip fabrication. The fabrication detail has been reported previously (Choi *et al* 2001). After drilling holes for fluidic interconnection at inlet and outlet, the microfluidic chip was bonded with the sensor chip using the UV adhesive bonding technique at room temperature (Zou *et al* 2007) to achieve the final device. The photographs of the fabricated device are shown in figure 3, which illustrate the microelectrode array, electrical connections and microchannels. The entire chip size is 1.5 cm × 2 cm, and the inlet and outlet channels have widths of 200 μm and depths of 100 μm. The reaction chamber has a width of 2 mm, length of 10 mm, depth of 100 μm, and volume of 2 μL.

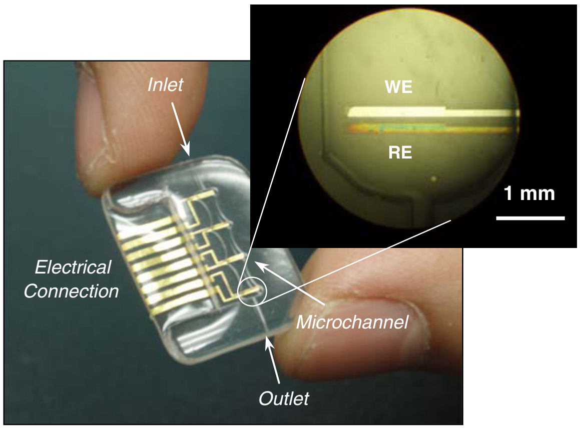


Figure 6. Photographs of the fabricated device and the microscopic image of the on-chip phosphate sensor composed of Co working electrodes (WE) and Ag/AgCl reference electrodes (RE).

The details of the Co working electrode and the Ag/AgCl reference electrode are clearly shown in figure 6 inset. Both electrodes have lengths of 1.5 mm, widths of 200 μm, and a spacing of 200 μm. As shown in figure 6, the new on-chip phosphate sensor using planar Co microelectrodes has been developed and fully characterized (Zou *et al* 2007). The feasibility of this electrochemical sensor to monitor inorganic phosphate compounds has been fully demonstrated. By incorporating mass-production microfabrication techniques and high throughput plastic micromachining, the proposed on-chip phosphate sensor with the integrated microfluidic chip can be batch fabricated at very low cost and high yield compared to the conventional bulk Co-wire-based sensor, while maintaining excellent performance. The miniaturized sensing system is especially suitable for large-scale field deployment for mass environmental data collections. Moreover, the proposed on-chip microsensor is fully integrated with the polymer microfluidic system and can be easily developed as multi-analyte polymer lab-on-a-chips for a wide range of applications.

These Co-wire electrodes show a very good response to inorganic phosphate (KH_2PO_4 and NaH_2PO_4) in a very wide dynamic range from 5×10^{-5} to 5×10^{-2} M with a detection limit less than 10^{-5} M. The Co-wire electrode has high selectivity for phosphate ions with respect to many other common anions (Chen *et al* 1997). In this research, an on-chip Co microelectrode phosphate sensor was evaluated for its performance in comparison to traditional bulk Co-wire-based phosphate sensors. It is also evident that due to the miniaturized sensing size and reaction volume, the on-chip sensor reaches an equilibrium response rapidly, in approximately 1 min for 10^{-5} M and in less than 30 s for higher concentrations above 10^{-5} M. The on-chip sensor presents a steady-state response for more than 30 min in 10^{-5} M KH_2PO_4 solution, which is sufficient for disposable sensor applications. In addition, this sensor has high reproducibility which is another vital requirement for mass-produced microsensors. For example, injections of 10^{-3} M phosphate solution into the same phosphate sensor for ten times reveal good measurement reproducibility (i.e. 526 ± 4 mV or relative standard deviation (RSD) of 0.6%). Reasonably low chip-to-chip deviation has been obtained by measuring KH_2PO_4 at 10^{-3} M on four different sensors with variances of 2.5% RSD for KH_2PO_4. The proposed on-chip sensor also exhibited high selectivity for $H_2PO_4^-$ (e.g. $K_{i,j}(Cl^-) = 4.1 \times 10^{-3}$, $K_{i,j}(NO_3^-) = 8 \times 10^{-4}$, $K_{i,j}(SO_4{}^{2-}) = 8.2 \times 10^{-4}$, $K_{i,j}(I^-) = 1.1 \times 10^{-2}$) which is comparable to bulk Co-wire-based phosphate sensors.

4.2. *Oxidation–reduction potential LOC sensor*

The determination of oxidation–reduction potential (ORP or redox potential) is of great importance in the field of water quality monitoring since solutions can be graded as oxidizing or reducing based on measurements of the ORP value. There is an increasing demand for measurement of ORP in solutions, both in industry and in environmental research (Jang *et al* 2005). For example, ORP values can provide significant information for effective disinfection in pool, spa and potable water since they are related to the kill time of *E. coli* bacteria in water (Almasi and Pescod 1996). Similarly, electrolyzed oxidizing water has been reported to have strong bactericidal effects on many pathogenic bacteria (Hsu 2005). ORP measurements in microbial systems are also of interest since ORP is primarily determined by the energy-yielding reactions of bacterial cells and is, therefore, a parameter associated with a dynamic process (Bishop and Yu 1999). Microscale *in situ* measurements of ORP at a specific location where biodegradation of toxic organics occurs in soil, biofilm, sediment or other systems can be used to explain many of the complex reactions which take place in the system being tested, because many chemical or biological reactions are correlated with the ORP–time relationship.

In potentiometry, the potential difference of ion-selective electrodes, such as pH, K^+, NH_4^+, NO_2^- and NO_3^-, is generally determined by selectively transferring the ion to be measured from the sample solution to the liquid ion exchange (LIX) membrane. The potential difference developed across the LIX membrane is directly proportional to the logarithm of the ionic concentration in the sample solution. Thus, in order to determine the ionic concentration of an unknown solution, it is only necessary to measure the potential difference in standard solutions, construct a straight-line calibration graph by plotting millivolts versus the logarithm of the ionic concentration, and then read off the unknown ionic concentration from the measured voltage. On the other hand, instead of using the LIX membrane of ion-selective electrodes, a noble metal (platinum or gold) can be used as a solid-state membrane in ORP electrodes. The redox potential generated at the solid-state membrane varies with the chemicals in the solution. When coupled with an Ag/AgCl reference electrode, nominal redox potentials for the pH 7 and pH 4 quinhydrone reference solutions, as recommended by the American Society for Testing and Materials (ASTM), should be 92 and 268 mV, respectively, at 20 °C; 86 and 263 mV, respectively, at 25 °C (Jang *et al* 2005). As shown in figure 7, the measured ORPs using the three kinds of redox potential solutions were typically slightly lower than those of the nominal redox potential (NRP). However, ASTM suggests that the measured redox potentials should be within ±10 mV of the nominal redox potentials for a good redox electrode. Thus, all of the measurements would be deemed acceptable. Additionally, the response times (seconds) for stabilization of the ORP LOC at 90% of the final stable reading were only a few seconds.

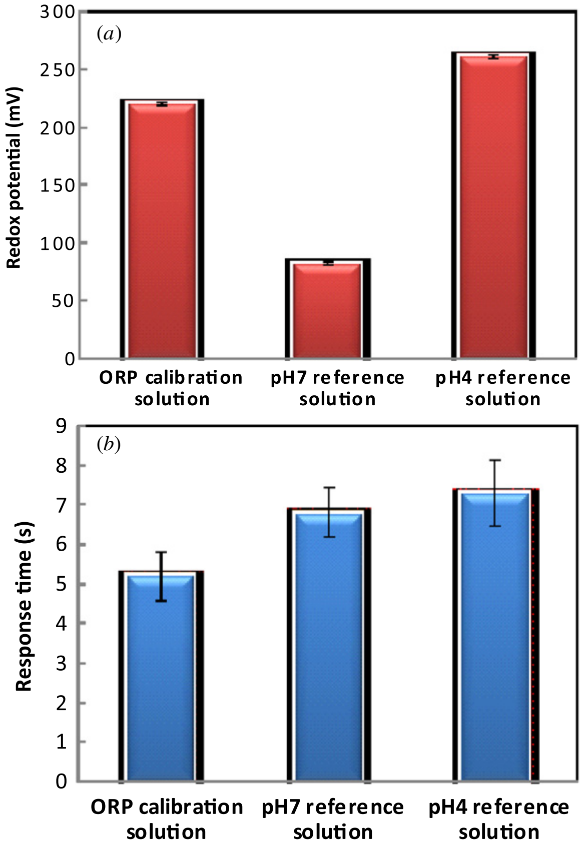


Figure 7. Measured redox potentials (*a*) and response times (*b*) by the ORP chip sensor with Ag in 1M KCl and Ag/AgCl (1M KCl) reference electrodes (number of measurement, $n = 10$).

The rapid development of microdevices for chemical analysis has been greatly promoted by the progress of MEMS techniques, and such microfabricated sensing platforms have attracted much attention by scientists and engineers since their simple measuring principles and portability make them suitable for on-site analysis. Clearly, such an LOC platform has paved the way for new and improved environmental monitoring devices, which apply ORP as one of the online control parameters in various plants.

4.3. *Ion-selective (nitrate and pH) LOC sensors*

A novel approach we are working on is the development of the 'smart' membrane, which is considered to be one of

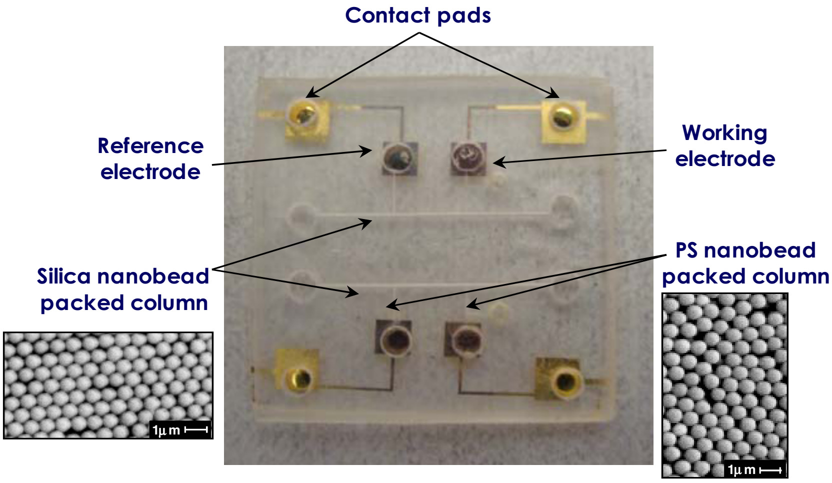


Figure 8. Photograph of the dual ion-selective lab chip and the ESEM images of the heterogeneous nanobead packed column.

the most difficult tasks in the development of ion-selective microelectrodes (Johnson *et al* 2008). For the development of potentiometric nitrate and pH LOC sensors, the nanobead packing technique can be applied to fabricate self-assembled nanobead hetro (nBH) columns in a polymer chip cartridge. When making planar nitrate and pH microelectrodes and Ag/AgCl reference microelectrode, using MEMS fabrication technologies, the main problems are how to hold the liquid ion exchange (LIX) membrane to permit ion selectivity and how to contain the internal KCl solution to make a reliable miniature reference electrode. Most planar miniature solid-state reference electrodes coated by electrodeposition or screen-printing techniques have been constructed without any internal electrolyte. Such planar solid-type microelectrodes resulted in only short-term stability, poor reproducibility, and poor lifetime (Valdes-Ramirez *et al* 2005, Suzuki *et al* 1999). For these reasons, various fabrication methods have been suggested for producing miniature electrodes of different types (Liao and Chou 2006), but none has achieved any commercial significance. Based on several methods described in the literature (Valdes-Ramirez *et al* 2005, Suzuki *et al* 1999) to overcome potential drift problems, the next most promising method of construction of miniature ion-selective and reference microelectrodes would be a liquid-junction electrode containing LIX and saturated KCl solutions. One way to realize the liquid-junction electrode is to make a self-assembled nBH column to hold LIX or electrolyte.

For fabrication of the nBH column on a chip, the patterned COC chip substrate in our labs is pretreated with O_2 plasma for 2 min to produce hydrophilicity on the surface of the microchannel (Lee *et al* 2007). Pretreated open microchannels showed high enough hydrophilicity to drive the silica colloidal suspension to the end of the channel by capillary force. The colloidal silica solution (800 nm, 0.1 wt%, Bang's Laboratories, Inc., Fishers, IN, USA) was heated (60 °C) and gently stirred (80 rpm) to prevent slow precipitation of the aggregated silica particles. The room temperature and humidity should be controlled during the self-assembly bead packing process to ensure repeatable results. Once the colloidal silica particles reach the end of the capillary channel, spontaneous three-dimensional packing of the silica beads starts from the end of the microchannel due to the slow evaporation of water. The packing process continues toward the end of the empty microchannel at the bottom. The packing speed is controlled by the opening of the beaker. The designed column can be packed in a relatively easy and rapid manner, and it takes less than 2 min for the bead packing process to be completed in a micro-volume channel of 0.0125 mm^3.

As shown in figure 8, the hydrophilic or hydrophobic nanobeads were partially packed in a designated region by capillary electrophoresis. As a result, the newly developed nBH column was composed of a hydrophilic silica bead-packed area for electrolyte loading and a hydrophobic polystyrene bead-packed area for a LIX membrane which is permeable for target ions but impermeable for interferring species. Based on our experience with self-assembly bead packing technology, we produce various sizes and shapes of nBH columns on polymer lab chips for practical on-site electrochemical analyses. The nBH column developed from this work can provide a new electrochemical sensing platform with a high sensitivity and excellent ion selectivity for environmental monitoring.

Higher magnification of the top surface clearly shows a hexagonal packing structure. From these results, more controlled pore size and/or structure of a self-assembled nanobeads packed column can be produced by characterization and optimization of the self-assembly process. The combination of the width of the microchannel, size of the beads, and the target length is critical for the quality of the bead assembly process. One novel approach here is to utilize polystyrene nanobeads with hydrophobic surfaces to build the LIX membrane connected to a hydrophilic silica nanobead packed column. The highly porous nBH column is capable of generating various ISE membranes by filling the void spaces with an appropriate LIX membrane solution. According to the nano pore size and distribution, as well as the nanobeads' hydrophilic or hydrophobic properties, the holding capacity of LIX membrane solutions and electrolyte in the pores is shown to be different, which is one of challenging problems we will need to overcome in the future.

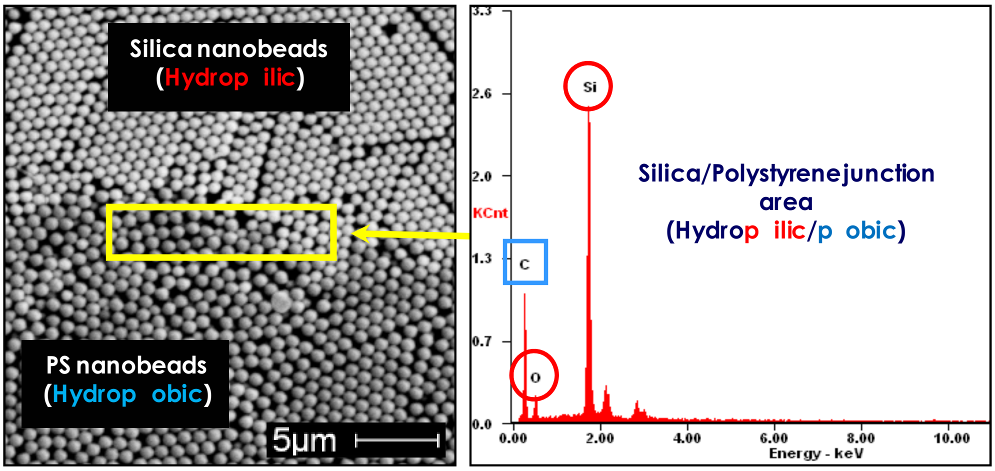
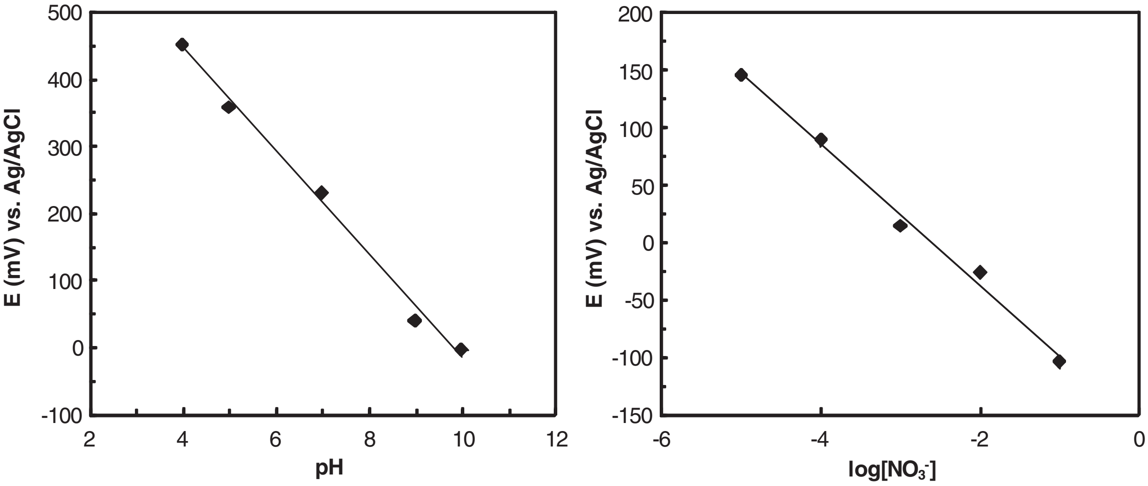


Figure 9. Material identification of the nBH column based on EDS.



(*a*) (*b*)

Figure 10. Potentiometric responses of the (*a*) pH and (*b*) nitrate ion-selective lab chip.

In order to insure adequacy of the self-assembled nanobead heteroassembly, energy dispersive spectrometry (EDS) was performed over the nBH column as shown in figure 9. Si, oxygen and carbon peaks were uniformly shown at the junction area. This clearly confirmed that a heterogeneous nanobead packed column was successfully constructed on a chip.

To assess the electrochemical detection performance of the ion-selective sensor chip for pH and nitrate, potentiometric responses were measured. As shown in figure 10(*a*), the measured results for the pH chip due to variations of pH show good sensitivity with a slope of -77.0 mV/pH unit and good linear response within the pH range of 4–10. Figure 10(*b*) shows that the potentiometric response of the nitrate ion-selective sensor chip was linear with a Nernstian slope of -61.4 mV/decade within the NO_3^- concentration range of 10^{-5} to 10^{-1}. The response times of the pH and nitrate sensors were less than 5 and 7 s, respectively.

4.4. Heavy metal LOC sensor

To estimate the detrimental role of heavy metals in various natural media, it is important to determine the concentration of heavy metals in soil and groundwater. Mercury-based electrodes are generally reported to be the most sensitive working electrodes for measuring various levels of trace heavy metals (Wang *et al* 2000), due to their advantageous electrochemical properties. As shown in figure 11, a mercury-based on-chip sensor was developed in our group to detect heavy metals (Zhu *et al* 2005). The mercury droplet was successfully generated in a microfluidic system under the control of thermopneumatic actuation and surface tension.

However, because of mercury's toxicity, expensive disposal procedures are required to limit release of mercury to the environment and to reduce human exposure. For these reasons, a more environmental-friendly heavy metal sensor needs to be developed. Bismuth (Bi) is an environmentally friendly element, with a very low toxicity and widespread

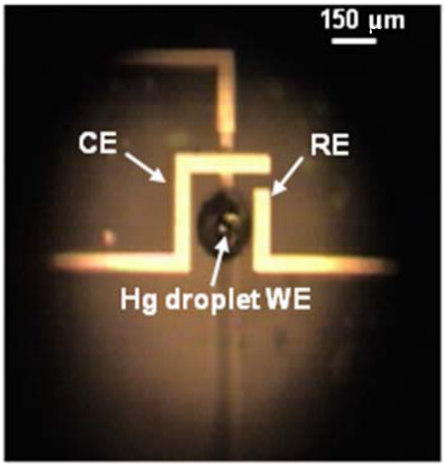


Figure 11. Picture of on-chip generation of the mercury microelectrode (Zhu *et al* 2005).

pharmaceutical use (Baldrianova *et al* 2006, Kokkinos *et al* 2008, Wang *et al* 2000). Generally, for bismuth-based analytical systems, Bi(III) ions (0.25–1 ppm) are directly added into the sample solution and then simultaneously deposited on the carbon substrate along with target heavy metals. However, in some cases, this method is not applicable for on-site heavy metal measurement in natural environments because it introduces additional Bi ions. One way to overcome this problem is the development of a thin-film chip sensor which is able to rapidly measure heavy metals in water. The use of a thin-film chip sensor for measuring heavy metals seems quite promising, as it has many advantages over other conventional methods, including a simple structure, high sensing performance, and compatibility with other advanced technologies such as MEMS techniques. The Bi-coated film electrode has received considerable attention due to its very low toxicity as opposed to mercury and advantageous electrochemical properties, and insensitivity to dissolved oxygen (Baldrianova *et al* 2006, Gouveia-Caridade *et al* 2006, Wang 2005, Hocevar *et al* 2002, 2005, Arribas *et al* 2006). Accordingly, Bi film electrodes were prepared in our labs by plating Bi ions onto a polymer substrate.

4.4.1. Square wave anodic stripping voltammetry. It is widely accepted that anodic stripping voltammetry is one of the most sensitive, convenient, and cost-effective analytical methods for detection and determination of heavy metals in water, whether from rivers, lakes, process streams or drinking sources. The square wave anodic stripping voltammetry (SWSV) sensing method was used to follow the heavy metal stripping process at the Bi working electrode because, in previous studies, it offered higher detection sensitivity than either linear sweep or differential pulse voltammetry for rapid analysis of heavy metals (Zhu *et al* 2005, Wang 1985). This technique generally utilizes a three-electrode system: a working electrode through which a potential is applied, a reference electrode and an auxiliary electrode through which current flows. In this configuration, any species which undergo electrochemical oxidation or reduction at the potential applied are detected at the working electrode.

The fabrication of the chip involves two processes: a photolithography and metal patterning step for the sensor chip, and UV LIGA (Ahn *et al* 2004b, Zou *et al* 2008) and polymer injection molding for the microfluidics chip. As shown in figure 12, for the sensor chip, a gold layer of 100 nm and a Bi layer of 100 nm were deposited on the 3 inch blank COC substrate using an e-beam metal evaporator (Temescal FC1800, BOC Edwards Temescal, CA, USA). A 2 μm thick Shipley 1818 photoresist layer was spin-coated and patterned on a COC substrate by photolithography. The electrodes were etched by Bi (2% H_2SO_4 and 2% H_2O_2) and gold (TFA) etchants to form the designed electrode structures. The Ag/AgCl ($\sim$1 μm) layer was deposited as the reference electrode on the gold seed layer using electroplating. For the microfluidics chip, a 220 μM thick negative SU-8 photoresist layer was coated on the 3 inch blank nickel disk and exposed to UV light through a chrome mask, and then developed to pattern the channel structures. A nickel electroplating process was followed to grow 200 μM thick nickel structures on the SU-8 patterned nickel disk. After removing the SU-8, the nickel disk with patterned channel structures was used as a master mold to replicate the COC polymer microfluidic chips by injection molding (BOY 22A, BOY Machines Inc., PA, USA). After drilling holes for fluidic interconnection at the inlet and outlet using a micro drill bit, the microfluidics chip was bonded with the sensor chip using the UV adhesive bonding technique at room temperature to make the final lab chip.

The photographs of the fabricated chip are shown in figure 13, and illustrate three individual sensors with planar electrode arrays, electrical connections, and microchannels. The entire chip size is 4 cm × 2.5 cm, and the sensing chamber has a width of 3 mm, length of 15 mm, depth of 200 μm, and total volume of around 10 μL. All electrodes have a length of 3 mm, width of 500 μm, and a spacing of 500 μm. The array of three sensors was easily microfabricated on one chip and was used for simultaneous measurements to obtain an average measurement value on one chip. The size of the chip fit the chip slots on the fluidic motherboard. When the chips are loaded into the analyzer, the fluidics inlet and outlet on the chip connect to the microchannels on the fluidics motherboard of the analyzer through O-rings, and the electrical contact pads on the chips are contacted to spring-loaded connectors (Mill-Max Mfg. Corp., NY, USA) on the PCB board in the analyzer to form the electrical connection.

5. A portable and automatic analyzer

Throughput, precision, convenience, and data handling capability of the analysis could be greatly improved if sampling and laboratory analysis of the contaminated environmental samples could be integrated and automated with a small piece of analytical equipment. The automated on-site monitoring platform could produce a large number of screening results in a short time, which offers significant advantages over classic technologies. Such a total system can be developed using microfabricated biochips and portable analyzers. The analytical on-site monitoring system based on lab chips presented here has the ability to detect contaminants at low

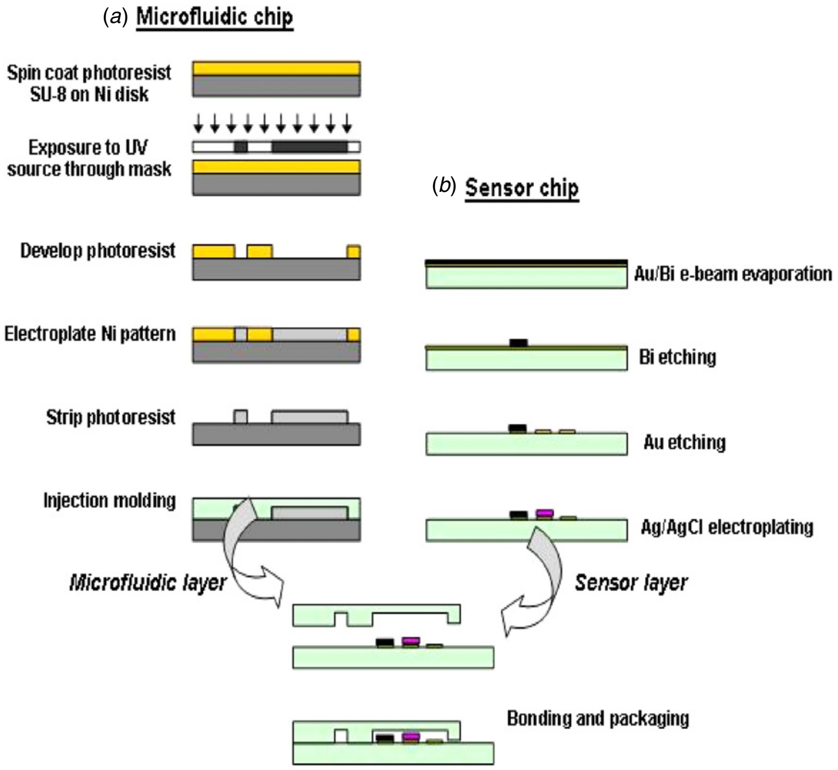


Figure 12. Schematic procedure of microfabrication processes of the heavy metal chip sensor.

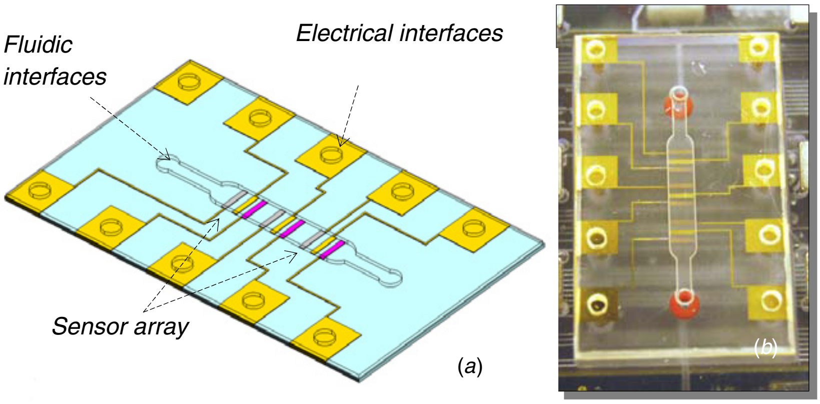


Figure 13. Polymer lab chip: (*a*) schematic view of the polymer lab chip with an array of three sensors and (*b*) photograph of the fabricated chip.

concentrations in a single, quick analysis without any prior sample pre-concentration or pretreatment steps.

The prototype of the integrated analyzer system with lab chips was developed as shown in figure 14 (Zou *et al* 2009). The design of the analyzer is comprised of a plastic fluidics motherboard with a microchannel network, valves, and a pump; printed circuit board (PCB) for control circuits; a wireless communication module; a potentiostat; and LabVIEW programs with a data acquisition (DAQ) card. The analyzer was loaded with an array of seven disposable LOCs. The size of the analyzer is 30 cm × 23 cm × 7 cm, and the weight is around 3 kg. The total power consumption of the analyzer at idle status is 0.24 W or 2.64 W, depending on whether the wireless module was turned off or on, and the power consumption during pumping is 1.44 W (wireless off) or 3.84 W (wireless on). Latching-type magnetic solenoids valves, which only consumed power when they changed the valve status, were specially chosen to reduce power consumption. A Ni-MH rechargeable battery-pack was used to provide power.

In this work, an array of lab chips was incorporated into the automated analyzer with customized user interface to perform the continuous measurement for on-site applications. As an example, in figure 15, SWASV was automatically performed to measure a 125 μg L^{-1} Cd (II) standard solution in a phosphate buffer solution (PBS, pH = 7.43). The measurements were performed serially by all three individual sensors on one chip for the same sample. In figure 15, clear Cd (II) reduction

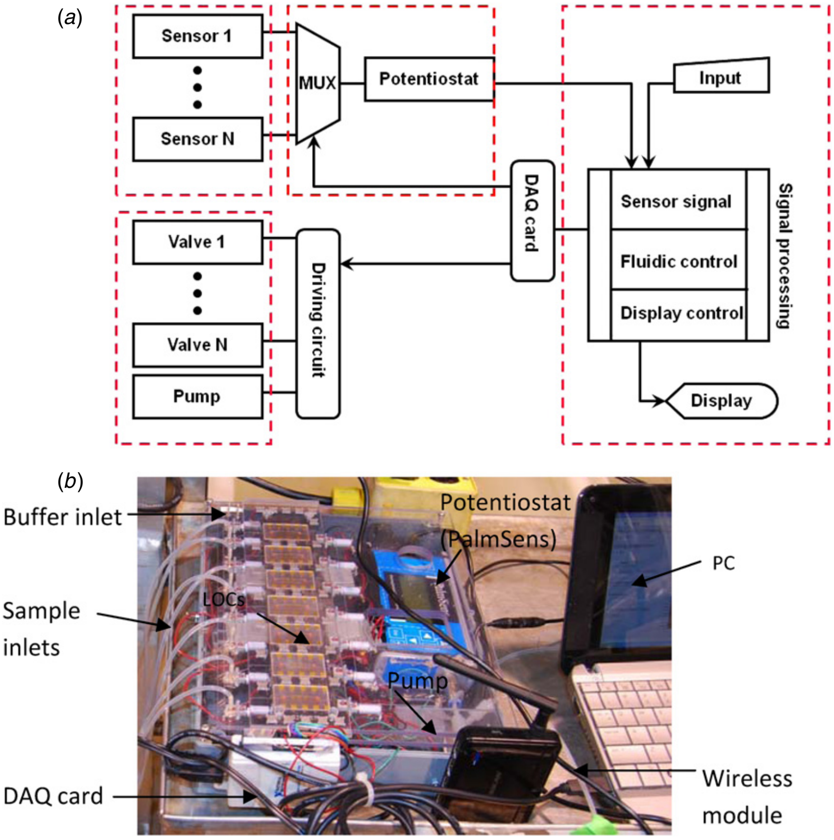
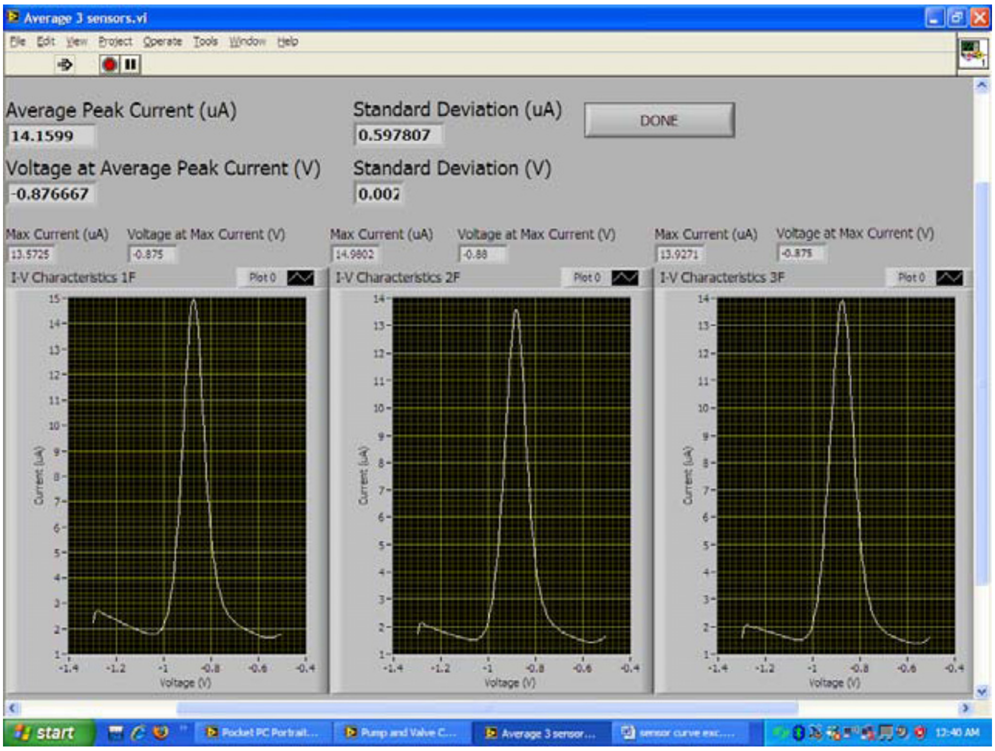


Figure 14. System block diagram (*a*) and photograph (*b*) of the automated portable analyzer system.



Average Peak Current (uA)
14.1599

Standard Deviation (uA)
0.597807

Voltage at Average Peak Current (V)
-0.876667

Standard Deviation (V)
0.007

Max Current (uA) 13.5725 Voltage at Max Current (V) -0.875

Max Current (uA) 14.9802 Voltage at Max Current (V) -0.88

Max Current (uA) 13.9271 Voltage at Max Current (V) -0.875

Figure 15. Screen shot of SWASV measurement of the 125 ppb Cd (II) in PBS buffer (pH = 7.43) which is automatically performed by three sensors on one chip using the analyzer with the customized LabVIEW program. SWASV conditions: E_{dep}: −1.2 V for 30 s, E_{sw}: 25 mV, E_{step}: 5 mV, f: 100 Hz, flow rate during deposition: 350 μL min^{-1}.

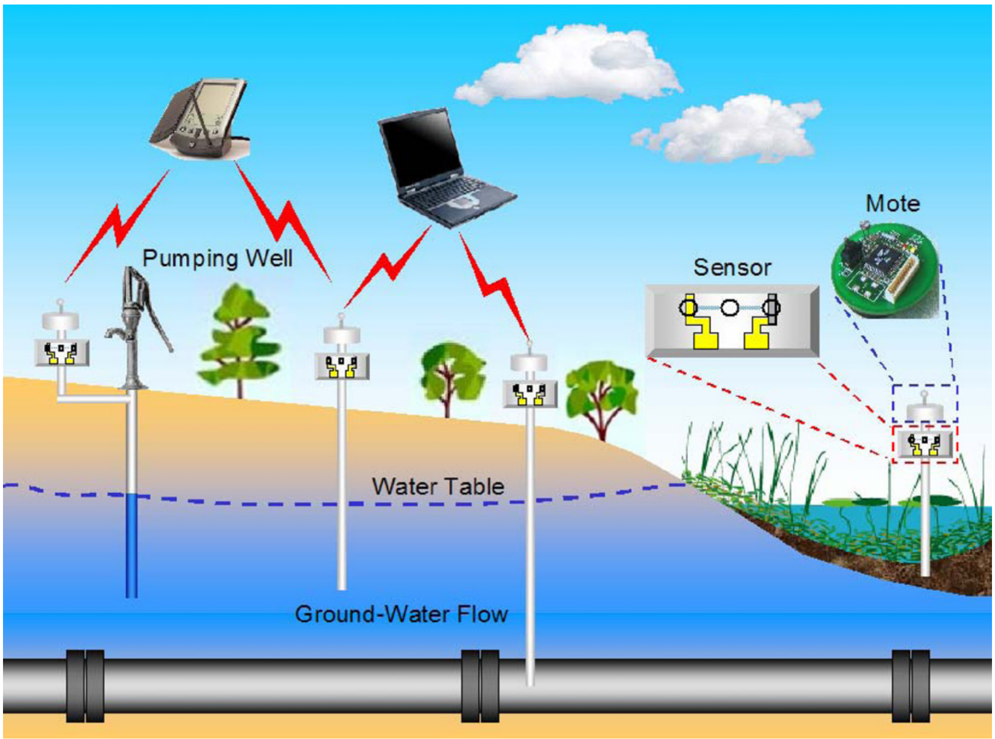


Figure 16. Schematic application of the proposed portable integrated chip sensor system that monitors the environment for multiple analytes connected to individual surface-based stations that can be interrogated with conventional computing devices.

peaks are displayed on the ultra-mobile personal computer (UMPC) screen with a low standard deviation on both the peak current height and peak current potential obtained from all three sensors. The average value (14.2 μA) and standard deviation (0.6 μA) of peak currents were calculated for calibration. All data were stored on the PDA (personal digital assistant)/UMPC for further analysis.

In practice, the idealized condition could be disturbed because under most actual conditions there are multi-ions present in the test solutions. These ions may interfere with the measurements and contribute to the overall voltage output from the electrode, resulting in that the observed detection limits are somewhat lower than those observed with optimized liquid contacts. A possible approach for improving the sensitivity and selectivity of an analyzer involves separation technologies to concentrate and purify the samples. One such development involves the use of a nanobead-packed column to remove potential interferents from unfiltered samples. With further development, it may be possible to develop an ideal portable and automatic analyzer with an increased accuracy and dynamic range.

6. Concluding remarks

There is a large demand for the development of a point-of-care (POC) environmental instrument, and the demand will be further increased due to cost savings from on-site analysis and, more importantly, enforcement of environmental legislation, necessitating reliable monitoring of the environment for the presence of compounds which may adversely affect human health and local ecosystems. However, most of the environmental monitoring sensor systems commercially available today are either prohibitively costly or highly inflexible; they use invasive sampling that can alter chemical and biological conditions at the observation site; or they have electrodes that must be dipped into the water containing the pollutants. Systems with this type of sensor have had very low user satisfaction when operated under a continuous, long-time mode due to the electrode sensor becoming fouled or encrusted by bacteria, ions or other charged particles in the water. Ironically, as the demand for environmental analysis grows, analytical and research laboratories increasingly use and dispose environmentally toxic reagents. By contrast, the portable monitoring system developed from our studies is probably one of the most promising ways to solve these problems, due to its numerous advantages: (a) it will be free of problems associated with cost since the lab chip microfabrication technologies enable not only miniaturization but also batch fabrication, which leads to the production of inexpensive sensors; (b) it would be free of fouling or clogging problems, resulting from deposition of iron and manganese oxides as well as scaling of poorly soluble salts, because it is a disposable polymer lab chip; (c) it will also allow for very accurate results without disturbing the test samples; (d) it can be used to reduce the consumption of reagents and sample, and resulting waste production (reducing the amount of used chemicals will have an impact not only on the environment but also on the economy as the number of assays steadily increases); (e) it will not require a highly trained staff, making it more useful from an economic point of view; and (f) it will offer strong potential for environmental scientists to more effectively investigate and understand diverse environmental phenomena, including the fate and transport of contaminants in soil and groundwater. It is clear that understanding the transport of contaminates in this complex and coupled bio-geochemical environment will provide insights into the mechanisms of remediation. As a result, 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. As shown in figure 16, 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 water contaminant levels with less effort and cost.

Acknowledgment

This research was supported by a grant from the National Institute of Environmental Health Sciences (NIEHS), under the Superfund Basic Research Program (SBRP) Individual Research Projects (R01) (grant no 5R01ES015446).

References

Ahn C H, Choi J W, Beaucage G, Nevin J H, Lee J B, Puntambekar A and Lee J Y 2004a Disposable smart lab on a chip for point-of-care clinical diagnostics *Proc. IEEE* **92** 154–73

Ahn C H, Jin-Woo C, Beaucage G, Nevin J H, Jeong-Bong L, Puntambekar A and Lee J Y 2004b Disposable smart lab on a chip for point-of-care clinical diagnostics *Proc. IEEE* **92** 154–73

Almasi A and Pescod M B 1996 Wastewater treatment mechanisms in anoxic stabilization ponds *Water Sci. Technol.* **33** 125–32

Alvarez-Romero G A, Palomar-Pardave M E and Ramirez-Silva M T 2007 Development of a novel nitrate-selective composite sensor based on doped polypyrrole *Anal. Bioanal. Chem.* **387** 1533–41

Arhonditsis G, Eleftheriadou M, Karydis M and Tsirtsis G 2003 Eutrophication risk assessment in coastal embayments using simple statistical models *Mar. Pollut. Bull.* **46** 1174–8

Arribas A S, Bermejo E, Chicharro M and Zapardiel A 2006 Voltammetric detection of the herbicide metamitron at a bismuth film electrode in nondeaerated solution *Electroanalysis* **18** 2331–6

ATSDR 1999 *Toxicological Profile for Cadmium* (Atlanta, GA: Department of Health and Human Services, Public Health Service)

Bagal-Kestwal D, Karve M S, Kakade B and Pillai V K 2008 Invertase inhibition based electrochemical sensor for the detection of heavy metal ions in aqueous system: application of ultra-microlelectrode to enhance sucrose biosensor's sensitivity *Biosens. Bioelectron.* **24** 657–64

Baldrianova L, Svancara I, Vlcek M, Economou A and Sotiropoulos S 2006 Effect of Bi(III) concentration on the stripping voltammetric response of *in situ* bismuth-coated carbon paste and gold electrodes *Electrochim. Acta* **52** 481–90

Berenzen N, Schulz R and Liess M 2001 Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms *Water Res.* **35** 3478–82

Bishop P and Yu T 1999 A microelectrode study of redox potential change in biofilms *Water Sci. Technol.* **39** 179–85

Bishop P L 2007 The role of biofilms in water reclamation and reuse *Water Sci. Technol.* **55** 19–26

Bizsel N and Uslu O 2000 Phosphate, nitrogen and iron enrichment in the polluted Izmir Bay, Aegean Sea *Mar. Environ. Res.* **49** 101–22

Caruso B S *et al* 2008 Metals fate and transport modelling in streams and watersheds: state of the science and USEPA workshop review *Hydrol. Process.* **22** 4011–21

Chen Z, Marco R D and Alexander P W 1997 Flow-injection potentiometric detection of phosphates using a metallic cobalt wire ion-selective electrode *Anal. Commun.* **34** 93–5

Choi J-W *et al* 2001 A plastic micro injection molding technique using replaceable mold-disks for disposable microfluidic systems and biochips *Proc. 5th Int. Conf. Micro Total Analysis Systems 2001* pp 411–2

Choi Y W, Minoura N and Moon S H 2005 Potentiometric Cr(VI) selective electrode based on novel ionophore-immobilized PVC membranes *Talanta* **66** 1254–63

de Beer D and Schramm A 1999 Micro-environments and mass transfer phenomena in biofilms studied with microsensors *Water Sci. Technol.* **39** 173–8

De Marco R, Pejcic B and Chen Z 1998 Flow injection potentiometric determination of phosphate in waste waters and fertilisers using a cobalt wire ion-selective electrode *Analyst* **123** 1635–40

Engblom S O 1999 Determination of inorganic phosphate in a soil extract using a cobalt electrode *Plant Soil* **206** 173–9

Erie J C, Butz J A, Good J A, Erie E A, Burritt M F and Cameron J D 2005 Heavy metal concentrations in human eyes *Am. J. Ophthalmol.* **139** 888–93

Fewtrell L 2004 Drinking-water nitrate, methemoglobinemia, and global burden of disease: a discussion *Environ. Health Persp.* **112** 1371–4

Fitch M W, Murphy J B and Sowell S S 1999 Biological fixed-film systems *Water Environ. Res.* **71** 638–56

Fortin D, Davis B, Southam G and Beveridge T J 1995 Biogeochemical phenomena induced by bacteria within sulfidic mine tailings *J. Ind. Microbiol. Biotechnol.* **14** 178–85

Gouveia-Caridade C, Pauliukaite R and Brett C M A 2006 Influence of Nafion coatings and surfactant on the stripping voltammetry of heavy metals at bismuth-film modified carbon film electrodes *Electroanalysis* **18** 854–61

Gupta G and Torres N 1998 Use of fly ash in reducing toxicity of and heavy metals in wastewater effluent *J. Hazard Mater.* **57** 243–8

Hezard T, Durand G, Rakib M, Viers P, Roy S, Guigues N, Brach M, Heitzmann J P, Eberle P and Bellouard F 2009 Metal on-line voltammetric analyzer for on-site monitoring of iron speciation in acid mine drainage waters: development and characterization *Electroanalysis* **21** 1401–10

Ho Y S, Huang C T and Huang H W 2002 Equilibrium sorption isotherm for metal ions on tree fern *Proc. Biochem.* **37** 1421–30

Hocevar S B, Svancara I, Vytras K and Ogorevc B 2005 Novel electrode for electrochemical stripping analysis based on carbon paste modified with bismuth powder *Electrochim. Acta* **51** 706–10

Hocevar S B, Wang J, Deo R P and Ogorevc B 2002 Potentiometric stripping analysis at bismuth-film electrode *Electroanalysis* **14** 112–5

Horn H 1994 Dynamics of a nitrifying bacteria population in a biofilm controlled by an oxygen microelectrode *Water Sci. Technol.* **29** 69–76

Hsu S Y 2005 Effects of flow rate, temperature and salt concentration on chemical and physical properties of electrolyzed oxidizing water *J. Food Eng.* **66** 171–6

Huang X P, Huang L M and Yue W Z 2003 The characteristics of nutrients and eutrophication in the Pearl River estuary, South China *Mar. Pollut. Bull.* **47** 30–6

Ibanez-Garcia N, Mercader M B, da Rocha Z M, Seabra C A, Gongora-Rubio M R and Chamarro J A 2006 Continuous flow analytical microsystems based on low-temperature co-fired ceramic technology. Integrated potentiometric detection based on solvent polymeric ion-selective electrodes *Anal. Chem.* **78** 2985–92

Jaffrezic-Renault N and Dzyadevych S V 2008 Conductometric microbiosensors for environmental monitoring *Sensors* **8** 2569–88

Jang A, Lee J H, Bhadri P R, Kumar S A, Timmons W, Beyette F R, Papautsky I and Bishop P L 2005 Miniaturized redox potential probe for *in situ* environmental monitoring *Environ. Sci. Technol.* **39** 6191–7

Jang A, Szabo J, Hosni A A, Coughlin M and Bishop P L 2006 Measurement of chlorine dioxide penetration in dairy process pipe biofilms during disinfection *Appl. Microbiol. Biotechnol.* **72** 368–76

Jang A, Yoon Y H, Kim I S, Kim K S and Bishop P L 2003 Characterization and evaluation of aerobic granules in sequencing batch reactor *J. Biotechnol.* **105** 71–82

Johnson R D, Gaualas V G, Daunert S and Bachas L G 2008 Microfluidic ion-sensing devices *Anal. Chim. Acta* **613** 20–30

Jones J A A 1999 Climate change and sustainable water resources: placing the threat of global warming in perspective *Hydrol. Sci. J.* **44** 541–57

Kersebaum K C, Matzdorf B, Kiesel J, Piorr A and Steidl J 2006 Model-based evaluation of agri-environmental measures in the Federal State of Brandenburg (Germany) concerning N pollution of groundwater and surface water *J. Plant Nutr. Soil Sci.* **169** 352–9

Knobeloch L, Salna B, Hogan A, Postle J and Anderson H 2000 Blue babies and nitrate-contaminated well water *Environ. Health Persp.* **108** 675–8

Kokkinos C, Economou A, Raptis I and Efstathiou C E 2008 Lithographically fabricated disposable bismuth-film electrodes for the trace determination of Pb(II) and Cd(II) by anodic stripping voltammetry *Electrochim. Acta* **53** 5294–9

Kuswandi B 2003 Simple optical fibre biosensor based on immobilised enzyme for monitoring of trace heavy metal ions *Anal. Bioanal. Chem.* **376** 1104–10

Lee S H 2008 Polymer lab-on-a-chip with functional nano/micro bead-packed column for biochemical analysis *PhD Thesis* University of Cincinnati

Lee S H, Lee D, Kim W, Park J, Horiike S and Ahn C H 2007 A fully packed capillary electrophoretic (CE) microchip for rapid on-site sampling and analysis *Pittcon 2007 (Chicago, IL)*

Li B K and Bishop P L 2004 Oxidation-reduction potential changes in aeration tanks and microprofiles of activated sludge floc in medium- and low-strength wastewaters *Water Environ. Res.* **76** 394–403

Liao W Y and Chou T C 2006 Fabrication of a planar-form screen-printed solid electrolyte modified Ag/AgCl reference electrode for application in a potentiometric biosensor *Anal. Chem.* **78** 4219–23

Liu G D, Wu W L and Zhang J 2005 Regional differentiation of non-point source pollution of agriculture-derived nitrate nitrogen in groundwater in northern China *Agr. Ecosyst. Environ.* **107** 211–20

Liu L and Lu G H 2008 Groundwater nitrate contamination risk induced by irrigation from polluted river water *J. Hydrol. Eng.* **13** 371–7

Mak W C, Chan C Y, Barford J and Renneberg R 2003 Biosensor for rapid phosphate monitoring in a sequencing batch reactor (SBR) system *Biosens. Bioelectron.* **19** 233–7

Martin T A and Ruby M V 2004 Review of *in situ* remediation technologies for lead, zinc, and cadmium in soil *Remediation J.* **14** 35–53

Meruva R K and Meyerhoff ME 1996 Mixed potential response mechanism of cobalt electrodes toward inorganic phosphate *Anal. Chem.* **68** 2022–6

Mukhopadhyay R 2007 When PDMS isn't the best *Anal. Chem.* **79** 3248–53

Mukhopadhyay R 2009 Hard-soft microfluidic device bypasses drawbacks of PDMS *Anal. Chem.* **81** 5108–

Mulligan C N, Yong R N and Gibbs B F 2001 Remediation technologies for metal-contaminated soils and groundwater: an evaluation *Eng. Geol.* **60** 193–207

Nelson D C, Revsbech N P and Jorgensen B B 1986 Microoxic-anoxic niche of Beggiatoa Spp—microelectrode survey of marine and fresh-water strains *Appl. Environ. Microbiol.* **52** 161–8

Olguin E J, Sanchez G and Mercado G 2004 Cleaner production and environmentally sound biotechnology for the prevention of upstream nutrient pollution in the Mexican coast of the Gulf of Mexico *Ocean Coast. Manag.* **47** 641–70

Ong K G, Grimes C A, Robbins C L and Singh R S 2001 Design and application of a wireless, passive, resonant-circuit environmental monitoring sensor *Sensors Actuators* **93** 33–43

Pizeta I, Billon G, Fischer J C and Wartel M 2003 Solid microelectrodes for *in situ* voltammetric measurements *Electroanalysis* **15** 1389–96

Powlson D S, Addisott T M, Benjamin N, Cassman K G, de Kok T M, van Grinsven H, L'hirondel J L, Avery A A and van Kessel C 2008 When does nitrate become a risk for humans? *J. Environ. Qual.* **37** 291–5

Revsbech N P and Jorgensen B B 1986 Microelectrodes—their use in microbial ecology *Adv. Microb. Ecol.* **9** 293–352

Revsbech N P, Jorgensen B B, Blackburn T H and Cohen Y 1983 Microelectrode studies of the photosynthesis and O-2, H2s, and Ph profiles of a microbial mat *Limnol. Oceanogr.* **28** 1062–74

Rogers K R 2003 *Biosensors for Environmental Monitoring* (Las Vegas, NV: US EPA)

Santamaria P 2006 Nitrate in vegetables: toxicity, content, intake and EC regulation *J. Sci. Food Agric.* **86** 10–7

Sawyer C N, McCarty P L and Parkin G F 1994 *Chemistry for Environmental Engineering* (New York: McGraw-Hill)

Singleton M J, Esser B K, Moran J E, Hudson G B, Mcnab W W and Harter T 2007 Saturated zone denitrification: potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations *Environ. Sci. Technol.* **41** 759–65

Strebel O, Duynisveld W H M and Bottcher J 1989 Nitrate pollution of groundwater in Western-Europe *Agr. Ecosyst. Environ.* **26** 189–214

Suzuki H, Shiroishi H, Sasaki S and Karube I 1999 Microfabricated liquid junction Ag/AgCl reference electrode and its application to a one-chip potentiometric sensor *Anal. Chem.* **71** 5069–75

Szitanyi Z, Nemes C and Rozlosnik N 1996 Metallothionein and heavy metal concentration in blood *Microchem. J.* **54** 246–51

Tariq S R, Shah M H, Shaheen N, Jaffar M and Khalique A 2008 Statistical source identification of metals in groundwater exposed to industrial contamination *Environ. Monit. Assess.* **138** 159–65

Valdes-Ramirez G, Alvarez-Romero G A, Galan-Vidal C A, Hernandez-Rodriguez P R and Ramirez-Silva M T 2005 Composites: A novel alternative to construct solid state Ag/AgCl reference electrodes *Sensors Actuators* B **110** 264–70

Verma N and Singh M 2005 Biosensors for heavy metals *Biometals* **18** 121–9

Wagner M, Klein C L, van Kooten T G and Kirkpatrick C J 1998 Mechanisms of cell activation by heavy metal ions *J. Biomed. Mater. Res.* **42** 443–52

Wang J 1985 *Stripping Analysis: Principles, Instrumentation and Applications* (New York: VCH)

Wang J 2005 Stripping analysis at bismuth electrodes: a review *Electroanalysis* **17** 1341–6

Wang J, Lu J M, Hocevar S B, Farias P A M and Ogorevc B 2000 Bismuth-coated carbon electrodes for anodic stripping voltammetry *Anal. Chem.* **72** 3218–22

Xiao D, Yuan H -Y, Li J and Yu R-Q 1995 Surface-modified cobalt-based sensor as a phosphate-sensitive electrode *Anal. Chem.* **67** 288–91

Yu T and Bishop P L 1998 Stratification of microbial metabolic processes and redox potential change in an aerobic biofilm studied using microelectrodes *Water Sci. Technol.* **37** 195–8

Zhang T C and Pang H 1999 Applications of microelectrode techniques to measure pH and oxidation-reduction potential in rhizosphere soil *Environ. Sci. Technol.* **33** 1293–9

Zhang Z B, Cai W J, Liu L S, Liu C Y and Chen F Z 2003 Direct determination of thickness of sea surface microlayer using a pH microelectrode at original location *Sci. China* B **46** 339–51

Zhu X S, Gao C, Choi J W, Bishop P L and Ahn C H 2005 On-chip generated mercury microelectrode for heavy metal ion detection *Lab Chip* **5** 212–7

Zine N, Bausells J, Teixidor F, Vinas C, Masalles C, Samitier J and Errachid A 2006 All-solid-state hydrogen sensing microelectrodes based on novel PPy[3,3′-Co(1,2-$C_2B_9H_{11})_2$] as a solid internal contact *Mater. Sci. Eng.* C **26** 399–404

Zou Z J, Jang A, MacKnight E T, Wu P-M, Do J, Shim J S, Bishop P L and Ahn C H 2009 An on-site heavy metal analyzer with polymer lab-on-a-chips for continuous sampling and monitoring *IEEE Sensors J.* **9** 586–94

Zou Z W, Han J Y, Jang A, Bishop P L and Ahn C H 2007 A disposable on-chip phosphate sensor with planar cobalt microelectrodes on polymer substrate *Biosens. Bioelectron.* **22** 1902–7

Zou Z W, Lee S and Ahn C H 2008 A polymer microfluidic chip with interdigitated electrodes arrays for simultaneous dielectrophoretic manipulation and impedimetric detection of microparticles *IEEE Sensors J.* **8** 527–35