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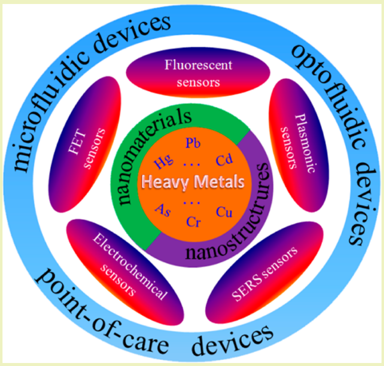
# Nanostructured Sensors for Detection of Heavy Metals: A Review

Ming Li, Honglei Gou, Israa Al-Ogaidi, and Nianqiang Wu*

Department of Mechanical and Aerospace Engineering, West Virginia University, Morgantown, West Virginia 26506-6106, United States



**ABSTRACT:** Heavy metal pollution is one of the most serious environmental problems, which undermines global sustainability. Many efforts have been made to develop portable sensors for monitoring heavy metals in the environment. Incorporation of nanomaterials and nanostructures into sensors leads to significant improvement in the performance of devices in terms of sensitivity, selectivity, multiplexed detection capability and portability. In addition, small molecules, DNA, proteins and bacteria have been integrated with inorganic materials to selectively bind heavy metals as the molecular recognition probes. This review presents a recent advance in optical, electrochemical and field-effect transistor sensors for heavy metal detection. The optical sensors are focused on colorimetric, fluorescent, surface-enhanced Raman scattering and surface plasmon resonance devices. In addition, optofluidic devices which integrate optical components with microfluidic chips are discussed. Furthermore, nanoparticle-modified electrodes, microelectrode (or nanoelectrode) arrays and microfluidic electrochemical sensors are summarized. This paper highlights the strategies for design of nanostructured sensors and the benefits from the use of nanomaterials and nanostructures.

**KEYWORDS:** *Heavy metal, Optical sensor, Electrochemical sensor, Nanomaterial, Biosensor, Microfluidics, Optofluidics*

Heavy metals enter the environment due to increasing industrial activities. Heavy metals such as Hg, As, Pb, and Cd are highly toxic and carcinogenic even at a trace level.[1,2] They are nonbiodegradable and can accumulate in the food chain, which poses a severe threat to the environment and human health. Heavy metal pollution becomes a concern for global sustainability. It is therefore essential to monitor heavy metals in the environment, drinking water, food, and biological fluids. Conventional methods for heavy metal measurement include atomic absorption spectroscopy, inductively coupled plasma/mass spectrometry, inductively coupled plasma/atomic emission spectrometry, ultraviolet−visible spectroscopy, etc.[3−6] Although these techniques are highly sensitive and selective, they require tedious sample preparation and preconcentration procedures, expensive instruments, and professional personnel.[7] Moreover, they cannot be used as portable devices for on-site detection. In contrast, sensors have great potential in high throughput detection of multiple heavy metals on-site. Rapid development of nanotechnology has provided new opportunities for improving the performance of sensors in terms of sensitivity, limit of detection, selectivity, and reproducibility, and also enabled miniaturization with assistance of lab-on-chip (LOC) technology.[8,9]

This paper reviews the recent progress in nanostructured sensors for heavy metal detection. Sensors are summarized based on different signal transduction mechanisms, including optical, electrochemical, and field-effect transistor (FET) sensors. The optical sensors include fluorescent, colorimetric, surface plasmon resonance (SPR), and surface-enhanced Raman scattering (SERS) sensors. Special emphasis is put on the role of nanomaterials and nanoarchitecture in sensors. In addition, this paper highlights the integration of sensors with microfluidic chips, which is an important direction toward applicability of sensing devices to real-world samples.

## FLUORESCENT SENSORS

Fluorescence sensing is based on analyte-induced changes in the physicochemical properties of fluorophores including fluorescence intensity, lifetime, and anisotropy, which are related to charge transfer or energy transfer processes.[10] Researchers have put a spotlight on the Förster resonance energy transfer (FRET) process when developing fluorescent sensors for heavy metal detection. FRET occurs through the dipole−dipole interaction between an energy donor and an acceptor, following a $1/d^6$ separation distance dependence.[11−13] The effective interaction distance (Förster distance) typically is $\sim$6 nm. Typically, FRET requires the spectral overlap between the emission band of donor and the absorption band of acceptor.[14] Ono has pioneered the development of FRET sensors for heavy metal detection.[15,16] Ono has linked an organic dye (fluorophore) and a quencher to two ends of a molecular beacon to form a FRET sensor, as shown in Figure 1. The presence of $Hg^{2+}$ ions induced a hairpin structure due to the formation of the $T-Hg^{2+}-T$ sandwich structure, which brought the quencher close to the fluorophore and thus enabled the FRET process, leading to fluorescence quenching

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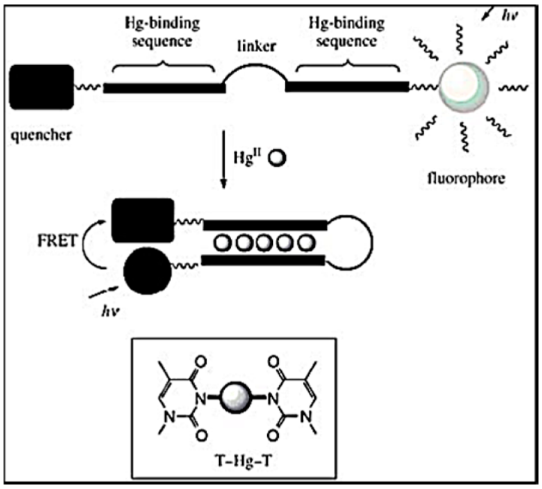

**Figure 1.** Sketch showing the molecular beacon-based FRET sensor; the presence of Hg$^{2+}$ ions leads to quenching of fluorescence emission of fluorescein.[16].

of the organic dye. This sensor achieved a limit of detection (LOD) of 40 nM toward Hg$^{2+}$ in a buffer solution and excellent selectivity toward Hg$^{2+}$ ions coexisting with other metal ions. Ono's work has opened a new avenue for development of fluorescent heavy metal sensors.

Organic dyes usually are used as the fluorophores in FRET sensors due to the commercial availability of toolboxes, well-established labeling protocols, and small size that minimizes possible steric hindrance. However, organic dyes suffer from photobleaching and have narrow excitation spectra.[17,18] Inorganic quantum dots (QDs), graphene oxide (GO), carbon dots, metallic clusters, and upconversion luminescent nanoparticles (NPs) have been emerging as alternative fluorophores due to their unique properties. For example, QDs possess broad excitation profiles, narrow and symmetric emission spectra, high photostability, high quantum efficiency, size-dependent fluorescence emission peaks, and potential multiplexed detection capability.[19−23] Carbon dots and graphene QDs are free of heavy metal elements, possess excellent biocompatibility, and have attractive surface functionality.[24−26] Owing to their good biocompatibility, polymer nanomaterials and carbon dots are of interest to fluorescent sensors used in in vivo and in vitro detection of heavy metals. In addition, upconversion NPs are explored for in vivo sensing due to their low background interference by the near-infrared excitation source.[27−29]

When the organic quencher is replaced with a gold NP in a fluorophore-quencher system, the energy transfer mechanism is dependent on the plasmonic nature of the Au NP. If the Au NP exhibits surface plasmon resonance (SPR), the energy transfer from the fluorophore (an organic dye or a quantum dot) to the gold NP follows the FRET mechanism.[30] When the Au NP has no SPR, the energy transfer mechanism is switched from FRET to nanometal surface energy transfer (NSET).[30] Unlike FRET, NSET is originated from the dipole-induced interband electronic transition in a thin layer of a semi-infinite metal hemisphere, which does not require the resonant interaction between electrons.[30,31] The NSET process follows a $1/d^4$ separation distance dependence and exhibits a much longer effective interaction distance (∼30 nm).[31−34] Au NPs are excellent fluorescence quenchers over a broad range of wavelengths and long distances in comparison with organic acceptors.[30,32,33] Au NP quenchers have several appealing features.[30,35−37] (i) Au NPs have no defined dipole moment as do organic molecules. Hence, the energy transfers in any orientation relative to the Au NPs. (ii) Au NPs have large absorption cross sections near the plasmon resonance. (iii) Au NPs have no fluorescence emission, avoiding the cross-excitation that usually occurs in organic dyes. (iv) Au NPs have low toxicity.

Recently a QD−DNA−Au NP ensemble was created by utilizing the NSET mechanism.[38] As shown in Figure 2, the complementary oligonucleotide strands were linked to the QDs and Au NPs, respectively. When Hg$^{2+}$ ions were present in the aqueous solution that contained the oligonucleotide-conjugated QDs and Au NPs, Hg$^{2+}$ ions selectively bound to thymine in the oligonucleotides, forming the DNA helix. As a result, the QDs and Au NPs were brought into a close proximity, leading to NSET from the QD to Au NPs. Consequently, the fluorescence emission of the QD was quenched by the Au NPs. This nanosensor exhibited a LOD of 0.4 and 1.2 ppb toward Hg$^{2+}$ in the buffer solution and in river water, respectively, and excellent selectivity toward Hg$^{2+}$ over various metal ions. In addition, a Rhodamine B−Au NP-based NSET probe has been reported for monitoring mercury in water, fish, and contaminated soil. This inexpensive and battery-operated sensor exhibited excellent sensitivity (2 ppt) and selectivity for Hg$^{2+}$ over Cu$^{2+}$ and Pb$^{2+}$.[39]

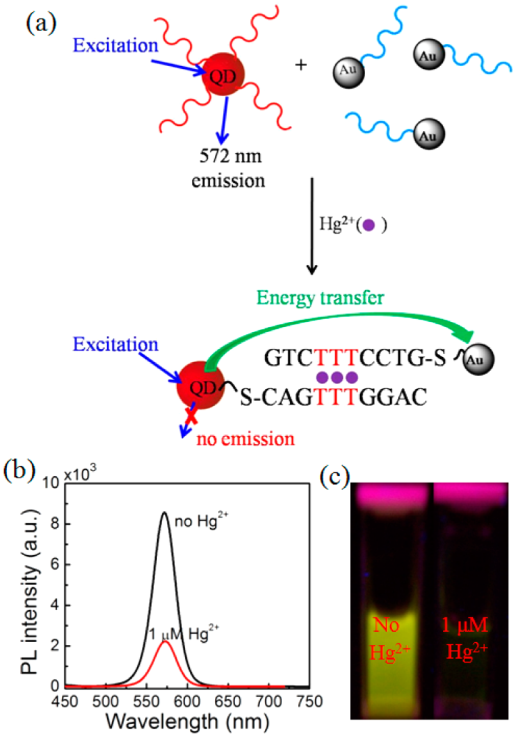

**Figure 2.** (a) Sketch showing the QD−DNA−Au NP ensemble sensor for Hg$^{2+}$ detection. (b) Fluorescence emission spectra. (c) Photograph of the QD/DNA/Au NP solution under 365 nm laser excitation in the absence and presence of 1 $\mu$M Hg$^{2+}$.[38]

Graphene oxide (GO) is an interesting fluorescence quencher alternative to the Au NPs in NSET sensors.[40−42] GO is inexpensive and can be produced massively. It has excellent water solubility and a facile surface-functionalization feature due to its inherent oxygen-containing functional groups.[43−46] Moreover, it has super fluorescence quenching capability. Hence, GO is attractive for sensors based on energy transfer.[47−51] For example, a fluorescent QD−aptamer−GO sensor was designed based on the NSET mechanism in order to detect $Pb^{2+}$ ions (Figure 3).[40] The QD-labeled aptamer, which was employed as the molecular recognition probe, was physically adsorbed onto the GO surface via the strong $\pi-\pi$ stacking interaction between the single-stranded DNA and GO. Fluorescence of the QDs adsorbed on the GO was quenched due to the NSET from the QDs to GO. The presence of $Pb^{2+}$ in the assay led to the formation of a G-quadruplex/$Pb^{2+}$ complex that had very weak affinity with the GO sheet. Consequently, the QDs conjugated with the G-quadruplex/$Pb^{2+}$ complex was detached from the GO sheets, which turned on the fluorescence emission of QDs. This sensor exhibited a LOD of 90 pM and excellent selectivity over a wide range of metal ions. In addition, a GO−DNAzyme-based biosensor was developed for detection of $Pb^{2+}$,[52] in which the GO also acted as a quencher for an organic fluorophore.

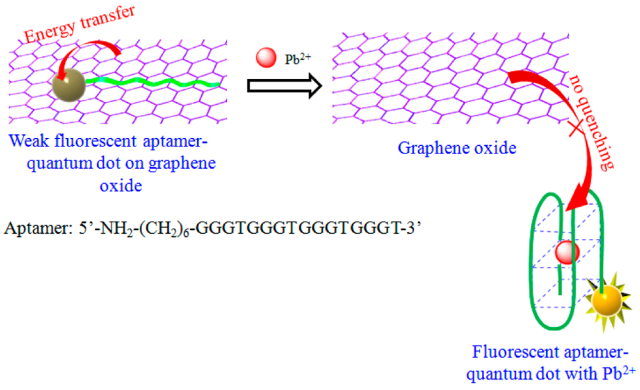

**Figure 3.** Sketch showing a "turn-on" fluorescent QD−aptamer−graphene oxide sensor for $Pb^{2+}$ detection.[40]

Besides conventional fluorescent sensors based on FRET and NSET, chemiluminescent sensors have attracted interest for detection of heavy metals.[53,54] In the chemiluminescent resonant energy transfer (CRET) sensors, luminescence of the donor is excited via a chemical reaction without the need of an external light source, which can simplify the design of the sensor system and reduce the noise background.

In addition to the energy transfer processes, the electron transfer process has also been employed for designing fluorescent sensors for heavy metal detection.[47,55,56] The electron transfer proceeds via different mechanisms including the Dexter interaction, intramolecular photoinduced electron transfer, and interfacial electron transfer. The rate constant of electron transfer exponentially decays with the distance from the nuclei, and the electron transfer is efficient at separation distances less than 1 nm.[57−59] By utilizing the electron transfer mechanism, a QD-based fluorescent sensor was developed for simultaneous detection of $Hg^{2+}$ and $Ag^{+}$.[55] This sensing assay initially contained the free-standing nucleic acid-functionalized QDs, which emitted fluorescence under light excitation. When $Hg^{2+}$ or $Ag^{+}$ appeared in the assay, the metal ions were sandwiched in the rigid hairpin structure of DNA, which enabled electron transfer from the QD to $Hg^{2+}$ ions along the duplex DNA channel, quenching the fluorescence of the QD.

It is worth noting that GO can act as not only a quencher (acceptor)[40] but also a fluorophore (donor).[43,56] Unlike QDs such as CdS, CdSe, and PbS, GO not only contains a nontoxic element but also possesses inherent carboxylic acid that can be easily conjugated to amine-functionalized DNA and antibody molecules. Therefore, GO is a promising alternative to QDs as a fluorophore in sensors. For example, the aptamer-functionalized GO has been employed as a fluorophore in a sensor based on the electron transfer mechanism for label-free detection of $Hg^{2+}$ in the aqueous solution.[56] Although this GO-based sensor was designed and constructed in a very simple way, it was a very effective sensor. The sensor showed a LOD as low as 0.92 nM for $Hg^{2+}$ in an aqueous solution.

## ■ PLASMONIC SENSORS

**Nanoparticle-Based Colorimetric Sensors.** SPR arises from collective oscillation of free conduction electrons in the noble metal in resonance with the incident electromagnetic radiation.[60,61] For example, 20 nm sized monodispersed Au NPs in the aqueous solution exhibit a strong SPR peak in the absorption spectrum, and the aqueous solution shows a "red wine" color. When the Au NPs aggregate to some degree, the color of the solution will change due to a shift of the SPR peak. On the basis of this principle, colorimetric sensors are constructed for detection of various analytes.[62−67] This type of sensor provides direct, visual, and rapid detection of analytes, which minimizes the costs. Hupp has pioneered the development of colorimetric sensors for heavy metal detection.[68] So far, the colorimetric detection method has been used for monitoring various heavy metals such as $Hg^{2+}$, $Pb^{2+}$, $Cu^{2+}$, and $As^{3+}$.[69−78] Liu et al. have demonstrated the colorimetric detection of $Hg^{2+}$ using the oligonucleotide-functionalized Au NPs (Figure 4).[76] The selectivity toward $Hg^{2+}$ was attributed to selective binding of $Hg^{2+}$ to the T−T mismatches, leading to aggregation of Au NPs. Aggregation of Au NPs resulted in a color change due to coupling of SPR of the Au NPs in close proximity. This sensor reached a LOD of 3 $\mu$M toward $Hg^{2+}$. Although the colorimetric detection method is simple and rapid, its LOD is relatively high and requires the preconcentration step to detect trace metals. In addition, it remains a

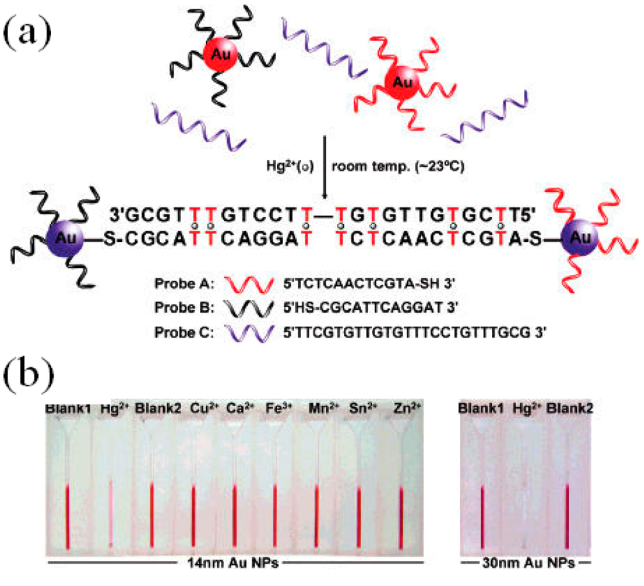

**Figure 4.** (a) Sketch showing a colorimetric sensor for $Hg^{2+}$ detection. (b) Color response of the 14 nm (bottom left) and 30 nm (bottom right) Au NPs after addition of metal ions ($Hg^{2+}$, $Cu^{2+}$, $Ca^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Sn^{2+}$, $Zn^{2+}$; 10 $\mu$M each).[76]

challenge to apply the colorimetric sensors to real-world sample matrices such as human blood and plasma.

**Chip-Based SPR Sensors.** The position and intensity of the SPR peak are sensitive to change in the surrounding environment (i.e., refractive index) and coupling with the nearby plasmonic field.[79−81] According to this principle, transmission localized surface plasmon resonance (T-LSPR) spectroscopy can be performed from a plasmonic substrate that can selectively capture metal ions. The resulting SPR peak varies upon the change in the refractive index during capture of heavy metals on the substrate.[80]

## ■ SERS SENSORS

SERS sensors have been extensively used for chemical and biological sensing and medical diagnostics,[82,83] but only a few papers have reported the heavy metal detection.[84−91] Although SERS as a molecular spectroscopy can provide the spectral fingerprint signatures of analytes, it is unable to directly detect heavy metal ions. A solution to this problem is to functionalize plasmonic nanostructures with an organic ligand that binds specifically to heavy metal ions. As mentioned above, aggregation of Au NPs can be used for colorimetric detection. On the other hand, aggregation of Au NPs can lead to coupling of plasmonic fields of neighboring NPs, forming "hot spots" for SERS enhancement. On the basis of this principle, Chen et al. have developed a SERS sensor for detection of $As^{3+}$. In this sensor, glutathione, which can selectively bind to $As^{3+}$ ions through the As−O bond, was conjugated onto the Ag NPs, and 4-mercaptopyridine (4-MPY) was used as the Raman reporter.[85] In the presence of $As^{3+}$ ions, binding of $As^{3+}$ with glutathione induced the aggregation of Ag NPs, which made the Raman reporter molecules located in "hot spots", enhancing the SERS signal from 4-MPY. This SERS sensor was simple but achieved a LOD as low as 0.76 ppb with excellent selectivity over various metal ions. However, there is still concern with the stability and reproducibility of the colloid-based SERS sensors.

SERS provides both the information regarding the presence of the chemical element and its chemical form, which is a critical aspect in the toxicological study of metal ions because different complex forms of heavy metals exhibit distinct toxicity toward human or animals. Accordingly, SERS has been employed for direct detection of $UO^{2+}$, $TcO^{4-}$, $CrO_4^{2-}$, $NpO_2^{+}$, $NpO_2^{2+}$, $WO_4^{2-}$, etc.[88−90] Irudayaraj et al. have demonstrated SERS sensing and imaging of toxic chromate in bacteria.[91] Their results showed that SERS exhibited high sensitivity and was able to distinguish two stable valence forms of chromate in cells.

## ■ OPTOFLUIDIC SENSORS

Optofluidic devices refer to the miniaturized microfluidic systems that integrate optical sensors with microfluidics, which brings a number of unique characteristics.[92−96] Optofluidic devices enable real-time detection within a minimized sample volume and facilitate multiplexed detection of analytes in a single chip simultaneously.[96] In microfluidic SERS devices, the SERS substrates are incorporated into the microfluidic channels in two ways.[92] (i) The metal NPs, which act as the SERS substrates, are suspended in the fluid passing through the channel. (ii) A metal nanostructure is fixed on the bottom of a microfluidic channel as the SERS substrate. Choo has combined the droplet-based microfluidics with the SERS transducer for detection of $Hg^{2+}$ (Figure 5).[97] The Rhodamine B molecules were preadsorbed on the Au NP surface, which can be replaced by $Hg^{2+}$ due to stronger affinity between the Au NPs and $Hg^{2+}$ ions. As a result, the SERS signal from Rhodamine B varied as a function of the $Hg^{2+}$ concentration. Another interesting feature of the microfluidic SERS devices is their ability to monitor and separate heavy metals.[98] However, the number of active sites and accessibility of analytes to the SERS active sites are reduced in the microfluidic SERS devices. This challenge can be addressed by either incorporation of photonic crystals into the device or preconcentration of analytes.[99,100] Another approach is to use an optofluidic resonator for SERS excitation in the microfluidic environment.[101,102] The high-intensity field at the optical resonator can function as a high-power excitation source for SERS and, thus, enhance the Raman signal.

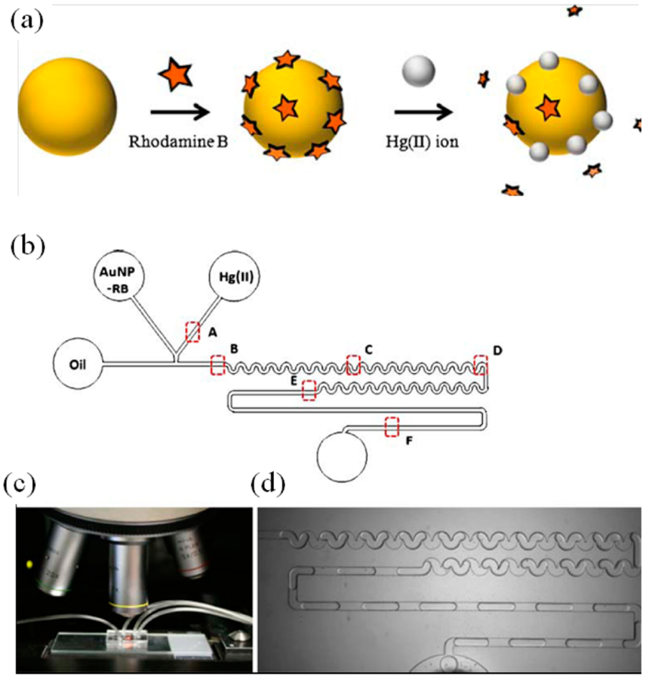

**Figure 5.** (a) Illustration of the $Hg^{2+}$ sensing principle based on the replacement of the dye molecules with the reduced $Hg^{2+}$ ions on the surface of Au NPs. (b) Microchannels for droplet generation. (c) Microfluidic chip under the microscope. (d) Photograph of the device in operation. [97]

## ■ ELECTROCHEMICAL SENSORS

Compared to optical sensors, the sensing signal of electrochemical sensors is collected through conducting wires instead of optical detectors. Hence, electrochemical sensors can be easily packed into a compact system. In addition, because heavy metals have the defined redox potential, the selectivity toward specific heavy metal ions can be achieved by bare electrodes without the need of a molecular recognition probe. Several techniques are employed in electrochemical sensing, including voltammetry, amperometry, potentiometry, impedemetry, and conductometry.[103−105] In particular, the anodic stripping voltammetry (ASV) method is readily amendable for determination of heavy metals. ASV analysis typically involves two steps:[106−108] (i) electrochemical deposition or accumulation of heavy metals at a constant potential to preconcentrate the analyte onto the electrode surface, and (ii) stripping or dissolution of the deposited analyte from the electrode surface.

The mercury-based electrode was the first one that received attention for electrochemical detection of heavy metals because it brought high sensitivity, good reproducibility, and a wide cathodic potential range for heavy metal detection.[109,110] However, owing to its high toxicity, the mercury-based electrode has been replaced by the environmentally friendly bismuth electrode that exhibits similar electrochemical behavior in many aspects.[111,112] Unfortunately, the bismuth electrode has a relatively narrow cathodic potential range and suffers from the instability in air due to its natural oxidation.[113] Besides the mercury- and bismuth-based electrodes, glassy carbon, gold, and boron-doped diamond electrodes have also been used in ASV analysis of heavy metals.[114,115] A detection level of 68 nM has been achieved in 0.1 M $KNO_3$ using a boron-doped diamond electrode with the differential pulse voltammetric technique.[114]

Although bulk electrodes have been used in ASV analysis of heavy metals,[114−118] the LOD and the sensitivity of bulk electrodes cannot meet the need for detection of trace heavy metals. Bulk electrodes (or macro-electrodes) have several problems: (i) a large overpotential required for analyte deposition, (ii) a high stripping potential needed for analyte detection, (iii) the interference of other coexisting metals, and (iv) the interference due to supporting electrolyte ions. An effective way to solve these problems is surface-modification of bulk electrodes with nanoparticles.[119,120] Another route is to use the micro- or nanoelectrodes.

**Nanoparticle-Modified Electrodes.** Nanoparticle-modified electrodes possess higher surface area, improved electron transfer rate, increased mass-transport rate, lower solution resistance, and higher signal-to-noise ratio.[107,119,121] Au NPs have been used to modify bulk electrodes.[122,123] It has been demonstrated that Au NP-modification of glassy carbon electrodes eliminated the memory effect and interferences of other ions from intermetallic compounds. Moreover, the Au NP-modified glassy carbon electrode significantly lowered the LOD toward $Hg^{2+}$.[116] It has also been reported that the sensitivity of Au NP-modified electrodes was an order of magnitude higher than that of the macro-electrode counterpart when used for $As^{3+}$ detection.[124] Also, the stripping peak of $As^{3+}$ was narrower and more symmetric for the Au NP-modified electrode, which mitigated the effect of copper interference on the arsenic detection. Recently, a Bi nanoparticle-modified electrode was employed for heavy metal detection.[125] The results showed that the sensitivity and LOD of the electrode were improved with a decrease in particle size of the Bi nanopowder, which was ascribed to a higher electroactive surface area.

In addition, carbon nanomaterials such as carbon nanotubes (CNTs), carbon nanofibers, and graphene have been explored as the electrode materials for detection of heavy metals.[126−130] For electrochemical sensing applications, CNTs and graphene have many advantages including a large surface area, small size, excellent electron transfer ability, and easy surface-modification. In addition, CNTs have been recognized as excellent sorbents for heavy metal ions.[131] Therefore, it is promising to construct electrochemical sensors using the CNT- or graphene-modified electrodes for heavy metal detection because they are capable of simultaneous preconcentration and detection of multiple heavy metal ions. A single-walled CNT electrode was used to simultaneously measure the $Cd^{2+}$ and $Pb^{2+}$ ions in a 0.02 M HCl solution with the square-wave stripping voltammetry method. It showed a LOD of 2.2 ppb for $Cd^{2+}$ and 0.6 ppb for $Pb^{2+}$. However, the stripping peak current dropped severely after day-to-day operation.

Because the bare CNTs are hydrophobic, the CNTs are typically modified with functional groups to make them hydrophilic. The surface-functionalization also alters the affinity with heavy metals, which could be utilized for selective detection of heavy meals. For example, the CNTs covalently functionalized with thiacalixarene (TCA) were immobilized on a glass carbon electrode to detect trace $Pb^{2+}$ ions with the differential pulse ASV method.[132] In this electrode, the CNTs enhanced the electron transfer, and the TCA enabled the selective accumulation of $Pb^{2+}$ ions at the electrode due to its excellent selective recognition. As a result, this electrode exhibited excellent selectivity and high sensitivity for electrochemical detection of $Pb^{2+}$ ions over a range from $2 \times 10^{-10}$ to $1 \times 10^{-8}$ M with a LOD of $4 \times 10^{-11}$ M. In addition, the cysteine-modified CNTs that were cast on the glassy carbon electrode were used for ASV measurement of $Pb^{2+}$ and $Cu^{2+}$.[133] The sensor utilized the high affinity of cysteine toward some heavy metals and exhibited the LOD of 1 ppb for $Pb^{2+}$ and 15 ppb for $Cu^{2+}$.

Nanostructured electrodes have also been constructed by the composites that combine two to three materials together to provide synergistic contribution from individual components.[134−137] Nanostructured metal oxides are typically not involved in the redox process in heavy metal detection, but they can combine with other active materials to improve the sensing performance.[134] Polymers have also been integrated with CNTs and graphene for heavy metal detection.[130]

**Microelectrode and Nanoelectrode Arrays.** Even trace-level (ppb) heavy metals pose a threat on humans and environment. This places a demand on low LOD for sensors. An effective way to improve the LOD and sensitivity of electrochemical sensors is to employ microelectrode arrays (MEAs) and nanoelectrode arrays (NEAs).[138−140] MEA and NEA refer to a collection of microelectrodes and nanoelectrodes. They have advantages over conventional macroelectrodes,[138,141,142] including (i) a high signal-to-noise ratio because the noise level depends on the active area of the electrode, while the signal depends on the total geometric area of the diffusion field, (ii) no need for convection due to the enhanced mass transport, (iii) a small current that enables stripping analysis to be performed in a high resistive media, which makes the supporting electrolyte unnecessary and hence reduces the interference effects, and (iv) small overpotential required for analyte deposition and stripping.

An array of 256 gold microelectrodes in a diameter of 5 $\mu$m with an interelectrode distance of 100 $\mu$m was used for detection of $Hg^{2+}$.[143] This microelectrode array exhibited excellent anti-interference capability due to the short deposition time and low overpotential required for ASV analysis. ASV measurement of $Hg^{2+}$ ions usually encounters the problem of interference from coexisting chemical species such as chloride due to the formation of insoluble calomel ($Hg_2Cl_2$). It was interesting that this microelectrode array was able to measure $Hg^{2+}$ in 0.1 and 1 M chloride media in the range from $5 \times 10^{-8}$ to $1 \times 10^{-6}$ M, avoiding the interference of the calomel formation on the voltammetric peak of interest. In addition, Feeney et al. have developed an Ir-based microelectrode array consisting of 25 microdiscs in a diameter of 10 $\mu$m and an interelectrode distance of 100 $\mu$m, which was used for the determination of 20−100 $\mu$g/L Cd in the aqueous solution. Furthermore, the vertically aligned CNT array was also used for

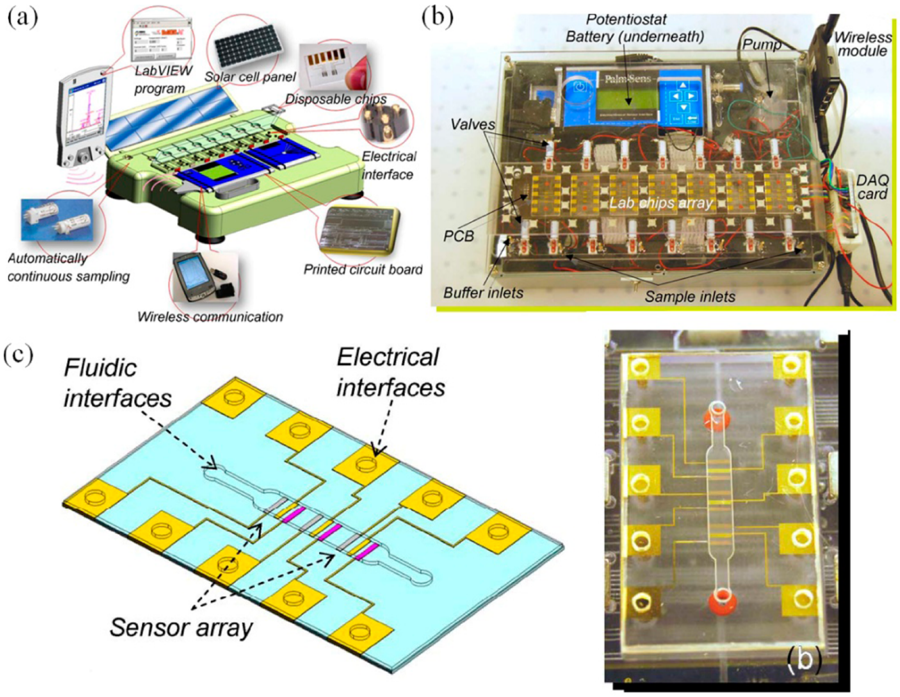

**Figure 6.** (a) Sketch showing a polymer lab-chip based system for automatic, continuous, on-site sampling, and sensing of heavy metals. (b) Photograph of the analyzer. (c) Polymer lab-chip with array of three sensors: (left) schematic view and (right) the photograph.[147]

detection of heavy metals because the CNTs feature unidirectional electron transport, high electrical conductivity, and excellent strength.[127,131] However, The CNT array suffers from the high background current and absence of functional groups for heavy metal chelation. To overcome the drawbacks, Lin et al. have sealed the side-walls of CNTs in epoxy to mitigate the current leakage and to reduce the electrode capacitance, leading to a low background current from aligned CNT forest.[144] This voltammetric sensor with the CNT-NEA coated with a bismuth film showed a high signal-to-noise ratio and achieved a LOD of 0.04 $\mu$g/L for $Cd^{2+}$.

**Microfluidic Electrochemical Devices.** Electrochemical sensors can be incorporated into the microfluidic channel to form a LOC device,[145,146] in which three electrodes (reference, counter, and working electrodes) can be integrated into a single microfluidic chamber so that the total assay process is performed by electrochemical techniques. Microfluidic electrochemical devices offer significant advantages, including (i) miniaturization of devices, (ii) enabling detection with a small sample/reagent volume, (iii) on-chip preparing sample and preconcentrating the analytes, (iv) allowing multiplexed detection in a single chip, and (v) shortening the assay time. The microfluidic electrochemical devices can be used as portable devices for field-deployable applications,[147−152] Zou et al. have developed a portable analytical system using an array of disposable polymer LOCs and a continuous flow sensing method (Figure 6).[147] This analytical system enabled the long-term, automatic, continuous water sampling, and on-site measurement of heavy metals. More importantly, this system has a capability of monitoring multiple heavy metals using the ASV method. This system is capable of on-site measuring the Cd concentration change in the soil pore and groundwater samples.

## FET SENSORS

FET sensors have been used for detection of heavy metals by utilizing the interaction between the analyte and semiconductor resistor.[153] FET sensors are capable of real-time label-free detection of heavy metals. One-dimensional and two-dimensional semiconducting nanomaterials are especially attractive to the FET sensors because they have very high surface-to-volume ratio, leading to high sensitivity.[154,155] A Si nanowire was used to construct a FET sensor for detection of heavy metals.[154] This FET sensor exhibited high sensitivity with a LOD of $10^{-7}$ M for $Hg^{2+}$ and $10^{-4}$ M for $Cd^{2+}$. Another example was a selective and sensitive FET sensor constructed with single-walled carbon nanotubes (SWCNTs), which was based on the conductance change due to the selective redox reaction between SWCNTs and $Hg^{2+}$.[156] Reduction from $Hg^{2+}$ to $Hg^0$ by the SWCNTs is thermodynamically favorable, while reduction of other metal ions with SWCNTs is unfavorable due to their negative potentials. This sensor exhibited a LOD of 10 nM, with a wide detection range from 10 nM to 1 mM, and excellent selectivity toward $Hg^{2+}$ over other metal ions in both aqueous solution and drinking water.

Nanomaterials usually require the surface functionalization for specific detection of metal ions.[153] Specific detection of mercury with a FET sensor has been demonstrated by self-assembly of a 1-octadecanethiol monolayer onto graphene due to high affinity of thiol to mercury.[157] However, the LOD was high (~0.5 $\mu$M). In order to improve the sensitivity, Chen et al. have used the thioglycolic acid (TGA)-functionalized reduced graphene oxide (rGO) to construct a FET sensor.[158] This FET sensor achieved a LOD of $2.5 \times 10^{-8}$ M and responded to the analyte within a few seconds with excellent selectivity over other metal ions. The improved sensing performance was attributed to the chelation interaction of heavy metal ions with the carboxyl group of TGA, rather than the selectively binding of thiol group to mercury. In addition, the protein-functionalized rGO was employed in a FET sensor to detect various metal ions with high sensitivity.[159]

## CONCLUSIONS AND PERSPECTIVES

Enormous efforts have been made to develop heavy metal sensors in several aspects: (i) explore different signal transduction mechanisms, resulting in various optical, electrochemical, and FET sensors; (ii) utilize or develop different molecular recognition probes, including small organic molecules, DNA, aptamer, proteins, bacteria, and metals; and (iii) incorporate nanomaterials and nanostructures into sensors, leading to significant improvement in the sensing performance, especially LOD and sensitivity. However, most of the work has only demonstrated a proof-of-concept for sensors that could detect heavy metals in buffer solutions or artificial matrices. It remains a significant challenge to apply the sensors to complex real-world samples for real-time on-site detection of heavy metals. These challenges include but are not limited to the following. (i) The chemical and biological species in the real-world samples (such as river water, blood, and urine) have a severe interference on the sensing signals. Under some circumstances, the transducer of sensors suffer from fouling of organics or inorganics, leading to malfunction of the sensor. (ii) Most of the sensors reported previously were only able to detect free ions of heavy metals. Unfortunately, many heavy metals in the real-world samples are present in the form of metal−organic complexes instead of free ions. Consequently, the samples must be pretreated in a central laboratory prior to sensing. The advantages of sensors over conventional analytical techniques are their portability and on-site detection ability. If sample pretreatment has to be performed in a central laboratory prior to sensing, the enthusiasm with sensors will be severely diminished. An effective solution to these problems is to develop LOC devices that integrate sensors with microfluidics on a single chip. The microfluidic module in the LOC chip can be used to digest metal−organic complexes to release free metallic ions and separate the metallic ions from the sample matrix prior to sensing.

## AUTHOR INFORMATION

### Corresponding Author

*Fax: +1-304-293-6689. E-mail: nick.wu@mail.wvu.edu.

### Notes

The authors declare no competing financial interest.

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