Recent Advances in Mercury Detection: Towards Enabling a Sensitive and Rapid Point-of-Check Measurement

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Abstract

Heavy metals emission, in particular, mercury is ever increasing due to global urbanization and industrialization. Due to increasing number of health problems related to heavy metals contamination, monitoring it becomes a crucial task for authorities and environmentalists. Therefore, the development of a nanobiosensing technique that can detect mercury ions as low as 1 ppb for its limit of quantification is necessary to provide sufficient information to ensure a sustainable and healthy environment for the community around the world. Herein we reviewed reported studies on the mercury ions bioavailability and recent progress of its detection methods. Comparison of detection methods based on electrochemical and optical technique has been made. There are two main technological gaps that need to be filled, which are sensitivity and practicality of measurement at point-of-check for in-situ analysis.

Introduction

Heavy metals are non-biodegradable elements that fall in a group of natural constituents of the earth. Their specific gravity is greater than 5.0, and it has relatively high atomic weight [1,2]. Many industrial applications nowadays involve the use of heavy metals for their production which includes mining activities (Cu, Cd, Co, Cr, Ni, Pb, Zn, Hg and As) [3-6], power generation industries (As, Cd, Pb and Hg) [7], smelting industries (Cd, Pb, Cu and Zn) [8,9] battery production (Pb, As, Cd, Ni, Hg) [10], dyes and textile manufacturing industries (Al, Co, Ni, Ti, Pb, Cd, Zn, Cu, Cr, Fe and Mn) [11,12], biosolids and manure (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Ti, Sb) [13], as well as fertilizer production (As, Cd, Pb, Cu, Cr and Zn) [14-16], and other applications. Artisanal and small-scale gold mining that use amalgamation as the gold extraction technique, for example, is rampant across Africa and South America. This sector alone is estimated to produce 1000 tonnes of Mercury per annum [17]. If these industries are not tightly regulated, the level of heavy metals will be elevated tremendously in the environment and can cause severe problems to the biosphere [17,18]. Accumulation of heavy metal contaminants can be found in water, sludge, air, and soil. Indirect ingesting of these metals will cause bioaccumulation in living organisms and eventually will lead to biomagnification, a phenomenon of metal ions intensification in higher trophic levels [19,20].

A small amount of heavy metals including Arsenic, Chromium, and Cadmium have preferential metabolic functions. Obviously, however, it can be detrimental if it exceeds the maximum permissible limit (MPL) that is also known as the toxic dosage [21-25]. Lead, for example, is often associated with damage to the human kidney [26,27] and central nervous system [28,29]. It can also cause anemia [30], hypertension [31,32], immunotoxicity, and oxidative stress [33]. Very recently, it is suggested that the presence of lead causes a sympathovagal imbalance that leads to a high blood pressure [34]. Copper is another well-known heavy metal that is essential for human metabolism activities. Its hemeostasis is maintained by CsoR protein [35], and in the form of ceruloplasmin it aids the transport of iron to the cells [36,37]. Overexposure to this element, however, will lead to dermal toxicity [38], respiratory problem [39] and can lead to kidney damage through the up-regulation of tumor gene suppressor p53 and apoptotic gene caspase-3 [40]. Mercury, one of the top ten elements or chemicals that pose concerns in public...
health, is known as neurotoxicant. Exposure to a low level of Mercury can cause neurological and behavioral disorder [41], heart disease [42], and permanent damage to vital organs. Exposure to > 1.6 ppb (or μg/kg body weight) and > 4.0 ppb of mercury can cause neurocognitive effects and kidney damages, respectively [43,44]. This is even lower toxic dosage compared to As, Cd, and Pb for similar adverse effects.

Heavy metal exposure not only affects human health but it also can disrupt an ecosystem. In the open ocean, 99% of plastic debris that pollutants like mercury are clinging onto is untraceable [45]. The estuarine biota is often in danger as industrial discharges from factories is usually released into the nearby river. In a recent study, neurotoxic MeHg was found to be bioaccumulated in the pelagic food web, in which will become human food sources later on [46]. When this contaminated food is consumed, it will result in food poisoning among the walks of life on earth. The consumption of contaminated food is a major pathway of human exposure to heavy metals as compared to inhalation and dermal contact.

Among all heavy metals, mercury is the most detrimental due to its neurotoxic and nephrotoxic properties. Mercury was started to be used as separating agent to separate fibers of fur from the pelt in a felting industry, and as a preservative for seeds and vaccine. Recent data shows that inorganic mercury was excreted as waste from coal-burning power plant, waste incinerators, alkali-chlor factory and gold mining industry. Human exposure to mercury can occur via eating MeHg-contaminated food (fish, shellfish, and other aquatic life), dental amalgam procedure, usage of inorganic mercury products (medication, germicidal soap and skin cream), occupational exposure (mining or mercury-related industry) and usage of any other mercury-based products (thermometer, sphygmomanometer, fluorescent light bulbs and batteries).

Mercury is commonly bioavailable (Figure 1) and enters the food web as methylmercury (MeHg) which can be magnified as it passes up the food chain. However, the natural form of mercury that often excreted out from the industrial area is inorganic mercury, mercury (II) ions, and elemental mercury. Elemental mercury, Hg⁰ exist in the form of volatile liquid from soil-air flux and other anthropogenic releases that can remain suspended in the atmosphere for one to two years long [47,48]. It can be converted to Hg²⁺ via oxidation and eventually will reside in the water sources or soil through the rain. This Hg²⁺ will then be converted to methylmercury through methylation process by anaerobic sulfur reduction bacteria which includes Desulfovibrio desulfuricans, Desulfobulbus propionicus, Desulfococcus multivorans, Desulfobacter sp., and Desulfo bacterium sp. [49].

The abundance of mercury in the environment is inevitable since the natural ecosystem produces it as well [50]. The concentration of Mercury in the ocean, precisely at the level shallower than 100 m was tripled from its pre-anthropogenic condition to 0.6 pM [51]. The increment of their concentration especially when localized raised concern among environmentalists and population as a whole. According to the World Health Organization (WHO), the MPL for almost all heavy metals in the environment must be below 0.2 ppm. Further, the MPL of mercury ions, have to be below 0.002 ppm.

Due to increasing industrial and mining activity globally, we are now facing a silent threat from mercury contamina-
tion. The anthropogenic release of mercury was estimated at 2320 tonnes/year in 2010 [52]. Meanwhile the United Nations Environmental Programme (UNEP) and the Arctic Monitoring and Assessment Programme (AMAP) approximated the anthropogenic mercury emissions globally has an uncertainty range of 1010-4070 tonnes/year. Artisanal and small-scale gold mining (ASGM) were identified as the major anthropogenic sources (37%) and the dental amalgam emitted through cremation were identified as the minor anthropogenic sources (0.2%) [53]. Figure 2 below shows a mercury emission percentage based on different sources of emission.

There are several cases worth mentioning to show that contaminated water is one of the main sources where mercury contamination could affect most lives. Water sources are the reservoir where all the inorganic mercury would reside before it will be methylated and would be magnified in the higher organism hierarchy as it passes up a food chain. In general population, dietary intake is the most common pathway for mercury contamination. The classic case of Minamata Bay Japan, in 1953 witnessed 2252 victims poisoned with MeHg (5.61 ppm to 35.7 ppm) in their marine product [54]. In 1964, the similar case happened in Niigata with approximately 700 victims through the same exposure of the contaminated marine products [55]. In 2006, China faced a threat from mercury pollution due to their mining and coal-burning industry. National Bureau of Oceanography (NBO) of China stated that there is approximately 77 ton of Hg were released into the coastal area through river annually [56,57].

Development of Nanosensors to Detect Heavy Metals

Conventional methods

The determination of heavy metals in the environment is essential in order to find a targeted area for remediation process [58-60]. Several detection techniques have been developed and commercially available, including atomic absorption spectrometry (AAS) [61-63], Synchrotron X-ray fluorescence (SXRF) spectrometry [64], microwave plasma atomic emission spectroscoPY (MP-AES) [65,66], inductively coupled plasma mass spectrometry (ICP-MS) [67,68], and cold vapour atomic fluorescence spectrometry (CVASF) [69] and thermal decomposition mercury analyser (TDMA) [70]. These techniques provide sensitive and accurate result in determining the level of heavy metal in the environment. Most of these techniques, however, require a sophisticated machine with the time-consuming procedure, and generally, need expensive consumables which make it non-practical to be used for in-situ analysis at a mass and constant screening level. It is also worthy to note that digestion step under an extreme condition is usually required for most of these techniques to free the metal ions prior to the measurement. This makes the results to be questionable because the original environmental condition of those metals is disregarded [71]. These limitations lead to the needs of developing simple yet effective techniques to detect heavy metal contamination at the point-of-check of an environment. This is especially important and useful to be used as a front-line screening procedure.

Biosensing methods

In recent years, researchers have turned their attention towards biosensor techniques to be used to detect heavy metal contamination. Biosensors are a device that uses any biological based elements including protein, enzymes, cells and nucleic acids to detect the presence of molecules; in this case, metal ions. It has two main components; biological element as a receptor...
or capturing agent, and physicochemical element as a transducer [72]. In the middle between these two components lay a surface chemistry that plays an important role at the interfacial. The transduced signal of a biosensing method can be amplified, enhanced and more effective by manipulating the surface chemical properties of a particular sensing platform. The biorecognition elements typically are whole cells, enzymes, antibodies, peptides, small molecules, or nucleic acids that could react selectively and sensitively with the target analytes. Meanwhile, signal transduction converts the biological response resulting from the interaction with the target analytes into a distinct and quantifiable signal [73]. Ionic elements like Hg$^{2+}$ has at least two characteristics that can be leveraged as a sensing counterpart element; i) As a charge-bearing atom, electrochemical sensing principle can be used because its presence will alter current or impedance. ii) Affinity towards capturing agents can be used as a chemical switch to generate molecular structural transfiguration that subsequently produces an optical sensing signal. We tabulated some of the newly developed biosensing methods that utilized these two concepts (Table 1). The electrochemical-based biosensor has the advantage for the sensitivity, whereas the optical-based biosensor can easily be miniaturized, primarily through the usage of color recognition apps on the smartphone.

### Capturing agents

In environmental monitoring, most biosensors have been developed for detection of pesticides and heavy metals due to their occurrence in the environment [74]. Some groups have reported using whole cell [75], microorganisms such as bacteria, and algae to detect heavy metal ions [76]. Alternatively, biomolecules can be utilized as effective capturing agents. Tanaka, et al. utilized thymine-thymine (T-T) mismatches to capture mercury ions effectively at [77]. They showed that mercury ion out of all ions has a greater binding affinity with thymine mismatches and suggesting that Hg$^{2+}$ is a substitute for imino group and bind with the nitrogen of the thymine residue. Soft acidic metals like mercury have a strong affinity with sulfur, nitrogen and oxygen elements. Hence protein antibody has become a good capturing agent to establish a new biosensing platform [78]. In addition to that, monoclonal antibodies have been generated to specifically recognize heavy metals ions such as Hg$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Co$^{2+}$, and Ag$^+$ [79-82]. Chelating agents like Rhodamine- [83-87], calix[4]arene [87], thioether- [88], and aniline-derivative can be used to specifically capture mercury at relatively

<p>| Table 1: Detection of mercury ions, Hg$^{2+}$ using optical-based or electrochemical-based biosensor. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Sensor type                                     | Capturing agents                                                                 | LOD (ppb) | Detection time | Practical for in-situ analysis | References     |
| Optical-based biosensor                         | AunPs with 3-mercaptopropionic acid and adenosine monophosphate                  | 100       | Several minutes| Yes                          | Yu and Tseng [93] |
|                                                | Papain-functionalized gold-nanoparticles (P-AuNPs)                               | 40        | Several minutes| Yes                          | Guo, et al. [95] |
|                                                | Cysteine functionalized gold-nanoparticles (C-AunPs)                             | 20        | &gt; 10 minutes   | Yes                          | Chai, et al. [102] |
|                                                | AuNPs modified with quaternary ammonium group-terminated thiols                  | 6         | Several minutes| No                           | Liu, et al. [99] |
|                                                | Mercaptopropionic acid-homocysteine-PDCA-modified gold nanoparticles              | 5         | 15 minutes     | Yes                          | Darbha, et al. [103] |
|                                                | Aggregation-induced quenching of the fluorescence of 11-mercaptopoundecanoic acid (11-MUA) protected 20 nm AuNPs | 1         | 10 minutes     | No                           | Huang, et al. [86] |
| Electrochemical-based sensor                   | Heated carbon nanoparticles                                                     | 1         | 2 minutes      | No                           | Aragay, et al. [78] |
|                                                | Protein-functionalized reduced graphene oxide (rGO)                              | 0.2       | Few seconds    | No                           | Sudibya, et al. [104] |
|                                                | Bimetallic Au-Pt nanoparticles/organic nanofibers with anodic stripping voltammetry | 0.08     | 100 seconds    | No                           | Gong, et al. [105] |
|                                                | AuNPs-graphene hybrid nanocomposite                                             | 0.06      | 110 seconds    | No                           | Gong, et al. [106] |
|                                                | Gold nanoparticles/carbon nanotubes (Au-NPs/CNTs) composites with anodic stripping voltammetry | 0.06      | 2 minutes      | No                           | Xu, et al. [107] |
|                                                | Nanostructured magnesium silicate hollow spheres                                 | 0.07      | Several minutes| No                           | Xu, et al. [108] |
|                                                | Electrochemically reduced GO (ERGO)-based diode with N-[1-pyrenyl-sulfonamido]-heptyl]-gluconamide (PG) as the modifier | 0.02      | Several minutes| No                           | Fang &amp; Liu [109] |
|                                                | Layered titanate nanosheets                                                      | 0.005     | 80 seconds     | No                           | Yuan, et al. [110] |</p>
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<th>Name</th>
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Gold-nanoparticles (AuNPs)-based biosensor is another type of versatile colourimetric assay methods. It has high extinction coefficient which makes it suitable to act as a sensing platform [78, 94, 95]. Gold-nanoparticles possess an exploitable property due to its intrinsic surface plasmon resonance that strongly depends on the sizes of the particles. It is also possible to modify the AuNPs with various kinds surface functionalization to specifically serve as mercury ions capturing agent in sub-ppm concentration [96]. Moreover, the color produced by the AuNPs is various, depending on the distance between the particles. As the distance between particles decreases, it will promote plasmon coupling which will shift the plasmon-band to a lower energy level. This will result in a change of color of the AuNPs solution from red to blue (in the case of 20 nm and 40 nm); namely a redshift phenomenon [97]. AuNPs can aggregate and disperse, which can be regulated by the ligand that is functionalized onto its surfaces. For example, the analyte that can bind with the ligand on top of the AuNPs will make two or more particles to attract each other which result in a subsequent change of color. The concept is also rightly applicable vice versa. The capability of gold nanoparticles to be conjugated with different types of ligands through surface chemistry, open a room of exploitation for this biosensing platform.

**Bio-Conjugated Gold-Nanoparticles**

Bio-conjugated gold nanoparticle is a modification onto gold nanoparticles surface in order to make it selective and sensitive towards desired interactions. Due to the capability of gold-nanoparticles to be conjugat-

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**Mercuryfluor-1 (flourescent probe)**

![Mercuryfluor-1 (flourescent probe)](image1)

**N,N’-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane**

![N,N’-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclo-octadecane](image2)

**Terphenyl-based reversible receptor with pyrene and quinoline as the fluorophores**

![Terphenyl-based reversible receptor with pyrene and quinoline as the fluorophores](image3)
ed with many biomolecules such as DNA, protein, and enzyme, there were many studies carried out to improve utilization of AuNPs [78]. There are three ways to conjugate and functionalize gold nanoparticles. The most common approach is via covalent coupling between gold and thiol group to ligate molecules onto the gold-nanoparticles surface [98]. There is also physical absorption where electrostatic or hydrophobic interactions are used. However, since this is a weak interaction, any physiological change to the AuNPs solution including pH and temperature can lead to detachment of ligand molecules [99]. The third approach is using an explicit specificity of ligand molecules by tagging gold-nanoparticles with antigen and uses it for detection of antibody. This approach is a fundamental idea to build a platform to detect any antibody and widely used in medical field. Hung, et al. develop a biosensor using series of alkanethiols which capable of detecting mercuric (Hg²⁺), silver (Ag⁺) and lead (Pb²⁺) ions [100]. They used sulfur group in alkanethiols to bind strongly with gold-nanoparticles and let the -OH functional group attract mercury ions.

**Conclusion**

The optical-based sensor is a promising sensor to detect mercuric pollution due to its vivid signal that is directly observable through the naked eye. The qualitative signal can be analyzed quantitatively using colour recognition and processing apps that are commercially available, and in-situ detection can be done through a smartphone. Furthermore, with the availability of the internet-of-things and cloud server, polluted areas and regions can be mapped in a real-time manner. Hence an automated early warning system can be put in operation [101]. Nonetheless, the reported studies shown here suggest that the sensitivity of optical-based detection methods remains a challenge. Electronic and electrochemical sensors, on the other hand, are very sensitive methods. However, to make a practical POC device, the electrochemical devices that are currently used need to be miniaturized, and enabled the operation without a plug-in electrical current source. The combination of the ability to detect mercury in a dynamic range of a very low concentration (ppt-pb or pM-nM), and the POC compatibilities that allow in-situ analysis for mass screening, can open a new avenue of applications.

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**References**


