

**Project:** R21OH008900-Portable Analyzer for On-site Monitoring of Worker Exposure to Toxic Metals

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## Table of Content

1.0 Abstract.....	3
2.0 Highlights/Outcomes/Relevance/Impact .....	4
3.0 Scientific Report.....	5
3.1 Background.....	5
3.2 Accomplishments.....	5
3.2.1 Specific Aim 1.....	5
3.2.2 Specific Aim 2.....	8
3.2.3 Specific Aim 3.....	11
3.2.4 Additional results.....	15
4.0 Research Translation.....	16
5.0 Peer Reviewed Publications/Presentations/Patents.....	17

## 1.0 Abstract

Large numbers of industrial workers are regularly exposed to toxic heavy metals like cadmium (Cd), lead (Pb), and mercury (Hg), which are known to induce various diseases that are detrimental to human health. In order to monitor workers for multiple toxic metal exposures and to ensure that these exposures are below a threshold for inducing permanent damage to various organ systems, a portable analyzer for real-time, onsite, non-invasive monitoring of workers for toxic metal exposure will be developed using a novel approach developed by our research group at the Pacific Northwest National Laboratory (PNNL). The analyzer will be based on the built-in preconcentration of cadmium, lead, and mercury present in urine by the highly efficient sorbent materials, created at PNNL, prior to their voltammetric quantitation. Urine is universally recognized as one of the best non-invasive matrix to assess both acute and ongoing exposures to a broad range of many toxic metals. The sorbent materials are functionalized mesoporous silica (SAMMS) and functionalized magnetic iron oxide nanoparticles (FNP), both have extremely high surface area and organic functional groups that are selective and effective for direct preconcentration of the target metals. They are designed to be highly specific for the metal ions even in complex urine matrix without sample dilution or sample pretreatment. Thus our sensors do not require toxic mercury for metal ion preconcentration unlike conventional electrochemical sensors. Delivery of samples and reagents is based on programmable sequential injection with turbulent flow-onto a design that minimizes the sensor fouling caused by proteins. The analyzer will reduce the time and costs of monitoring worker exposure and clinical diagnosis. It represents the next-generation of metal ion analyzer because of its affordability, low power consumption, portability, full automation, and easy operation, which are better than the traditional analytical methods like AAS and ICP-MS. The portable analyzer will eventually enable (1) better identification of at-risk workers and development of appropriate control and intervention strategies, (2) understanding of exposure-response relationships, and (3) improvement of baseline data for a standard setting and risk assessment, all of which have been identified as important by the Exposure Assessment Methods (EAM) team commissioned by NIOSH's National Occupational Research Agenda (NORA).

## 2.0 Highlights/Outcomes/Relevance/Impact

The overall goal of this project is to develop portable and field-deployable metal analyzers capable of detecting multiple metal analytes in urine with a high degree of selectivity and sensitivity similar to the in-lab state-of-the-art ICP-MS. In the past three years (FY06-08, with a no-cost extension in FY09), funded by an extramural R21 grant from the CDC/NIOSH, we have developed electrochemical sensors that are applicable in “real world” samples, specifically human urine, without sample pretreatment and sample dilution.

In order to overcome the binding competition for target metal ions and the electrode fouling by proteins and metabolic wastes in urine, we have investigated two classes of nanomaterials: functional mesoporous silica (SAMMS) and functional superparamagnetic nanoparticles (NPs) for metal preconcentration. We have successfully optimized sensor preparation process and measurement conditions to achieve most sensitive, reliable, and robust measurements. We have also built two programmable portable metal analyzers, one for quantitative metal analysis and the other for low cost, rapid screening test of the urinary metals.

In terms of impact, our work has shown for the very first time that mercury-free electrochemical sensors can be used successfully in protein-rich biological samples like urine without sample pretreatment (e.g., acid digestion, large dilution) or the use of internal standards. Our electrochemical sensors based on metal preconcentration at functional nanomaterials has been very well accepted by other leading scientists: *“This is an extremely elegant piece of analytical work, neatly combining surface modification chemistry with magnetic particle separation and sensitive electroanalytical detection,”* Prof. Richard Compton, a leading Electroanalytical Researcher at Oxford University, told Chemistry World about our work. *“The detection limits are impressive and, in particular, the challenge of working quantitatively on the substrates studied cannot be over-emphasized.”* Our work involving magnetic nanoparticles has attracted a lot of interest (e.g., one article has been one of the most accessed of Q3 of 2007 at Environmental Science and Technology and another has been one of the most accessed in both Feb and March of 2008 at Analyst). The magnetic nanoparticles have great potential for metal preconcentration at analytical instruments; hence PNNL has filed a patent on the technology (Application# 11936405: Functionalized magnetic nanoparticle analyte sensor). Another class of sensor material, SAMMS-Nafion composite is a technological breakthrough in sensor materials for complex “real world” samples, thus PNNL has recently filed the U.S. patent on the technology (Application No. E-15953: Electrochemical sensor and methods for making and using same). A private company is investigating this technology for detecting metals as biomarkers of specific diseases (proprietary information).

Major accomplishments from this grant also include 7 peer-reviewed publications, two book chapters, 3 Invention Disclosure Reports, and two U.S. Patent applications (see **Section 5.0**). In addition, an R01 proposal entitled “Next-Generation Cadmium and Lead Analyzer: Workplace Exposure and Biomonitoring (PI: W. Yantasee)” has been submitted to CDC/NIOSH in June of 2008, which proposes to extend the sensors developed in this R21 project for monitoring of the metals in workplace (in air and on surface) in addition to in workers as well as to perform the field-testing of the devices under the Health Hazard Evaluation (HHE) program of NIOSH. The portable metal analyzer was nominated for 2008 NIOSH’s Director Award. The PNNL press release on the portable sensor technology based on functional nanomaterials have been posted on over 30 of science and technology websites including *Chemistry World*, *ChemEurope.com*, and *eponline.com*. The PI (Yantasee) received the 2007 Ronald L. Brodzinski Early Career Exceptional Scientific Achievement Award, which recognized her achievement in the development of the next-generation metal analyzer (with \$62,500 grant award).

### 3.0 Scientific Report

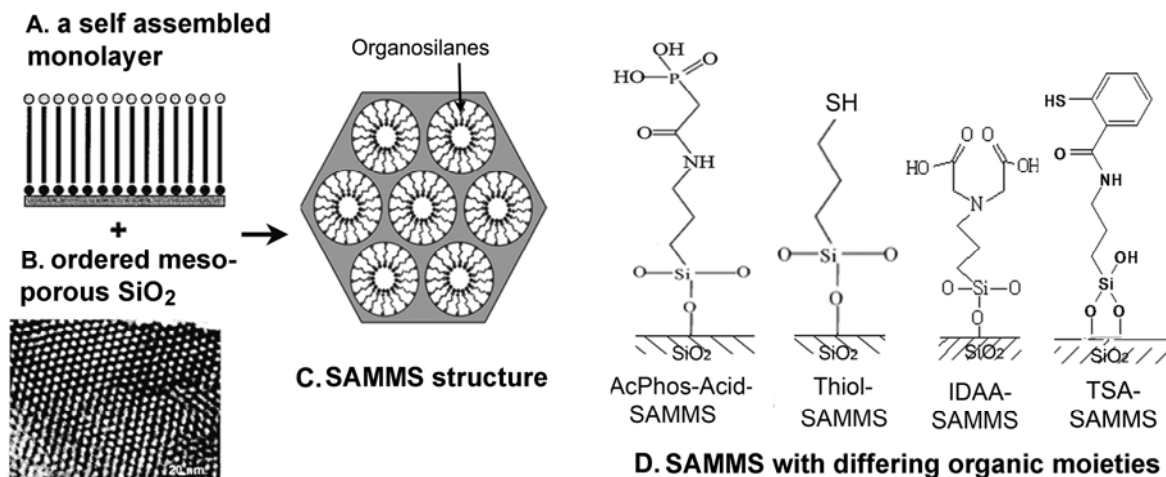
**3.1 Background.** This is the final report for a project that was initiated in September 2006 and completed in September 2009. In June of 2009, Dr. Yantasee, the original PI, has left PNNL to OHSU, and the project was transferred to Dr. Timchalk of PNNL as the new PI. The overall goal of this project is to develop metal analyzers capable of detecting multiple metal analytes with a high degree of selectivity and sensitivity similar to ICP-MS. The sensor is based upon the voltammetric analysis of metal ions with a built-in preconcentration of the target metal ions at the functionalized nanomaterials, specifically the self-assembled monolayers on mesoporous silica (SAMMS) or functionalized magnetic nanoparticles, making it sensitive and selective for the real-time and non-invasive analysis of toxic metal ions excreted into urine. The analyzer represents the next-generation of metal ion analyzer because it is more affordable, requires little power to operate, is highly portable, and is more easily operated than the more traditional analytical methods like AAS or ICP-MS.

To achieve the overall goal, the following specific aims were proposed:

1. To optimize adsorptive stripping voltammetric sensors consisting of a mixture of nanostructured functionalized silica materials (SAMMS) for the simultaneous analysis of Pb, Cd, and Hg in pure water.
2. To evaluate (1) the best sensors obtained in aim 1 for simultaneous analysis of Pb, Cd, and Hg contained in complex urine matrix and (2) the effects of proteins and chelating agents for the preconcentration of the metal ions by the selected SAMMS materials.
3. To build a fully-automated, portable metal analyzer prototype, optimize, evaluate, and validate it with urine samples containing metal ions at the occupational exposure levels.

**3.2. Accomplishments.** The accomplishments are summarized based on each specific aim as follows.

**3.2.1. Specific Aim 1: To optimize adsorptive stripping voltammetric sensors consisting of a mixture of nanostructured functionalized silica materials (SAMMS) for the simultaneous analysis of Pb, Cd, and Hg in pure water.**

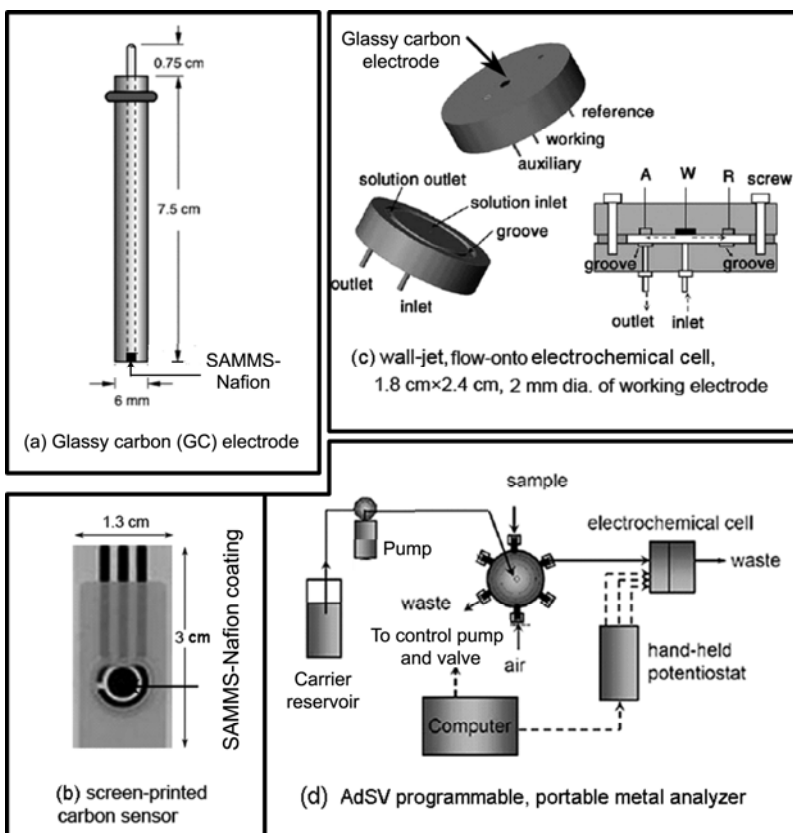


**Figure 1.** Schematic of self-assembled monolayers on mesoporous supports (SAMMS) and chemical structures of differing SAMMS.

**Sorbent material selection.** During the course of the R21 research grant (from FY2006-2008, with a no-cost extension to FY2009), we have developed and tested four SAMMS materials that are functionalized with acetamide phosphonic acid (AcPhos), thiol (SH), iminodiacetic acid (IDAA) and thioisalicylamide (TSA) as metal preconcentrators at electrochemical sensors. Their chemical structures are shown in **Figure 1(D)**. For Cd and Pb detection, we have found that SH-SAMMS and IDAA-SAMMS are best. For Hg detection, although SH-SAMMS may be best at preconcentrate Hg, the binding affinity is so great across the pH range including in

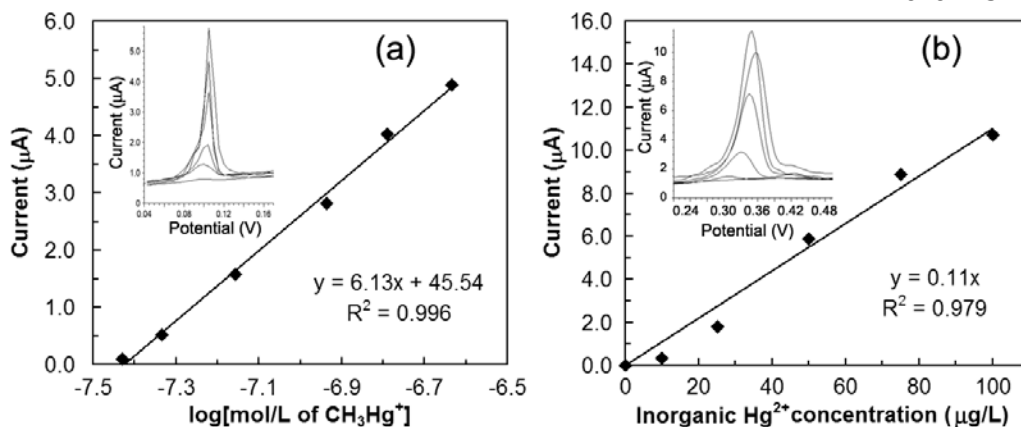
acid solutions. Thus once captured on SH-SAMMS, Hg cannot be released from the SAMMS (which is not conductive) to the conductive component of the electrode, hence cannot be detected sensitively. Thus TSA-SAMMS was chosen for the Hg sensors.

**Sensor platforms.** Different sensor platforms have been investigated in our lab for various applications (as shown in **Figure 2**), including (a) SAMMS-Nafion coated on glassy carbon rod electrode for batch evaluations, (b) SAMMS-Nafion coated on screen-printed carbon sensors (low-cost disposable sensors), and (c) electrochemical cell which is a major component of (d) a programmable, portable metal analyzer.



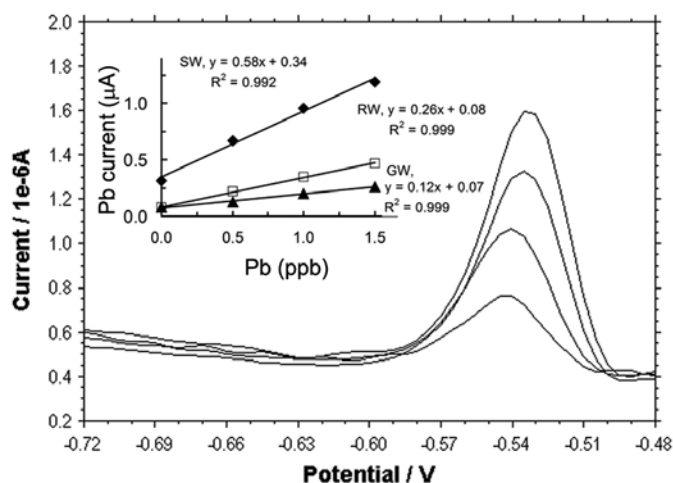
**Figure 2.** Different SAMMS-sensor platforms for metal analysis.

**Hg analysis in water.** **Figure 3** shows responses of (a) methyl mercury ( $\text{MeHg}^+$ ) and (b) inorganic  $\text{Hg}^{2+}$  measured in water using TSA-SAMMS-Nafion sensors as a function of the metal concentrations. The  $\text{MeHg}^+$  response is linear in a concentration range from  $3.7 \times 10^{-8}$  to  $2.3 \times 10^{-7}$  mol/L (or from 8 to 50  $\mu\text{g/L}$ ), and inorganic  $\text{Hg}^{2+}$  response is linear in a concentration range of 0 to 100  $\mu\text{g/L}$ . Lower concentration of  $\text{MeHg}^+$  (e.g., 1  $\mu\text{g/L}$ ) was detected by increasing the preconcentration time to 10 min. Hence, the current sensor platform is within the detection limit range of interest (e.g., EPA recommended benchmark dose for Hg is 5.8  $\mu\text{g/L}$  of blood). The lesson learned here is that SAMMS sensors can detect not only inorganic metal ions, but also organic metal ions (e.g., methyl mercury) because TSA-SAMMS can preconcentrate both ionic and organic Hg (but to a varied extent). Thus, SAMMS sensors will yield signal that is proportional to the total concentration of all metal species rather than inorganic species alone. Although this is not the focus of this R21 project, SAMMS sensors can be made to differentiate inorganic metal ions apart from organic metal ions by manipulating the SAMMS materials. For example, the pore size of SAMMS can be controlled to allow only free inorganic metal ions to reach the binding sites, while excluding the larger organic metal molecules, or hydrophobic alkyl groups can be added to the organosilanes of SAMMS to make it preferable to binding with hydrophobic organic metal ions rather than hydrophilic inorganic metal ions.



**Figure 3.** Responses of (a)  $\text{MeHg}^+$  measured after 5 min of preconcentration and (b) inorganic  $\text{Hg}^{2+}$  measured after 3 min of preconcentration, both at TSA-SAMMS-Nafion electrodes; insets show the corresponding voltammograms.

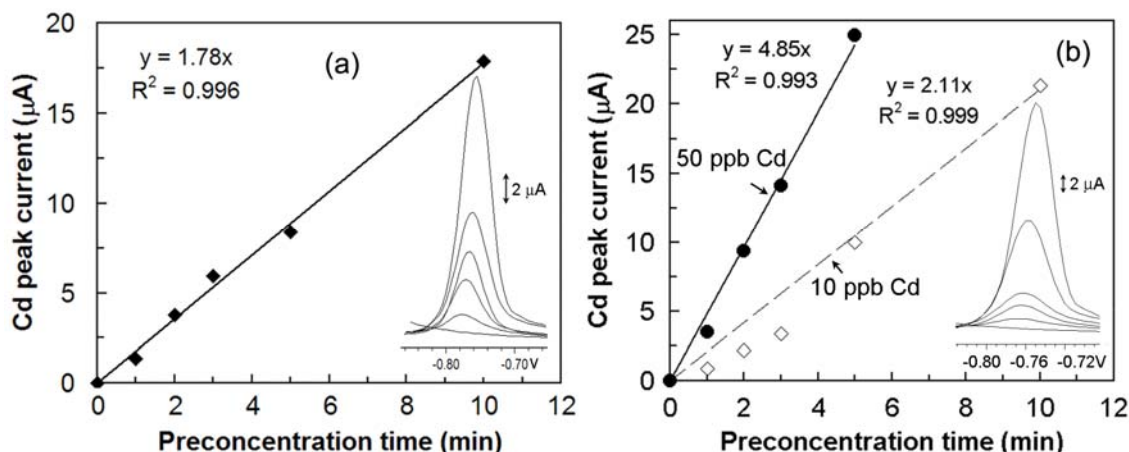
**Pb and Cd analysis in natural waters.** We have analyzed Pb in natural waters (for optimization studies) using two types of SAMMS (SH-SAMMS and IDAA-SAMMS) in two classes of electrodes (rod electrodes as in **Figure 2a** and disposable screen-printed electrodes as in **Figure 2b**). At SH-SAMMS-Nafion rod electrode, 0.5 ppb of Pb can be detected after 6 minutes in unfiltered river water, ground water and seawater and the linear calibration curves were obtained at as low as 0 to 1.5 ppb as shown in **Figure 4**. After only 3 minutes, 2.5 to 100 ppb of Cd could be detected in Cd spiked acetate buffer, and in river, ground, and sea waters. Even without sample pretreatment, the SH-SAMMS-Nafion sensors were exceptionally sensitive for Pb and Cd detection (with lower detection limit, LDL, of 0.5 ppb for Pb (6 min of preconcentration) and 2.5 ppb for Cd (3 min of preconcentration)), considering that the EPA's action level of the metals in public drinking water supplies is 15 ppb of Pb and 5 ppb of Cd. Until the SH-SAMMS-Nafion sensors, this low concentration range of metal ions in un-pretreated natural waters has been extremely difficult to detect using mercury-free electrodes. Others have reported the detection limits of Pb and Cd to be in low ppb levels at either mercury based electrodes or mercury-free electrodes, but the values were based on 3S/N and were measured in metal spiked buffered solutions rather than in real samples. For example, the detection limits based on 3S/N has been reported to be: 0.1 ppb of Pb and 22.5 ppb of Cd after 10 minutes at poly(pyrrole-malonic acid) film modified carbon electrode, 5 ppb of Pb at a Hg-film coated platinum electrode, and 24 ppb of Cd at the phytochelatin modified mercury drop electrodes, all measured in metal spiked buffered solutions.



**Figure 4.** Responses of 0 to 1.5 ppb of Pb in unfiltered seawater (SW) at a SH-SAMMS-Nafion electrode after 6 min preconcentration and the corresponding linear curve in the inset. Inset also shows the linear curve measured in unfiltered river water (RW) and groundwater (GW).

Owing to the rigid silica supports and appropriate pore structure (parallel, hexagonal) and pore size (5 nm), all SAMMS allows rapid metal sorption rate. For example, SH-SAMMS could remove over 99 wt % of 1 mg/L Pb from groundwater after 1 min of contact time, while it took over 10 and 120 min for Chelex-100 (EDTA based commercial resins) and GT-73 (thiol based commercial resins) to remove over 96% of Pb. This fast sorption rate makes it possible to have a short preconcentration time with an electrochemical sensor. **Figure 5** shows

the response versus preconcentration time of (a) 10 ppb Cd in acetate solution and (b) 10 and 50 ppb of Cd in filtered river water. In both systems, 10 ppb of Cd could be detected after only one minute of preconcentration. The increased signal as increasing preconcentration time has been observed in all SAMMS-based electrodes. Owing to the large binding site density of SAMMS, the electrodes is not easily saturated by the metal ions (e.g., as the preconcentration time increases from 0 to 30 minutes). Large binding site density of SAMMS also results in large linear range (e.g., linear ranges are obtained from few ppb to 1.6 ppm of Pb). At low concentration (e.g., 10 ppb Cd) in natural water, negative deviation from linear response is likely attributed to the slow transport of metal ions (that may bind with anions or organic molecules in natural water) to the electrode surface. At 50 ppb Cd in **Figure 5b**, linear response from 0 to 5 min is perhaps owed to the higher concentration gradient across the Nafion film. The deviation from linear response at in natural waters has also been found at mercury film electrodes for the detection of nickel in river water (from 0-0.35 ppm of Ni) and uranium in ground water (from 0-1.5 ppm of U).



**Figure 5.** Responses of (a) 10 ppb Cd in 0.01 M CH<sub>3</sub>COONa and (b) 10 ppb Cd and 50 ppb Cd in filtered river water, measured at a SH-SAMMS-Nafion electrode with varied preconcentration time. Inset shows the corresponding voltammograms of 10 ppb Cd.

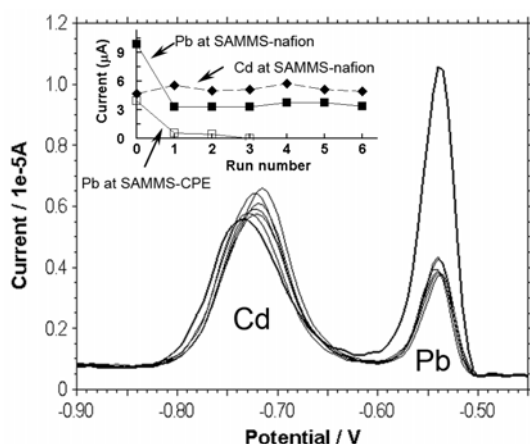
**3.2.2. Specific Aim 2: To evaluate (1) the best sensors obtained in aim 1 for simultaneous analysis of Pb, Cd, and Hg contained in complex urine matrix and (2) the effects of proteins and chelating agents for the preconcentration of the metal ions by the selected SAMMS materials.**

In this aim, we investigated the best sensors selected from Specific Aim 1 for the antifouling properties of the sensors in the presences of proteins, the chelating agents (e.g., surfactants and organic groups in real world waters), and the effects of solution pH on the metal measurements.

**Antifouling property of SAMMS-based sensors.** “Real world” samples like natural waters and urine contain surfactants, competing anions, organic substances, and proteins (normally present in mg/L to g/L concentrations) that bind very well with the target metals (normally present in μg/L concentration). Our results show that SAMMS materials can out-compete these species for the target metals, thus low ppb of Cd and Pb have been detected even in human urine. The binding sites on SAMMS are also not fouled by the proteins and organic groups. Therefore, the SAMMS materials have also been evaluated by our research group as chelators for decorporation of radionuclides and heavy metals in blood and gastrointestinal fluids (under two currently funded grants by NIAID grant grant<sup>#</sup> R01 AI074064 and NIEHS grant<sup>#</sup> R21 ES015620). In addition to competition for binding with the target metal ions, the organic substances and proteins are known to adsorb and form insulating layer on the electrode surfaces, leading to abnormally low signals for metal detection and short service time of the electrodes (described as “electrode fouling”). This is one of the reasons why single-use disposable electrodes instead of multiple use electrodes are normally employed in protein-rich samples (e.g., LeadCare® Blood Lead Analyzer from ESA Biosciences, Inc.).

For preventing protein fouling of electrodes, SAMMS-Nafion composite has been coated on glassy carbon electrodes (**Figure 2a**) and screen-printed carbon electrodes (**Figure 2b**). Nafion has been claimed to have

antifouling properties, owing to its ability to exclude molecules of large sizes. **Figure 6** shows the responses of the SH-SAMMS-Nafion electrode to 25 ppb of Cd and Pb in 0.01 M CH<sub>3</sub>COONa without albumin proteins (bovine serum albumin) (thick line) and for 6 consecutive runs after the addition of 20 mg/L of albumin (thin line) after 3 minutes of preconcentration. The addition of albumin did not affect the Cd signals but reduced the Pb signals by 65%, suggesting some competition between albumin and SH-SAMMS for Pb; this competition however will not affect the quantitative and sensitive analysis of Pb because standard addition method can be used in the same matrix. Nevertheless, Pb and Cd peaks remained stable after 6 consecutive measurements in samples containing albumin, indicating the antifouling at the SAMMS-Nafion composite electrode. On the other hand, a carbon paste electrode (CPE) modified with 10 wt.% SH-SAMMS was fouled after the first measurement in the same solution. The results clearly reveal the benefit of Nafion as an antifouling binder. In addition, the SAMMS-CPE yielded six-fold smaller signals for 25 ppb Pb and no signal for 25 ppb Cd even after 5 minutes of preconcentration (inset of **Figure 6**), compared to signals at SH-SAMMS-Nafion electrode. This is primarily due to the easier access of metal ions to the binding sites of SAMMS in the porous Nafion matrix than SAMMS that is embedded under the carbon paste matrix (which is a mixture of carbon graphite powder and mineral oil). SAMMS-Nafion composite is a technological breakthrough in sensor materials for complex “real world” samples, thus PNNL has recently filed the U.S. patent on the technology (E-15953: Electrochemical sensor and methods for making and using same).



**Figure 6.** Responses to 25 ppb of Cd and Pb in 0.01 M CH<sub>3</sub>COONa (thick line) and for 6 consecutive runs with the addition of 20 mg/L of albumin after 3 min of preconcentration at SH-SAMMS-Nafion electrode. Inset shows the responses at the SH-SAMMS-Nafion electrode (3 min of preconcentration) and at the SH-SAMMS-carbon paste electrode (CPE) (5 min of preconcentration).

**SAMMS-Nafion sensors versus Nafion sensors.** Having negative charges of sulfonic acid, Nafion is known to have some sorption affinity for metal cations and has been studied for metal preconcentration at electrode surfaces. However, the interaction between metal cations and Nafion are relatively weak, easily competed by other non-target cations, and highly pH dependent, thus limiting its effectiveness as a metal preconcentrator. In contrast, the SH-SAMMS has shown superior sorption properties for soft metal ions compared to commercial resin counterpart (e.g., GT-73). The distribution coefficients ( $K_d$  in mL/g) of SH-SAMMS to Cd and Pb in acetate solution, natural waters, and human urine are summarized in **Table 1**.

**Table 1.** The distribution coefficients<sup>a</sup> (mL/g) of Cd and Pb on SH-SAMMS in various matrices.

Matrices <sup>b</sup>	pH	Cd	Pb
Columbia River water	7.84	8700000	3100000
Hanford ground water	8.13	16000000	6000000
Sequim Bay seawater	7.65	1100000	1400000
0.01 M CH <sub>3</sub> COONa	6.64	4000000	1800000
Human urine	5.80	5100	NA

<sup>a</sup> The distribution coefficients were measured at L/S of 5,000 mL/g and initial metal ions of 100 ppb (each) with triplicates; except for human urine, L/S of 1,000 mL/g, initial Cd of 50 ppb;

<sup>b</sup> The natural waters were filtered through 0.45 micron filter before use.

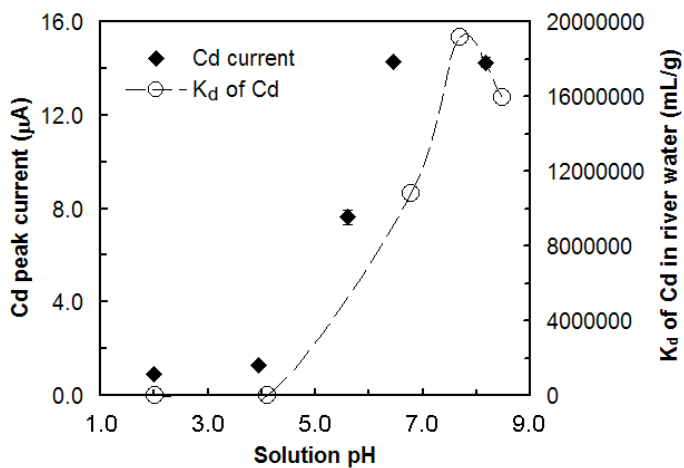
$K_d$  is a measurement of the binding affinity and is simply a mass-weighted partition coefficient between the SAMMS and the supernatant. The higher the  $K_d$  value, the more effective the sorbent material is at capturing and holding the target species. In general, sorbents with  $K_d$  values of  $10^2$ - $10^3$  mL/g are considered good and those with  $K_d$  above  $10^4$  mL/g are considered outstanding. From the  $K_d$  values, the SH-SAMMS is an outstanding sorbent for Cd and Pb in the four water matrices ( $K_d \sim 10^6$ - $10^7$ ) and a good sorbent for Cd in

human urine ( $K_d \sim 5,000$ ). Note that these high  $K_d$  of Cd and Pb were obtained in the presence of 100 ppb (each) of Co, Cu, As, Ag, Hg, and Tl, which did not interfere with the sorption of Cd and Pb. This is owed to the suitable interfacial chemistry and large surface area of SH-SAMMS ( $> 800 \text{ m}^2/\text{g}$ ), which results in large binding site density (2.8 mmol SH/g) and hence large metal loading (2.5 mmol Hg/g). As a result, **Table 2** shows that after 3 minutes of preconcentration, the SAMMS-Nafion sensor could detect 25 ppb of Cd in acetate solution and the three natural waters, while the Nafion sensor (without SH-SAMMS) could detect 25 ppb Cd sensitively in acetate solution but poorly in natural waters, where Cd ions may be bound to the various constituents of natural waters. SH-SAMMS also increases the detection sensitivity; the SAMMS-Nafion sensor could simultaneously detect 2.5 ppb of Cd and Pb in acetate solution after 3 minutes of preconcentration, while the Nafion sensor (without SAMMS) could detect only 10 ppb of Pb, but not 10 ppb of Cd. These results clearly show the advantage of SH-SAMMS for metal preconcentration at electrochemical sensors.

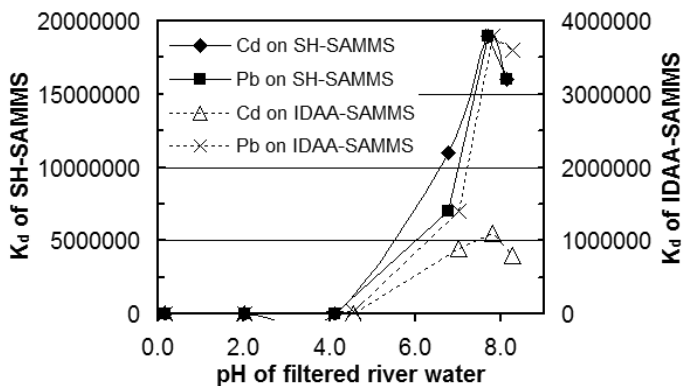
**Table 2.** Comparison of Cd and Pb signals, detected at Nafion- and SH-SAMMS-Nafion-sensors.

Matrix	Metal	Signal ( $\mu\text{A}$ )	
		Nafion sensor	SH-SAMMS-Nafion sensor
0.01 M NaAC	25 ppb Cd	12.81	18.40
Ground water	25 ppb Cd	0.03	16.83
River water	25 ppb Cd	0.20	14.28
Sea water	25 ppb Cd	0.09	5.92
0.01 M NaAC	2.5 ppb Cd/Pb	NA	0.04 (Cd), 0.24 (Pb)
0.01 M NaAC	10 ppb Cd/Pb	0 (Cd), 0.07 (Pb)	0.36 (Cd), 1.20 (Pb)

**Effect of solution pH on metal analysis.** Solution pH has profound effects on the preconcentration of metal ions at the SH-SAMMS-Nafion electrodes, which rely on the binding affinity between the metal ions and the thiol groups on SAMMS. **Figure 7** shows the effect of solution pH on the signals of 25 ppb Cd measured at a SH-SAMMS-Nafion electrode in pH-adjusted filtered river. **Figure 7** also shows the distribution coefficients ( $K_d$ ) of Cd on SH-SAMMS, also measured in the same matrix. The voltammetric signals of Cd depend on the affinity of SH-SAMMS that is immobilized on the electrode surface in capturing Cd, which in turn is a function of pH. For pH ranges from 0 to 4, the  $K_d$  values were small, corresponding to small Cd signal in that pH range. As pH increased from about 4 to 8, the  $K_d$  values increased significantly, and dropped off at pH above 8 (but still very high). This corresponds to a substantial increase in Cd signal from pH 4 to 6.5, which then remained constant from pH 6.5 to 8.2. Thus the optimal pH for Cd measurements was found in the range of 6 to 8, which is relevant to the pH of urine and natural waters. The shift in the effect of pH on Cd sensor signals compared to the observed  $K_d$  may be primarily a local pH issue (e.g., SH ligands buried in Nafion matrix may experience pH higher than pH of the bulk solution recorded as the  $K_d$  values were measured). The  $K_d$  trend of Pb on SH-SAMMS is similar to that of Cd (see **Figure 8**), thus it is reasonable to assume that the optimal solution pH for Pb detection would be about the same (e.g., from pH 6-8). **Figure 8** also show that the  $K_d$  values of Pb and Cd on IDAA-SAMMS follow the same trends as those on SH-SAMMS, suggesting that the optimal solution pH for IDAA-SAMMS based sensors should also be around 6 to 8. Note that although the  $K_d$  of SH-SAMMS is higher, IDAA-SAMMS is still considered an outstanding sorbent for Pb and Cd ( $K_d$  of  $10^5$ - $10^6$  at pH 6-8), and thus worth being investigated for developing electrochemical sensors for Pb and Cd.



**Figure 7.** Responses to 25 ppb Cd at a SH-SAMMS-Nafion electrode in pH-adjusted filtered river water. Second Y-axis shows the distribution coefficient ( $K_d$ ) of Cd on SH-SAMMS, measured in the same matrix, with L/S of 5,000 mL/g and initial Cd concentration of 100 ppb, in the presences of 100 ppb (each) of Co, Cu, As, Ag, Hg, and Tl.

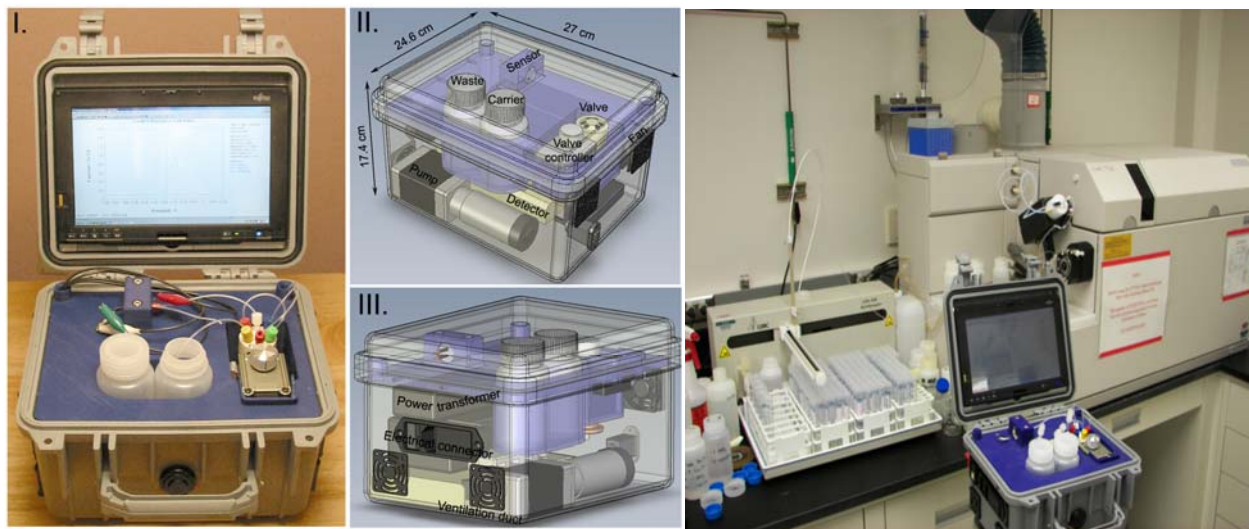


**Figure 8.** Distribution coefficients ( $K_d$ ) of Pb and Cd measured on SH-SAMMS (1<sup>st</sup>-Y-axis) and IDAA-SAMMS (2<sup>nd</sup>-Y-axis) in pH-adjusted filtered river water, L/S of 5,000 mL/g and initial metal concentration of 100 ppb, in the presences of 100 ppb (each) of Co, Cu, As, Ag, Hg, and Tl.

### 3.2.3. Specific Aim 3: To build a fully-automated, portable metal analyzer prototype, optimize, evaluate, and validate it with urine samples containing metal ions at the occupational exposure levels.

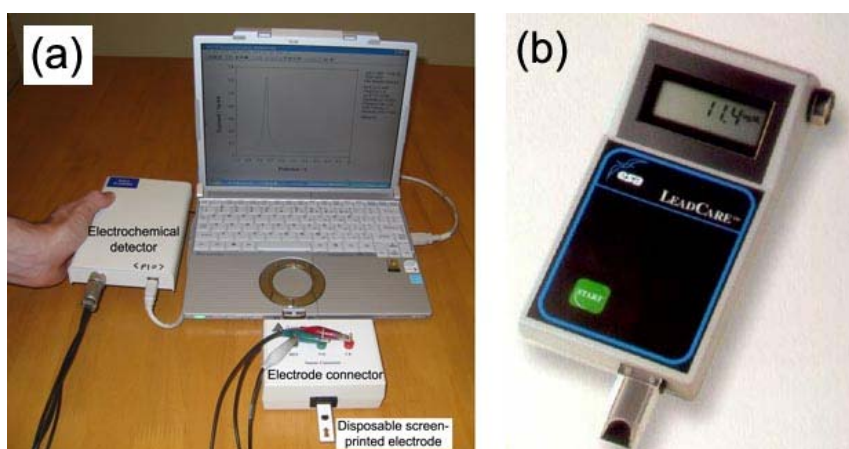
In this Specific Aim, we have built two prototypes for two intended applications as follows.

**Prototype of portable analyzer.** We have built a prototype device based on flow injection analysis (FIA) and stripping voltammetry as shown in **Figure 9**. A bit larger than a lunchbox, the device is field-deployable with plug-and-play features that allow different sensors to be easily exchanged for detecting a variety of heavy metal toxins. It requires sample in microL in volume and the flow rate is also in microL/s. The device requires about 1.5 times the power of a typical laptop computer. The automated system also routinely delivers reliable measurements within a rapid two-to-five minute analysis period. Early production cost estimates indicate that the device may be as much as 10 times less expensive than available ICP-MS, which are not field portable and require samples to be returned to the lab for time-consuming and more expensive analysis.



**Figure 9.** PNNL's portable metal analyzer (shown on the left) is compact in size compared to the state-of-the-art ICP-MS (shown in the background on the right).

**Prototype of disposable sensor system.** The second prototype is for screening of metals using disposable test strips. The test strips are created by coating of SAMMS-Nafion composite on disposable screen-printed carbon electrodes (SPE, see **Figure 2b**). The disposable strip is available commercially at low cost (< \$1 a strip) and consists of three built-in electrodes, including screen-printed carbon as counter and working electrodes and Ag/AgCl as reference electrode. However without SH-SAMMS-Nafion modification, the electrode strips are not sensitive at detecting urinary metals (e.g., 250 ppb Pb could not be detected at an unmodified electrode and 500 ppb Pb yielded signal equal to 12.5 ppb Pb at a SH-SAMMS-Nafion electrode). We have developed protocol for modifying the working electrode with SAMMS-Nafion. Briefly, the working electrode (e.g., the dark circle in **Figure 2b**) was drop-coated with 1  $\mu$ L of SAMMS-Nafion composite. The film was air-dried at room temperature for about 0.5-1 hr prior to using the electrode. The disposable test strips were then connected to a hand-held electrochemical detector (CH Instrument, Inc.) through an electrode connector (**Figure 10**) and a computer was used to control the detector and record the results.

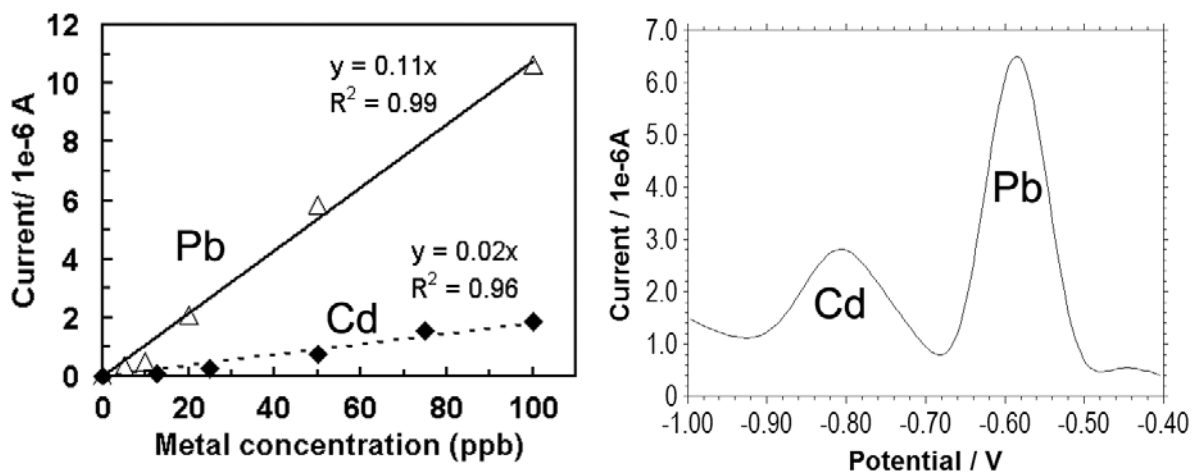


**Figure 10.** (a) PNNL's portable sensor system consisting of a disposable electrode strip, a hand-held electrochemical detector, an electrode connector, and a computer; all the components can ultimately be integrated into a single device similar to (b) the ESA LeadCare device (ESA Biosciences, Inc.).

After optimization studies in Specific Aims 1&2, the SAMMS-Nafion sensors were then evaluated for detecting metals in human urine as follows.

**Multiple metal detection.** Simultaneous detection of Cd and Pb in human urine could be accomplished with SH-SAMMS-Nafion coated on screen-printed carbon sensors (**Figure 2b**). Human urine was spiked with Cd and Pb to obtain concentrations from 0 to 100 ppb (each). **Figure 11(a)** show the linear calibration curves of Cd and Pb simultaneously detected in urine (5 minutes of preconcentration). Without the SH-SAMMS-Nafion modification, the electrodes could not detect urinary Pb as high as 250 ppb. **Figure 11(b)** shows the well-

defined voltammograms of urinary Cd (100 ppb) and Pb (50 ppb) (5 minutes of preconcentration). Both peaks do not overlap each other. In human urine that had not been pretreated, Pb was detectable at 5 ppb (three times lower than EPA's water drinking limit for Pb and thirty time lower than the biological exposure index (BEI) for urinary Pb) and Cd was detectable at 5 ppb (same with EPA's water drinking limit for Cd and BEI for urinary Cd) at both SH-SAMMS and IDAA-SAMMS sensors after 5 minutes of preconcentration. The detection limits of urinary Cd and Pb at SH-SAMMS-sensors are improved to 1 ppb after 10 minutes of preconcentration. The reproducibility (%R.S.D.) after 9 consecutive measurements of 50 ppb Pb in urine, detected with a single SH-SAMMS-Nafion screen-printed electrode is 2.6%, which is considered excellent for batch measurements. For 8 consecutive measurements of 25 ppb Cd in urine also on a single electrode, the %R.S.D. is 6.3%.



**Figure 11.** (a) Linear responses of simultaneous detection of urinary Cd and Pb and (b) voltammograms of urinary Cd (100 ppb) and Pb (50 ppb) at SAMMS-Nafion disposable sensors.

**Detection interferences.** Unlike other sensors that preconcentrate a target metal and detect it in a sample solution, SAMMS-based sensors preconcentrate a target metal in a sample solution, but detect it in a clean medium. Thus, an active species will only interfere with the analysis of the target metal if (1) it can out-compete the target metals for the binding sites on SAMMS and (2) once it adsorbs on SAMMS yields a signal that overlaps with that of the target metal in the stripping (detection) step. Natural waters and urine contain a number of confounding components including: proteins, organic substances, surfactants, anions, which all may compete with SH-SAMMS for the target metals. They also contain other cations that may compete with the target metals for the binding sites on SAMMS. With high content of proteins and metabolic wastes in urine,  $K_d$  of Cd in urine is still as high as 5,100 (**Table 1**). From previous competitive sorption studies, background ions, such as Na, Ba, and Zn, although present at high concentrations (i.e., 350 times higher concentrations than Pb), did not bind to the SH-SAMMS. The presence of other anions (i.e.,  $\text{CN}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ) did not significantly interfere either with the adsorption of Pb onto the SH-SAMMS. In summary, successful detection of low ppb Cd and Pb in natural waters and Cd in human urine indicates that SAMMS-Nafion sensors can overcome these interferences during the preconcentration. Once a species adsorbs on SH-SAMMS during the preconcentration step, it can interfere with the detection of the target metal if its signal overlaps with that of the target metal. Unlike at gold-based electrodes where overlapping of Cd and Pb signals is severe, at SAMMS-Nafion electrodes Cd and Pb signals do not overlap (see **Figure 11b**). TI has been reported by others to interfere with the detection of Cd due to its close proximity to the Cd peak (e.g., at slightly more negative potential than Cd). We have found that interference of TI to Cd signals was not observed until the TI:Cd mass ratio is larger than 2, while previous work observed the TI interference starting from a TI:Cd mass ratio of 0.08 at a silver electrode (Krasnodebska-Ostrega et al. 2005). This improved selectivity is a result of the much higher selectivity of SH-SAMMS for Cd ( $K_d$  of  $10^7$ ) over TI ( $K_d$  of  $10^2$ ) and consequently capture Cd far more preferentially than TI.

**Measurement reproducibility of SAMMS-Nafion sensors.** Reproducibility of the measurement has been determined for a single electrode and for many electrodes (termed inter-electrode reproducibility). It is represented by a relative standard deviation (%R.S.D.) from many consecutive measurements of the same metal concentration. For SH-SAMMS-Nafion coated rod electrodes (**Figure 2a**), %R.S.D. of 8 consecutive

measurements of 25 ppb Cd in 0.05 M sodium acetate after 3 minutes of preconcentration was 5% on a single electrode, which is considered outstanding for batch measurements. The reproducibility is normally improved in automated systems (which minimize human errors); we have achieved %R.S.D. of 2.4 at programmable Pb analyzer for 10 consecutive measurements of 10 ppb Pb in a sample containing 10% blood. This is very comparable to that of the gold standard ICP-MS, which has %R.S.D.  $\sim$  3. Good inter-electrode reproducibility of the rod electrodes was demonstrated by measuring the signals at 25 ppb Cd in 0.05 M sodium acetate measured with four electrodes, which yielded the signal ratios of 1.0, 1.1, and 1.2 (normalized with an average signal from the first electrode surface). For screen-printed electrodes (**Figure 2b**), the inter-electrode reproducibility of 10 electrodes used for measuring 25 ppb of Cd after 3 minutes of preconcentration was determined to be 10%. Note that these sensors are prepared by manual dip-coating of SAMMS-Nafion mixture at rod electrodes or manual drop-coating of the mixture at disposable screen-printed electrodes. Thus, the inter-reproducibility would likely to be much improved through a precise manufacturing method (e.g., spin-coating technique).

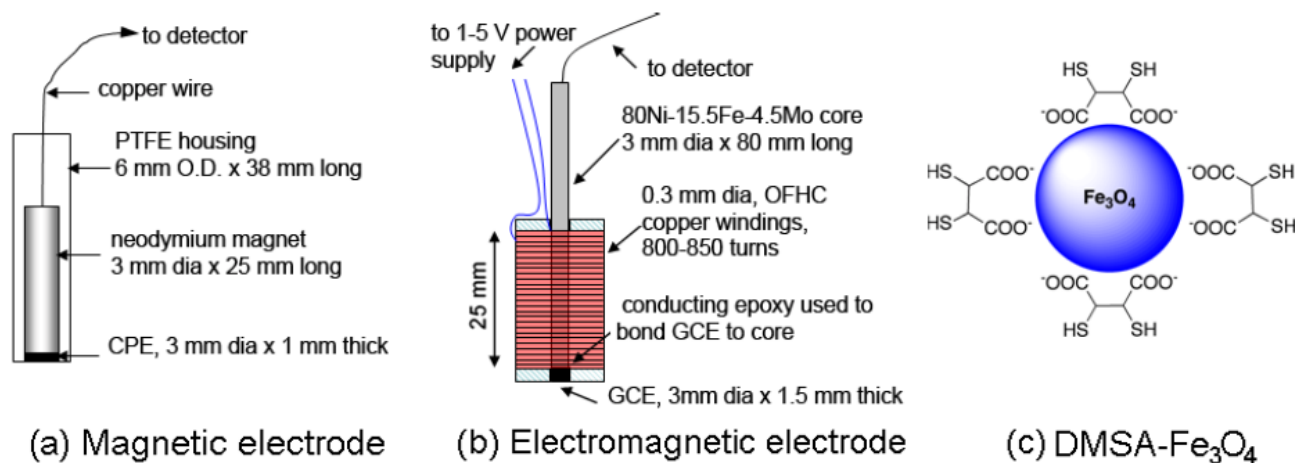
**Reliability and stability of SAMMS-Nafion sensors.** The perfluorinated backbone chains of Nafion are noted for their chemical and physical stability. However, when coated by itself on the electrode surface, from our experience Nafion film can peel off (wear and tear) from the surface. With a completely calcined silica support structure and surface functionalized with covalent crosslinked ligands, SH-SAMMS materials are also very stable across acid and alkaline conditions (silica is soluble at pH above 9). SAMMS is proven to strengthen the SAMMS-Nafion film (compared to the film of Nafion alone). This is expected because every time when a rigid structural phase is put into a squishy polymer phase (like glass fiber into epoxy to make fiberglass), the resulting composite becomes stiffer, stronger and less prone to creep. What's more, a low ppb concentration of metals could be detected at the SAMMS-Nafion sensors, suggesting that metal ions can reach the binding sites anchoring on the pore walls of SAMMS. This leads us to believe that Nafion does not clog the pores of SAMMS. As a result, the composite of Nafion and SAMMS provides a porous film with good mechanical and chemical stability resulting in robust and stable electrodes. The SAMMS-Nafion sensors are resistant to acid (used as electrolyte in the detection and cleaning steps); after immersing an electrode in 0.25 M HCl for 30 min, 60 min, 90 min, and 120 min, the ratio of 25 ppb Cd signals in 0.01 M sodium acetate measured with the same electrode was 0.99, 1.00, and 0.91 (normalized with the average signal after 30 min of immersion).

While the SAMMS-carbon paste electrode counterparts need daily surface renewal (when used with metal spiked buffered solutions) and after every single run (when used with protein-rich samples), the SH-SAMMS-Nafion composite electrodes have a long service life. For example, two rod electrodes (**Figure 2a**) were in service for 4 operational days with the total of 120 measurements for one electrode and 70 measurements for the other. During these measurements, the SAMMS-Nafion sensors are reliable and robust; for instance, **Figures 5-7** were measured with just a single electrode surface without renewal. Although the screen-printed carbon electrode (**Figure 2b**) is sold for a single use (at  $<$  \$1 a piece), once they are modified with SH-SAMMS-Nafion, they can be used for multiple measurements; e.g., often times, a single electrode can be used reliably for at least 30 measurements in urine without surface renewal. This owes to the antifouling of Nafion layer, the regenerability of SAMMS, and the chemical and mechanical stability of SAMMS-Nafion composite. Renewal of surface can be done with ease by wiping off the SAMMS-Nafion film with methanol, followed by drop coating of 1  $\mu$ L (or dip coating) of 10 w/v% of SAMMS and Nafion mixture on the surface. Then the electrode is left air-dried for 30-60 min and is ready to be used again.

In short, SAMMS-Nafion sensors offer a combined benefit of SAMMS as outstanding metal preconcentrators and Nafion as stable antifouling binder. The SAMMS-Nafion composite is a technological breakthrough that allows sensitive and reliable detection of metal ions in low ppb level in "real world" samples without sample pretreatment and electrode fouling.

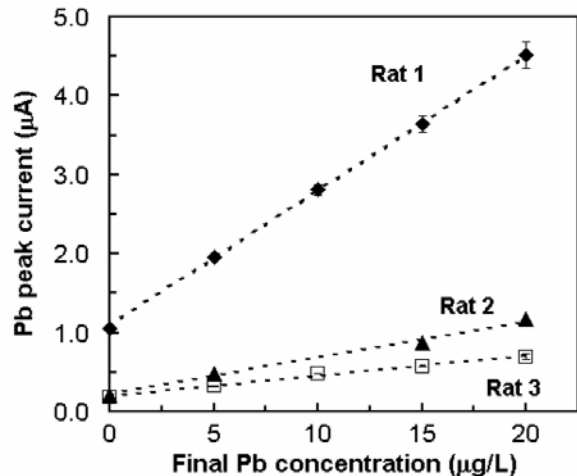
### 3.2.4. Additional results

In addition to SAMMS, we have investigated another class of novel materials, functional superparamagnetic nanoparticles (FNPs) for metal preconcentration. For direct, simple, and field-deployable monitoring of urinary Pb, electrochemical sensors employing superparamagnetic iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles with a surface functionalization of dimercaptosuccinic acid (DMSA) has been developed as show in **Figure 12**.



**Figure 12.** Schematics of (a) magnetic electrode and (b) electromagnetic electrode, which preconcentrate metal ions using (c) DMSA- $\text{Fe}_3\text{O}_4$  nanoparticles.

The metal detection involves rapid collection of dispersed metal-bound nanoparticles from a sample solution at a magnetic or electromagnetic electrode, followed by the stripping voltammetry of the metal in acidic medium. The sensors were evaluated as a function of solution pH, the binding affinity of Pb to DMSA- $\text{Fe}_3\text{O}_4$ , the ratio of nanoparticles per sample volume, preconcentration time, and Pb concentrations. The effect of binding competitions between the DMSA- $\text{Fe}_3\text{O}_4$  and urine constituents for Pb on the sensor responses was studied. After 90s of preconcentration in samples containing 25 vol.% of rat urine and 0.1 g/L of DMSA- $\text{Fe}_3\text{O}_4$ , the sensor could detect background level of Pb (< 1ppb) and yielded linear responses from 0 to 50 ppb of Pb, excellent reproducibility (%R.S.D of 5.3 for seven measurements of 30 ppb Pb), and Pb concentrations comparable to those measured by ICP-MS. **Figure 13** shows the signals of Pb from three urine specimens that were collected from 3 individual rats and spiked with Pb. It shows a clear evidence of the matrix effect on the Pb signals. Thus a standard addition method was used to obtain the concentration of Pb in the three urine specimens as shown in **Table 3**. The Pb concentration in whole urine was the absolute value of X-intercept that was corrected for the dilution factors (by 4 to 5 fold). The Pb concentrations obtained with the DMSA- $\text{Fe}_3\text{O}_4$  based sensors are compared very well with those measured by ICP-MS as summarized in **Table 3**.



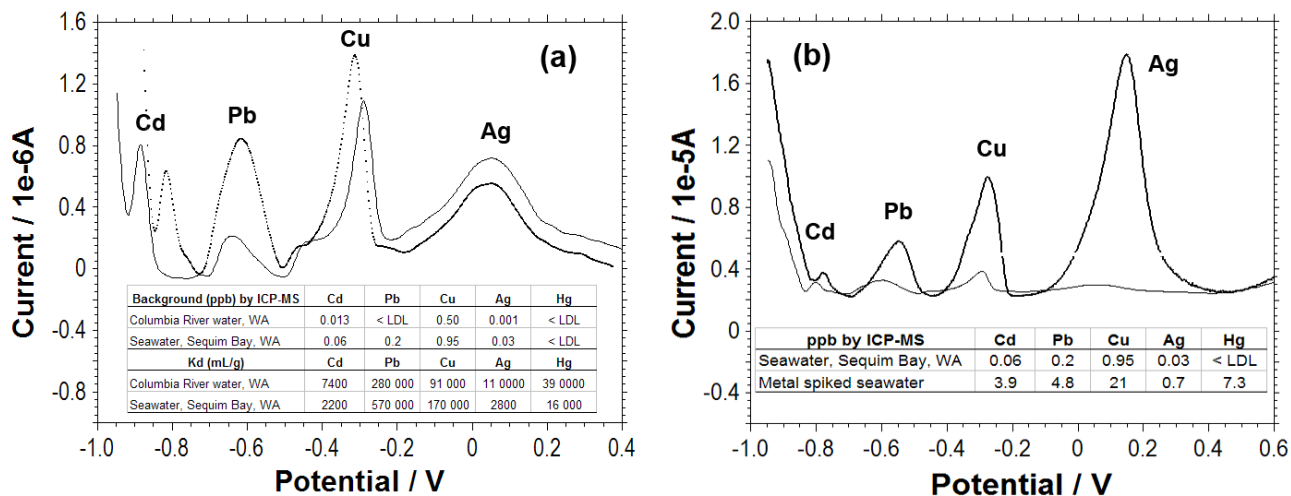
**Figure 13.** Pb signals by standard addition method measured at DMSA-Fe<sub>3</sub>O<sub>4</sub>-magnetic sensors in Pb-spiked samples contained 25 vol.% of urine from three individual rats.

**Table 3.** Pb concentrations in three rat urine specimens measured with DMSA-Fe<sub>3</sub>O<sub>4</sub> sensors and ICP-MS.

Parameters	Rat 1	Rat 2	Rat 3
Slope (µA/ppb Pb)	0.17	0.05	0.03
Y-intercept (µA)	1.08	0.23	0.20
ppb of Pb in diluted urine sample*	6.3	4.9	7.7
ppb of Pb in whole urine	25.1	19.7	38.5
ppb of Pb by ICP-MS analysis	25.0	20.1	41.4
% deviation from ICP-MS values	0.2	1.8	7.0

\* Specimens from Rat 1 and 2 were diluted for 4-fold and from Rat 3 for 5-fold

The sensor could also simultaneously detect background levels (< 1 ppb) of Cd, Pb, Cu, and Ag in river and seawater as shown in **Figure 14**, the low detections limits that are very comparable to ICP-MS.



**Figure 14.** Sensor measurements of (a) background metal ions in seawater (dash line) and river water (solid line) and (b) background metal ions (thin line) and metals spiked (thick line) in seawater, after 150s of preconcentration time. Inset shows the metal concentrations, measured with ICP-MS, and the distribution coefficients of multiple metal ions (S/L of 0.01 g/L of DMSA-Fe<sub>3</sub>O<sub>4</sub>, initial metal conc. of 500 ppb each, pH of 7.20 for river water and 7.64 for seawater).

In short, the magnetic nanoparticles have great potential for metal preconcentration at analytical instruments; hence PNNL has filed a patent on the technology (Application<sup>#</sup> 11936405: Functionalized magnetic

nanoparticle analyte sensor). However, the magnetic nanoparticles are newer, hence are less established and less understood than SAMMS (e.g., in terms of batch-to-batch variation and scaling up of their production, their performance after long term storage, and their dependence on solution's ionic strength). Much research is still needed to fully understand these aspects of the materials before their deployment.

## 4.0 Research Translation

Translation of research findings includes (3.1) prototype development, (3.2) institutional effort for commercialization and (3.3) communicating of the results and applications to the public.

**4.1. Prototype of portable metal analyzers.** We have built two prototype metal analyzers as shown in Figures 9 and 10. The first one is for quantitative analysis and the second one is for low-cost metal screening.

**4.2. Institutional effort for commercialization.** Battelle-PNNL has provided great support for our technology developed through this R21 grant. It has filed two U.S. Patent Applications on the technology; one for SAMMS-based sensors and the other for magnetic iron oxide based sensors (see Section 5.0). The PI (Dr. Yantasee) received the 2007 Ronald L. Brodzinski Early Career Exceptional Scientific Achievement Award from PNNL, which recognized her achievement in the development of the next-generation metal analyzer (with \$62,500 grant award).

**4.3. Communicating of the results to the public.** In addition to presentations and peer-reviewed publications (see Section 5.0), to communicate the results to the lay public, PNNL has issued a press release in Feb of 2008 on the portable sensor technology based on functional nanomaterials, which has been posted on over 30 of science and technology websites including *Chemistry World*, *ChemEurope.com*, and *eponline.com*. Example is <http://www.rsc.org/chemistryworld/News/2008/February/22020801.asp>

## 5.0 Peer Reviewed Publications/Presentations/Patents

### Publications:

1. Yantasee W, CL Warner, T Sangvanich, RS Addleman, TG Carter, RJ Wiacek, GE Fryxell, C Timchalk, and MG Warner. **2007**. "Removal of Heavy Metals from Aqueous Systems with Thiol Functionalized Superparamagnetic Nanoparticles." *Environmental Science & Technology* 41(14): 5114-5119. One of the most accessed articles of ES&T (Q3, 2007). <http://pubs.acs.org/cgi-bin/article.cgi?esthag/2007/41/i14/pdf/es0705238.pdf>
2. Yantasee W, Y Lin, K Hongsirikarn, GE Fryxell, RS Addleman, and C Timchalk. **2007**. "Electrochemical Sensors for the Detection of Lead and Other Toxic Heavy Metals: The Next Generation of Personal Exposure Biomonitor." *Environmental Health Perspectives* 115(12):1683-1690. <http://proquest.umi.com/pqdlink?index=20&did=1400553231&SrchMode=3&sid=1&Fmt=6&VInst=PROD&VType=PQD&RQT=309&VName=PQD&TS=1208196491&clientId=74975&aid=1>
3. Yantasee W, K Hongsirikarn, CL Warner, D Choi, T Sangvanich, MB Toloczko, MG Warner, GE Fryxell, RS Addleman and C Timchalk. **2008**. "Direct detection of Pb in urine and Cd, Pb, Cu, and Ag in natural waters using electrochemical sensors immobilized with DMSA functionalized magnetic nanoparticles." *Analyst*, 133: 348 – 355. One of the most accessed articles of *Analyst* in Feb and March, 2008. <http://www.rsc.org/ej/AN/2008/b711199a.pdf>
4. Yantasee W, B Charnhattakorn, GE Fryxell, Y Lin, C Timchalk, and RS Addleman. **2008**. "Detection of Cd, Pb, and Cu in non-pretreated natural waters and urine with thiol functionalized mesoporous silica and Nafion composite electrodes." *Analytica Chimica Acta* (620):55-63.
5. Harrer BJ, Yantasee, W, Fryxell, GE. Sensitive nanomaterials-based detection system rapidly measure heavy metals in liquids, *MRS Bulletin*, **2008**, 33:820.

6. Carter, TG, Yantasee, W; Sangvanich, T; Fryxell, GE; Johnson, DW; Addleman RS. New Functional Materials for Heavy Metal Sorption: Supramolecular Attachment of Thiols to Mesoporous Silica Substrates, *Chemical Communications*, **2008**, 5583 – 5585.

7. RS Addleman, W Yantasee, GE Fryxell, MG Warner, SA Fontenot, DW Johnson, JT Bays, CL Warner. Nanostructured materials for selective collection of trace-level metals from aqueous systems. DT Pierce, JX Zhao (eds). In *Trace Analysis with Nanomaterials*, 2009.

8. Warner MG, CL Warner, RS Addleman, and W Yantasee. 2009. "Magnetic Nanomaterials for Environmental Sensing." In *Magnetic Nanomaterials: NmLS-Series*, vol. 4, Wiley-VCH.

9. Samuels WD, NH LaFemina, V Sukwarotwat, W Yantasee, XS Li, and GE Fryxell. Chloromethylated activated carbon: A useful new synthon for making a novel class of sorbents for heavy metal separations, *Sep Sci Technol*. In Press

#### **Presentations:**

1. Yantasee W, GE Fryxell, C Timchalk, RS Addleman, and MG Warner. "Next-generation metal analyzers based on nanomaterials for biomonitoring and environmental monitoring." Presented by Wassana Yantasee (Invited Speaker) at 2008 NIOSH Direct-Reading Exposure Assessment Methods (DREAM) Workshop, Washington DC, November 13, 2008.

2. Yantasee W, GE Fryxell, RS Addleman, C Timchalk, and MG Warner. "Next-generation metal analyzers based on nanomaterials for biomonitoring and environmental monitoring." Presented by Wassana Yantasee (Invited Speaker) at IEEE Richland Section Sensors Chapter, WSU Tricities, WA, August 27, 2008

3. Warner MG, S Addleman, GE Fryxell, W Yantasee, CL Warner, AJ Tyler, JT Bays, J Davidson, and RM Ozanich. "Functionalization of Nanoporous and Nanoparticulate Materials for Removal and Detection of Biological and Chemical Contaminants.", Washington State University Seminar, Pullman, WA.

4. Warner MG, CL Warner, RS Addleman, T Droubay, MH Engelhard, JD Davidson, AD Cinson, MA Nash, and W Yantasee. "Synthesis of functionalized superparamagnetic iron oxide nanoparticles from a common precursor and their application as heavy metal and actinide sorbents.", 237th American Chemical Society National Meeting & Exposition, Salt Lake City, UT.

#### **Patent applications and invention disclosure reports (IDR):**

- US Patent application no. 11936405: Functionalized magnetic nanoparticle analyte sensor.
- US Patent application no. E-15953: Electrochemical sensor and methods for making and using same.
- IDR# 2464: Electrochemical sensors based on SAMMS-nafion composite
- IDR# 2206: Metal Sensors based on Functionalized Magnetic Nanoparticles
- IDR# 2085: Removal of Heavy Metals from Aqueous Systems with Thiol Functionalized Superparamagnetic Nanoparticles