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Automated portable analyzer for lead(II) based on sequential flow injection and nanostructured electrochemical sensors

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Abstract

A fully automated portable analyzer for toxic metal ion detection based on a combination of a nanostructured electrochemical sensor and a sequential flow injection system has been developed in this work. The sensor was fabricated from a carbon paste electrode modified with acetamide phosphonic acid self-assembled monolayer on mesoporous silica (Ac-Phos SAMMS) which was embedded in a very small wall-jet (flow-onto) electrochemical cell. The electrode is solid-state and mercury-free. Samples and reagents were injected into the system and flowed through the electrochemical cell by a user programmable sequential flow technique which required minimal volume of samples and reagents and allowed the automation of the analyzer operation. The portable analyzer was evaluated for lead (Pb) detection due to the excellent binding affinity between Pb and the functional groups of Ac-Phos SAMMS as well as the great concern for Pb toxicity. Linear calibration curve was obtained in a low concentration range (1–25 ppb of Pb(II)). The reproducibility was excellent; the percent relative standard deviation was 2.5 for seven consecutive measurements of 10 ppb of Pb(II) solution. Excess concentrations of Ca, Ni, Co, Zn, and Mn ions in the solutions did not interfere with detection of Pb, due to the specificity and the large number of the functional groups on the electrode surface. The electrode was reliable for at least 90 measurements over 5 days. This work is an important milestone in the development of the next-generation metal ion analyzers that are portable, fully automated, and remotely controllable.

Keywords: Automated portable analyzer; Sequential flow injection; Nanostructured electrochemical sensors

1. Introduction

Inorganic lead (Pb) compounds are known to be highly toxic especially to children whose intestinal absorption of Pb is more efficient than adults [1]. Although environmental exposure to Pb has been reduced considerably with the introduction of new regulations, Pb poisoning may still occur in susceptible sub-populations and due to occupational exposure [2]. Having recognized the potential danger from exposure to Pb, we have developed nanostructured materials for selectively sequestering of Pb [3] and conceptual-staged electrochemical sensors for the detection of Pb [4]. For non-invasive biomonitoring of Pb from occupational and environmental exposures, we have developed sensors and methods that minimize the electrode fouling from protein, thereby permitting the accurate detection of Pb in saliva [5]. These electrochemical sensors are developed as part of the next-generation metal ion analyzers that are portable and field-deployable unlike the current in-laboratory metal analyzers, such as ICP-MS and AAS.

To develop the next-generation prototype analyzer, we have integrated a sequential injection analysis (SIA) with the nanostructured electrochemical sensors. Since it was first introduced in 1990, SIA is gaining popularity because of its economical use of samples and reagents, robustness, inexpensiveness, and simple design of instrumentation [6–8]. The SIA has been coupled with spectrophotometers for simultaneous detection of ppm levels of metal ions specifically to those that can form complexes with dithizone [8].

In this work, an electrochemical sensor based on adsorptive stripping voltammetry (AdSV) is the detector of choice because of its many advantages [4]. Attributed to its built-in preconcentration step of AdSV, detection limits as low as 1 ppb of metal ions can be achieved. The preconcentration

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of metal ions at the electrode constructed with acetamide phosphonic acid self-assembled monolayer on mesoporous support (Ac-Phos SAMMS) and carbon paste utilized the excellent binding affinity and molecular recognition of the functional groups toward the target Pb ions without applying a potential. This results in excellent sensitivity and selectivity for the metal ion detection.

2. Experimental

2.1. Working electrodes

The SAMMS-modified carbon paste electrode was prepared by thoroughly mixing a 0.015 g quantity of Ac-Phos SAMMS with a 0.15 g quantity of CPO carbon paste (Bioanalytical Systems Inc., IN). The preparation and characterization of Ac-Phos SAMMS are described elsewhere [3]. Briefly, the mesoporous silica (MCM-41) which was used as the substrate had a surface area of 989 m^2/g and a nominal pore size of 5.0 nm. The functional group density was 2 mmol carbamoylphosphonic acid/g of Ac-Phos SAMMS. A drop (0.025 g) of mineral oil (Aldrich Co.) was added to the carbon paste/Ac-Phos SAMMS mixture and mixed until obtaining a uniformly wetted paste. The mixture was packed tightly into a cylinder (2 mm i.d. × 2 mm depth) hole at the center of a custom-made PEEK block and served as the working electrode (Fig. 1a). After the packing, the electrode surface was then smoothed. The unused electrode material was kept in a tightly closed container and stored at 4 °C for subsequent use. The electrode renewal was accomplished by replacing the old electrode material with the new one.

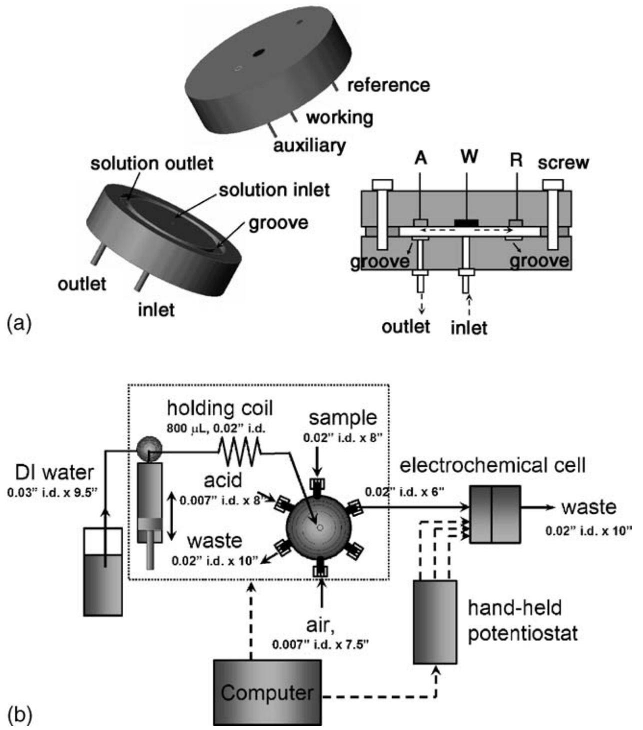

Fig. 1. Schematic of: (a) a wall-jet (flow-onto) electrochemical cell and (b) the portable sensor system based on the integration of computer-controlled sequential injector and electrochemical sensor.

2.2. Electrochemical flow cell

Fig. 1a illustrates the schematic of the electrochemical flow cell which has a wall-jet (flow-onto) configuration similar to that in the BAS's UniJet flow cell. The flow cell composed of two removable PEEK blocks and a sandwiched laser-cut Teflon gasket (UniJet 16 μm, Bioanalytical Systems Inc.) in between. The working electrode was embedded in one of the PEEK blocks, while the reference (Ag/AgCl wire) and auxiliary electrodes (Pt wire) were embedded in the second block. In the second PEEK block a groove was laid having a dimension of 0.035 in. wide × 0.035 in. deep and had an inner radius of 0.324 in. Solutions were injected onto the working electrode through the 0.019 in. i.d. inlet. The liquid flowed in the radial direction to the groove and then flowed out through the 0.031 in. i.d. outlet. All three electrodes were exposed to solutions during the operation. When not in use, the electrode was kept dry by flowing air through to remove all liquid.

2.3. User programmable sequential injection

The sequential injection system (MicroSIA, FIAlab Instruments Inc., WA) consisted of a six-port selection valve and a 24,000-step syringe pump with bi-directional valve. A computer was used to control both a hand-held potentiostat (Model CHI1232, CH Instruments Inc., TX) and the MicroSIA through RS232 connectors. The MicroSIA components included syringe 2.5 mL, 800 μL holding coil, and other sampling lines having dimensions as specified in Fig. 1b. Typical operating conditions are summarized in Table 1 including volumes of the sample and acid and their respective flow rate, which were delivered by the sequential injector. Automatic control of the sequential injector was done by a user-written program using various commands of FIAlab for Window 5.0 (FIAlab Instruments Inc., WA). The event procedure program allowed the controls of hardware configurations and timing in sequence and in repeated number of measurements as desired.

2.4. Electrochemical analysis

The analysis principle of the metal ion sensor and the protocol in batch analysis were described elsewhere [9]. For the stripping analysis in the sequential injection platform, Table 1 summarizes the necessary steps. Briefly, the metal ion in DI water (pH of 5–6) was first injected at 2 μL/s in a flow-onto manner to the working electrode, and the metal ions adsorbed on functional groups of SAMMS embedded in the electrode surface under open circuit potential. Then the system was rinsed to remove the unadsorbed metal ion. Next, 0.3 M HCl was injected at the flow rate of 2 μL/s to the

Table 1
Typical operating conditions

Step	Description	Sample and reagent	Volume used (μL)	Flow rate (μL/s)	Applied potential
1	Rinsing of sampling line and holding coil	DI water	3000	100	None
2	Preconcentration by adsorption at OCP	1–25 ppb metal ion in DI water	240/360	2	None
3	Removing unadsorbed metal ion	DI water	300	10	None
4	Desorption and cathodic electrolysis	0.3 M HCl	100	2	-1.0 V for 70 s
5	45-s quiet period and anodic stripping voltammetric detection	Holding acid from step 4	0	0	-0.8 to -0.2 V by DPV with a pulse of: 0.05-s width, 0.05-V amp, 0.2-s period
6	Rinsing acid off electrochemical cell	DI water	500	10	None

electrochemical cell to desorbed metal ions while a negative potential (-1.0 V) was immediately applied for 70 s to reduce the metal ion to elemental metal. The pump was then stopped and the differential pulse voltammetry (DPV) was performed by scanning potential from -0.8 to -0.2 V using the holding acid (0.3 M HCl) as the electrolyte. The anodic currents were recorded as a function of the scanning potential. The electrode was rinsed with DI water prior to subsequent runs.

Differential pulse voltammetry (DPV) technique performed at a hand-held potentiostat (Model CHI1232, CH Instruments Inc.) was used for the stripping analysis of metal ions. The DPV was operated at the pulse amplitude of 50 mV, pulse width of 0.05 s, sampling width of 0.025 s, and pulse period of 0.2 s. All measurements were made at room temperature and under an atmospheric environment. No de-aeration of samples and reagents were required. Control of the hand-held potentiostat for multiple measurements was achieved using the “repetitive runs” option of CHI1232 software which may be initiated by an external trigger sent from the sequential injector to the potentiostat for potentiostat-sequential injection system interfacing.

3. Results and discussion

3.1. Optimization of operating parameters

Operating parameters for the SAMMS-modified sensors have been evaluated in batch experiments including the potential, duration, and electrolyte conditions of the cathodic electrolysis and anodic stripping step [5]. In a sequential flow analyzer, the volume of sample or reagent and the flow rate played important roles in the sensitivity and accuracy of the analysis. Results showed that slower flow rate improved the mass transfer of metal ions from aqueous phase to the solid phase where the functional group was embedded. For example, 500 μL of 10 ppb Pb^{2+} sample when flowed through at the rate of 5 μL/s yielded as comparable peak current as 250 μL of 10 ppb Pb^{2+} sample when flow through at the rate of 2 μL/s. If the flow rate is too low (e.g., 1 μL/s) the preconcentration will take longer, leading to a longer total analysis time. Thus, 2 μL/s of flow rate was chosen for the preconcentration step.

Fig. 2 shows the signals of 10 ppb Pb^{2+} with varying adsorption times. The adsorption time was calculated as the sample volume (μL) divided by a constant flow rate (2 μL/s), minus the time required for the sample to reaching the electrode surface (20 s) via the connecting line. When the flow rate was kept constant, increasing the sample volume improved the Pb signals significantly initially and more slowly after 600 μL (or about 5 min). Since the long preconcentration time and the large sample volume were not desirable, 240 or 360 μL were chosen for subsequent studies. Nevertheless, the results show that the adsorption of Pb occurred instantaneously at the SAMMS-modified electrode. This corresponds to the previous kinetic study [3] that demonstrated that metal adsorption on SAMMS was a rapid process, thanks to the rigid and open-parallel pore structure and suitable interfacial chemistry of SAMMS.

SAMMS is composed of silica which is an electronic insulator, thus the desorption of the bound Pb^{2+} to the conductive carbon paste must occur for the electrochemical detection to be possible. Solution of 0.3 M HCl was found to be optimal in providing the necessary proton to displace the bound Pb^{2+} and the chloride ion for the proper function of Ag/AgCl reference electrode. With too low acid concentration, incomplete desorption may occur resulting in low signal, while with too high acid concentration, hydrogen evolution may occur resulting in high current that interfere which Pb^{2+} signal. The electrochemical cell with the flow-onto design permitted the flow of the acid directly onto the SAMMS-modified working electrode. Upon the onset of acid flow, a negative potential of -1 V was applied. As a result, desorption and cathodic electrolysis of Pb^{2+} occurred simultaneously with minimal

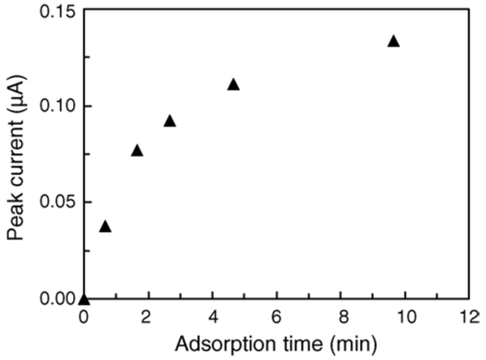

Fig. 2. Effect of preconcentration time (adsorption at open circuit) on the signals of 10 ppb Pb^{2+}, operating conditions as in Table 1.

Pb^{2+} lost with the acid stream. Simultaneous desorption and reduction of Pb^{2+} was proven previously in batch experiments using bulk acid solution [4] and minimal loss of Pb^{2+} was observed as long as the negative potential was applied to the working electrode immediately once being immersed in the acid solution.

Other parameters that have been optimized were related to cleaning of the electrochemical cell, the holding coil, and the sampling line. This optimization was a trade off between the cleaning time and completion of the cleaning. For example, complete removal of unadsorbed metal (Table 1, step 3) was necessary to ensure that only the metal ions that were selectively adsorbed by the functional groups of SAMMS contributed to the peak signal. Complete removal of the acid (Table 1, step 6) was also necessary otherwise the pH of the solution would be lower in the subsequent runs and would lead to lower adsorption (e.g., below pH 5) [3] and hence low metal signal [4].

3.2. *Evaluation of the system*

3.2.1. *Linearity*

The oxidation peaks of Pb were observed at −0.64 V. Fig. 3 shows a calibration curve in which the Pb signal was collected as a function of Pb concentration in the solution. The curve was adjusted for blank having a small peak for Pb (see Fig. 3, inset). At 3-min preconcentation time, excellent linearity ($R^2 = 0.995$) was obtained in the concentration range of interest (e.g., from 1 to 25 ppb). One ppb of Pb ion could be detected suggesting the high sensitivity of the device for Pb detection.

3.2.2. *Reproducibility and stability*

When a solid substrate electrode was used without physical polishing, "memory effect" at the electrode surface, may lead to the irreproducibility of the measurements [10]. Varying Pb concentrations from low (0 ppb Pb) to high (25 ppb Pb) and from high to low for a few cycles showed no significant "memory effect." In addition, rescanning after the stripping analysis showed no observed residual Pb peaks thus no electrode cleaning at positive potential was required. This is attributed to the stripping step being performed in acidic solution, thus once it was oxidized, Pb ions were completely removed and not re-adsorbed on the surface.

Without the "memory effect," the reproducibility of the measurements was achieved. Fig. 4 shows the voltammograms of 10 ppb of Pb^{2+} from seven consecutive measurements. At 2-min preconcentation period, the average Pb signal was 0.1 μA and the percent relative standard deviation (%R.S.D.) was 2.5%. Such %R.S.D. was extremely low compared to 5% R.S.D. (seven measurements) for the detections of 50 ppb Pb^{2+} after 2 min of preconcentration using batch operation [4]; the %R.S.D. is likely to be worse as Pb^{2+} concentration decreases. The extremely low %R.S.D. of 2.5% was attributed to automation of the analyzer, thus there was no human-error and/or batch-to-batch variation that may have contributed to large variability in the results.

The stability of the sensor was obtained after about five runs following the renewal of the electrode. In the first five runs, Pb signals consistently decreased due to depleting of the excess SAMMS from the electrode surface. However once stabilized, the sensor was stable for at least 90 measurements that were spread out over 5 days without surface renewal. The very strong covalent bonding of the functional groups to the silica substrate on SAMMS prevented electrode degradation due to lost of functional groups.

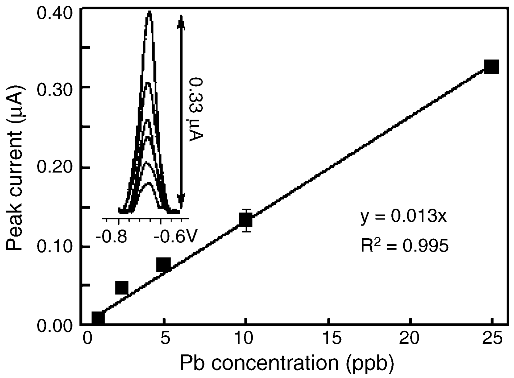

Fig. 3. Calibration curve for Pb detection after 3-min preconcentration period, other conditions as in Table 1. Inset shows the corresponding voltammograms.

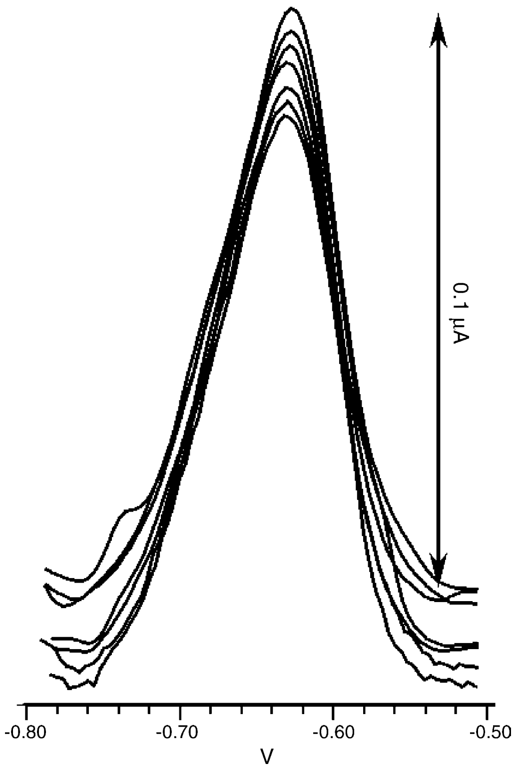

Fig. 4. Reproducibility of the signals of 10 ppb Pb^{2+} from seven consecutive measurements, 2-min preconcentration period, other conditions as in Table 1.

Table 2
Interference study for the detection of 10 ppb Pb^{2+}

Interference	Molar ratio per Pb	Pb peak area (nano-V.A) ± S.D.	Change (%)
None	0	10.03 ± 0.10	–
Ca	100	10.00 ± 0.28	−0.3
Ni	70	10.03 ± 0.38	0.0
Co	70	10.18 ± 0.83	1.5
Zn	70	10.07 ± 0.23	0.4
Mn	10	10.25 ± 0.31	2.2
Cd	1	11.65 ± NA	26.4
Hg	1	11.23 ± 0.36	12.0
Cu	1	18.26 ± 0.77	82.1

3.2.3. Interference study

Table 2 summarizes the interference effect of other metal ions on Pb detection. An ionic species can be an interference to the voltammetric detection of lead(II) if it can out-compete Pb(II) for the binding sites on SAMMS during the preconcentration step. From batch adsorption experiments, based on the distribution coefficient (K_d) values at pH 5.5 [3], Ac-Phos SAMMS had an affinity for metal ions in decreasing order as follows:

$$Pb^{2+} > Cu^{2+} > Mn^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+} > Ni^{2+} \sim Ca^{2+} \gg Na^{+}$$

Based on the affinity series, Ca, Zn, Ni, Co, and Mn could not complete with Pb for the binding sites. This corresponds to results reported in Table 2 where these metal ions did not interfere with Pb signals even when they were present at much higher molar concentrations (e.g., 100-fold for Ca, 70-fold for Zn, Ni, Co, and 10-fold for Mn).

On the other hand, cadmium (Cd) did interfere with Pb detection because of partial overlap of the oxidation peaks of both Cd and Pb. The hand-held CHI1232 potentiostat did not resolve both peaks as well as did the desktop potentiostat (i.e., CHI660A) as reported in our previous work [4]. Although the hand-held potentiostat is necessary for the portability of the analyzer, it has some compliance voltage limitation (vendor information) which led to the poor resolution of Pb and Cd peaks. Improvements in the compliance voltage for the CHI hand-held potentiostat are currently under development (vendor information) and when available will improve the simultaneous detection of Cd and Pb.

Copper (Cu) and mercury (Hg) were found to increase the sensitivity of the Pb signals. Minimization of the interference from Cu can be accomplished by depositing a thin-film of mercury prior to preconcentration of Pb at the electrode surface. For example, once 1 ppm of Hg in 0.5 M HCl was deposited on the electrode for 5 min prior to the preconcentration of Cu and Pb ions in a solution containing one-to-one molar ratio of Cu to Pb ion, the Cu interference was reduced by 60%. On the other hand, Pb and Cd did not interfere with copper detection [4]. Further study is needed to explain the copper interference phenomena.

4. Conclusion

This work is a significant milestone for the development of a fully automated and portable toxic metal analyzer. In the past, we reported the development and evaluation of nanostructured materials (Ac-Phos SAMMS) [3], and the applications of Ac-Phos SAMMS-modified electrodes in batch experiments [4]. This work integrated the sequential injection system and nanostructured electrochemical sensors and evaluated it for Pb detection. We also reported some limitations for the field applications where the detection interference from a certain metal species could occur. Nevertheless, the new results confirmed our previous work that SAMMS-modified sensors were highly selective and sensitive for target species (e.g., Pb). It was also robust and reliable in the sequential injection analyzer. The portable analyzer typically takes 6 min to analyze one sample and can be remotely controlled for field applications. Future study will be focused on the detections of other important toxic metal ions since the interfacial chemistry of SAMMS have been successfully tailored at PNNL to be specific for lanthanides, actinides, other heavy metal ions, and oxometallate, anions (e.g., chromate, arsenate, and pertechnetate) [11–20].

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