Technical Report

Ohio Lake Erie Protection Fund
Small Grant Program
Project Number: SG 385-10

"Improving Detection Limit of Phosphate Microsensor"
March 2010

Abstract

The main goal of this project was to improve the sensitivity (detection limit) of phosphate micro sensors for *in-situ* soluble reactive phosphorus (SRP) measurement. In this study, surface cobalt-based sensors were re-modified, characterized and tested to improve detection limits for phosphate. The effectiveness of surface modification on the performance of a modified cobalt-based microelectrode was fully examined for its characteristics: including detection limit, response time, selectivity, interference with ions (sulfate, nitrate, and ammonia) and dissolved oxygen (DO). The sensor was characterized and tested with various environmental samples with different concentration range to monitor field applicability of a sensor. To assess the performance of the sensors for real environmental applications, emphasis was also placed on monitoring phosphate release from Lake Erie sediments (internal SRP loading from sediments) with the sensors. After increasing phosphate sensing area and re-modifying surface, phosphate sensors showed increased detection capacity up to 1 ppb (mostly around 50-100 ppb) concentration of phosphate ion. Re-modified phosphate sensors showed improved sensitivity and could be applied to both water and sediment samples. However, signal interferences (especially with oxygen) needed to be considered for sample analysis with phosphate microsensors.

Activities and Timeline

Quarter 1: (March - May 2010): Modified sensor fabrication methods to improve sensor sensitivity (detection limit) and ruggedness for field application; collected samples from different sources (lake water, wastewater and sediments) and test sensor performances with environmental samples.

Quarter 2: (June - August 2010): Tested selectivity of sensors (interference tests with other ions such as sulfate, nitrate, nitrite, dissolved oxygen): collected water, wastewater treatment plant effluent and sediments samples; monitored and compared performance of phosphate microsensors with other conventional phosphate analysis methods.

Quarter 3: (September - November 2010): Monitored internal loading of soluble reactive phosphorus (SRP) from Lake Erie sediments under different environmental condition.

Quarter 4: (December - February 2010): Prepared final report and peer reviewed journal manuscript; communicated results with other institutes

Project Deliverables

Ding, X. and Seo, Y. Characterization and application of phosphate microsensors, 2nd Annual Midwest Graduate Research Symposium, March, 2011, Toledo, Ohio, USA

Ding X. and Seo, Y. Characterization and application of cobalt-based phosphate microsensors, AEESP Research and Education Conference, July, 2011 (Submitted and In review)

Ding X., Gruden, C, and Seo, Y., Application of phosphate microsensors to monitor phosphate release from the sediment, Chemosphere (In preparation)

Introduction

As a result of releasing anthropogenic polluted sewage effluent and agricultural fertilizers into the water or soil table, phosphorus is considered a major concern to universal environmental managers as it contributes to the eutrophication of the water body [1, 2].

The soluble reactive phosphorus (SRP) is largely comprised of the inorganic orthophosphate (PO4) form of phosphorus. SRP enters the environment from different sources such as fertilizers, detergents, and wastewater treatment plant discharge. While significant efforts have been made to control erosion of nutrient rich agricultural sediment loading into the Lake Erie watershed, controlling SRP from nonpoint pollution sources is still a significant concern as it is directly ingested by algae and accelerates eutrophication. Recent reviews of phosphorus loading to Lake Erie have shown that SRP loading deposition trends are greatly different from those of particulate loading. The Lake Erie Phosphorus Task Force also reported that algal proliferation trends in Lake Erie appear to match the trends of dissolved reactive phosphorus loading much more closely than those of total phosphorus or particulate phosphorus loading. Thus, a great need exists to carefully monitor SRP loading into water bodies to protect surface water, Ohio tributaries and Lake Erie from unwanted algae proliferation.

 To measure SRP, the ascorbic acid method described in Standard Methods has been widely used. However, in order to control or minimize SRP loading from various nonpoint sources, *in situ* monitoring of SRP can provide many advantages over traditional monitoring methods. Thus, the demand to develop fast, sensitive and versatile sensing devices for SRP monitoring has greatly increased.

In his previous study, PI successfully fabricated and characterized cobalt wire based phosphate sensor with small tip diameter (5-10 um). The sensor was successfully employed to monitor phosphate ion concentration changes in activated sludge floc under different redox condition for enhanced biological phosphorus removal (EBPR) process. However, the sensor showed limited field application capability beyond samples from wastewater with high phosphate concentration. The reliable detection limit of the phosphate sensor was to be high (500 ppb) for surface water samples, although sediment sample analysis as compared to conventional surface water has less than 50 ppb of SRP).
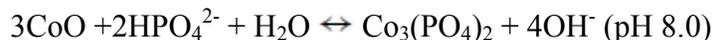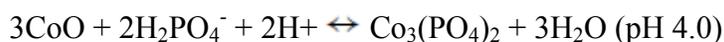
The main goal of this project was to improve the sensitivity (detection limit) of phosphate micro sensors for *in-situ* soluble reactive phosphorus (SRP) measurement. In this study, surface modified cobalt-based sensors were re-modified, characterized and tested to improve detection limits for phosphate (< 50ppb). A new fabrication method using polymer based sealing was also tested to provide increased durability for the phosphate sensors.

The effectiveness of surface modification on the performance of a modified cobalt-based microelectrode was fully examined for its characteristics: including detection limit, response time, selectivity, reproducibility, life time, interference with ions (sulfate, nitrate, and ammonia) and dissolved oxygen (DO). The sensor was characterized and tested with various environmental samples with different concentration range (lake water, wastewater, and sediment samples) to monitor field applicability of a sensor. To assess the performance of the sensors for real environmental applications, emphasis was placed on monitoring SRP release from Lake Erie sediments (internal SRP loading from sediments) with the sensors.

Materials and Methods

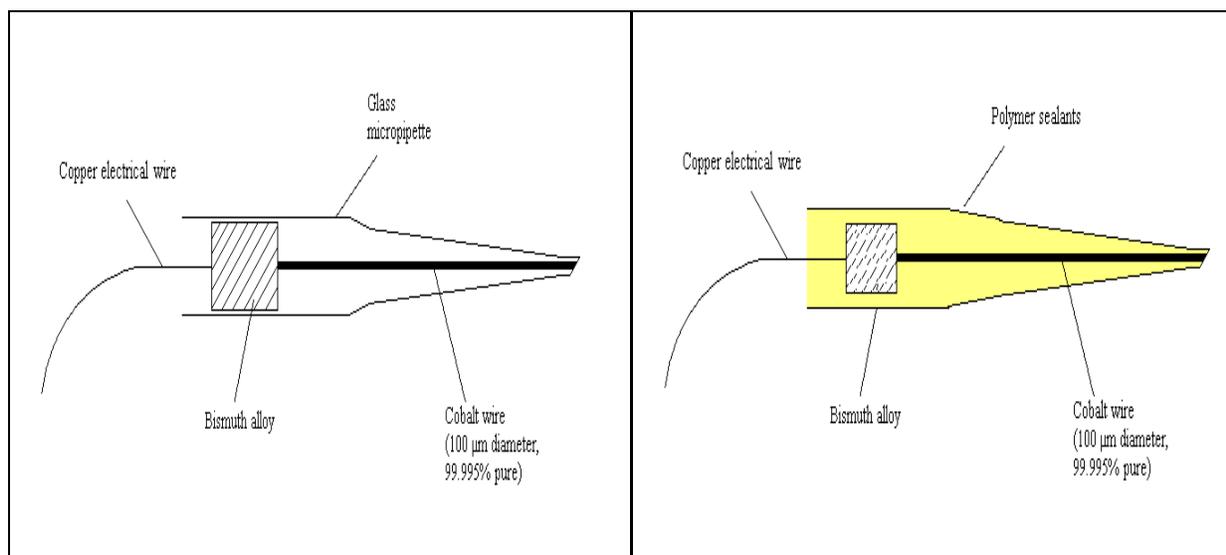### *Phosphate Sensor Fabrication*

The basic phosphate detection mechanism of the cobalt based phosphate sensor measure the voltage output response to different phosphate concentration. When a cobalt wire first contacts with water, a cobalt oxide film is formed on surface of the sensor tip. Subsequently, multiple reactions occur between cobalt oxide and phosphate and cobalt phosphate layer is formed.

$$3CoO + 2H_2PO_4^- + 2H+ \leftrightarrow Co_3(PO_4)_2 + 3H_2O \text{ (pH 4.0)}$$

$$3CoO + 2HPO_4^{2-} + H_2O \leftrightarrow Co_3(PO_4)_2 + 4OH^- \text{ (pH 8.0)}$$

$$3CoO + 2PO_4^{3-} + 3H_2O \leftrightarrow Co_3(PO_4)_2 + 6OH^- \text{ (pH 11.0)}$$

In order to construct the phosphate sensor, two fabrication methods were considered. First, previously adapted glass pipette barrels were used and tested for phosphate sensor fabrication. Alternatively, a new type of phosphate sensors was fabricated with polymer based sealants. For glass pipette based sensor fabrication, glass pipette barrels (O.D.: 1.2mm, I.D.: 0.69mm, 15cm length, Sutter instrument Co.) were purchased, heated and pulled over the flame. After pulling

barrels, a section of cobalt wire (0.1mm diameter, 99.995% pure, Aldrich Chemical Company) was inserted into the pulled glass micropipette. The micropipette was then melted in the middle part section using a trough heating filament (Sutter instrument Co.) to completely seal the cobalt wire in the glass pipette barrel. The tip of the sensor was then beveled using a diamond abrasive plate (Sutter instrument Co.) to hone the sensor to a 45° angle and expose the cobalt surface. Then the microelectrode was connected to an electrical wire by melting a small section of bismuth alloy (44.7% bismuth, 22.6% lead, 19.1% indium, 8.3% tin and 5.3% cadmium) [23]. Figure 1 shows the configuration of the phosphate microelectrode.

Figure 1. The structure of phosphate sensor: left) with glass pipette: right) with polymer sealant

To fabricate a new type of phosphate sensor utilizing polymer sealants, cobalt wires were dipped in a polymer solution and then the polymer layer was solidified and beveled to expose cobalt surface. During all the experiments, the potential between the working microelectrode and reference electrode was monitored using a pH /mill volt (mV) meter (Model 250, Denver instruments). An Ag/AgCl reference mini-electrode (MI-401, Microelectrodes Inc.) was used as the reference. The pH/ mV meter was connected to a computer and data was acquired with a software Balance Talk SL TM (Labtronics Inc.) spread logger to record responses in five second intervals.

After phosphate sensors were fabricated, the sensors were pretreated prior to calibration. In detail, sensors were first immersed into DI water to form a cobalt oxide (CoO) layer on the surface of sensor tips. After reaching a stable potential, the sensors were removed from DI water and immersed into 10^{-4} M KH_2PO_4 solution at pH 7 until a new steady-state potential was observed.

After the pretreatment, the phosphate sensors were calibrated. Several phosphate standard solutions were prepared with different concentrations. The experiments were conducted under condition of ambient oxygen levels and room temperature.

Ion interference tests

Considering the co-presence of other ions in natural environment and their interference on phosphate sensors performance, sulfate, nitrite, and nitrate ions were used to investigate potential ion interferences on the phosphate sensors. For each ion interference experiment, interfering ions were kept constant as phosphate ion concentrations were varied from 10^{-3}M to 10^{-6}M. Ion interference tests were also conducted by keeping the phosphate ion concentration constant while interfering ions were varied. Table 1 shows tested ions and the ranges of tested ion concentration. The concentrations of three interfering ions were selected based on a literature review [27]-[36].

Table. 1. Concentrations used for ion interference measurements.

Ion	Reagent	Concentration Range (mg/L)
Sulfate (SO_4^{2-})	$K_2 SO_4$	0.5 – 30
Nitrate (NO_3^-)	$Na NO_3$	0.3 – 2
Nitrite (NO_2^-)	$Na NO_2$	0.1 – 4

Sensor Applications to Environmental Samples

Performance of fabricated sensors was first evaluated with water samples. Tested samples include effluent from a wastewater treatment plant and lake water samples. Sensors were also tested with sediment samples. Sediment samples were collected from Lake Erie by Dr. Tomas Bridgeman's research group. The three sediment samples were taken from the same sampling

site in the Lake Erie in different month. Upon collection of the sediment samples, the sediment samples were taken, the sediment samples were sealed in plastic containers and transferred directly to a laboratory, where they were kept in a refrigerator for further sensor tests and phosphorus analysis. The total phosphorus (TP) and soluble reactive phosphorus concentrations in the three sediment samples were tested by Heidelberg Water Quality lab.

In order to monitor SRP release from sediments with the phosphate sensors, sediment samples were carefully transferred into a beaker and then saturated with sampled lake water. The depth of the sediment sample was approximately 3 cm and the depth of lake water was approximately 5 cm. The beaker was placed under a microscopy to increase sediment surface resolution for sensor application. The phosphate sensors were connected to a manipulator, which enhanced precise vertical movement of sensors. The position of the phosphate microsensors was slowly adjusted to avoid disturbing the sediment structure. During the profiling process, the Ag/AgCl reference electrode was kept in a static position. The concentration of phosphorus in lake water was measured using both phosphorus microelectrode and Ion Chromatography (IC-1200, Dionex Company) to verify the performance of the sensors.

Results and discussion

Phosphate microelectrode calibration

Figure 2 shows the calibration curve of a phosphate sensor. Eight different concentrations of standard phosphate solutions were prepared with a range of 10^{-8} to 10^{-1}M KH_2PO_4 for sensor calibration. After increasing the phosphate sensing area and re-modifying the surface, phosphate sensors showed increased detection. The detection threshold was reduced up to 1 ppb (mostly between 50 and 100 ppb) concentration of phosphate ion. Also, the phosphate microelectrodes linearly responded to phosphate concentration changes with a slope of -32.9 mV/ decade. Figure 3 shows the response changes of sensors over time. During the calibration, the response time of sensors for each standard point could be as low as 5 seconds and usually with an upper limit less than 1 min except to 10^{-8} M phosphate solution. The lowest test condition (10^{-8} M) required around 100 seconds for sensors to be stabilized.
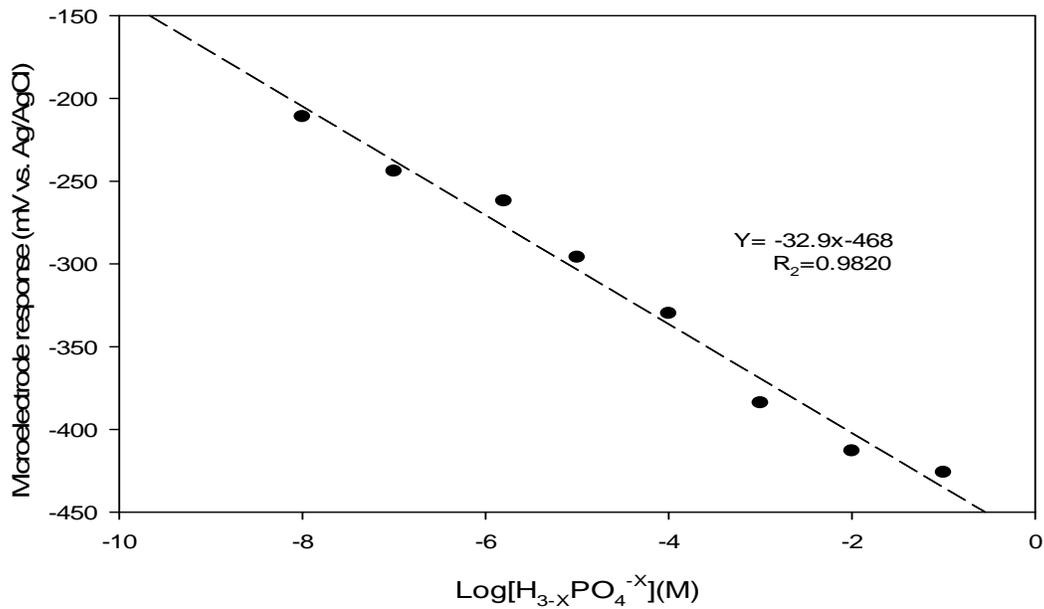
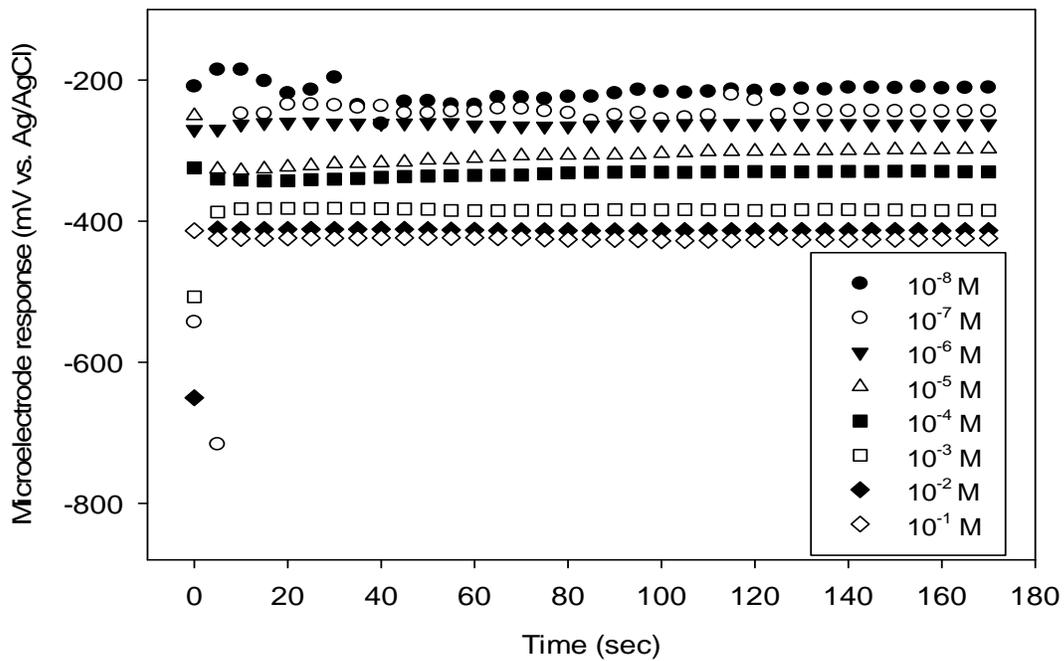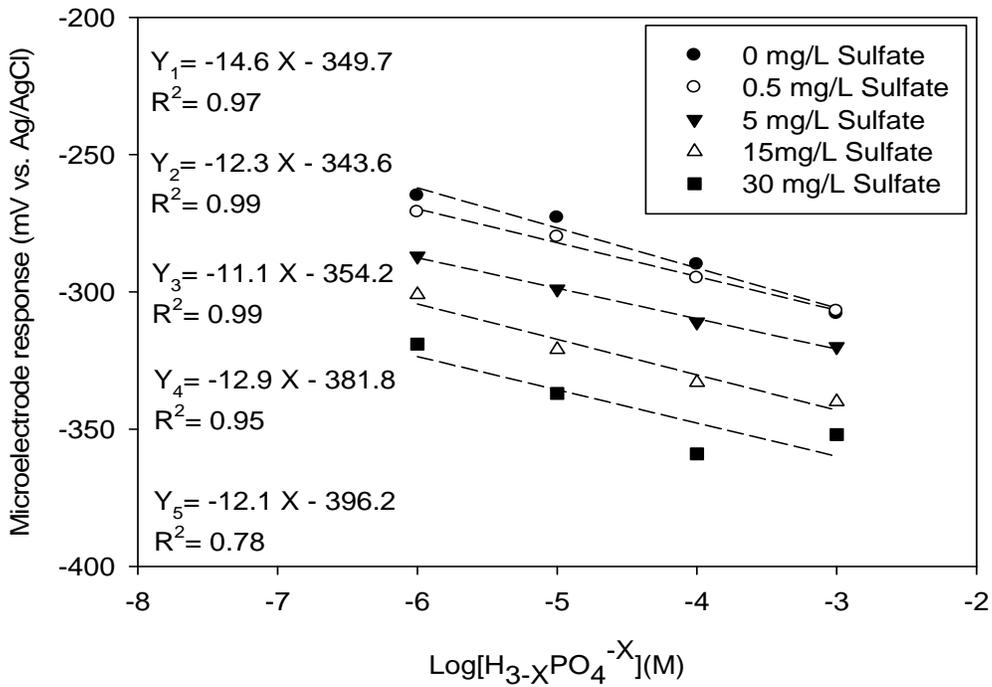**Figure 2. Calibration curve of a phosphate microsensor**

Figure 3. Response changes of sensors over time

Ion interference

Three ions (the sulfate, nitrite, and nitrate) commonly found in environmental samples were tested to evaluate the performance of phosphate microsensors under the presence of ions. The concentrations of three interfering ions were selected based on a literature review. Figures 4, 5, and 6 show the ion interference tests results. Compared to calibration curves generated in the absence of an interfering ion, the presence of ions changed the electrode signal responses toward phosphate ions. However, all calibration curves still exhibited a linear response to phosphate ion and the signal response shift resulting from tested ion interference appeared to be minor.

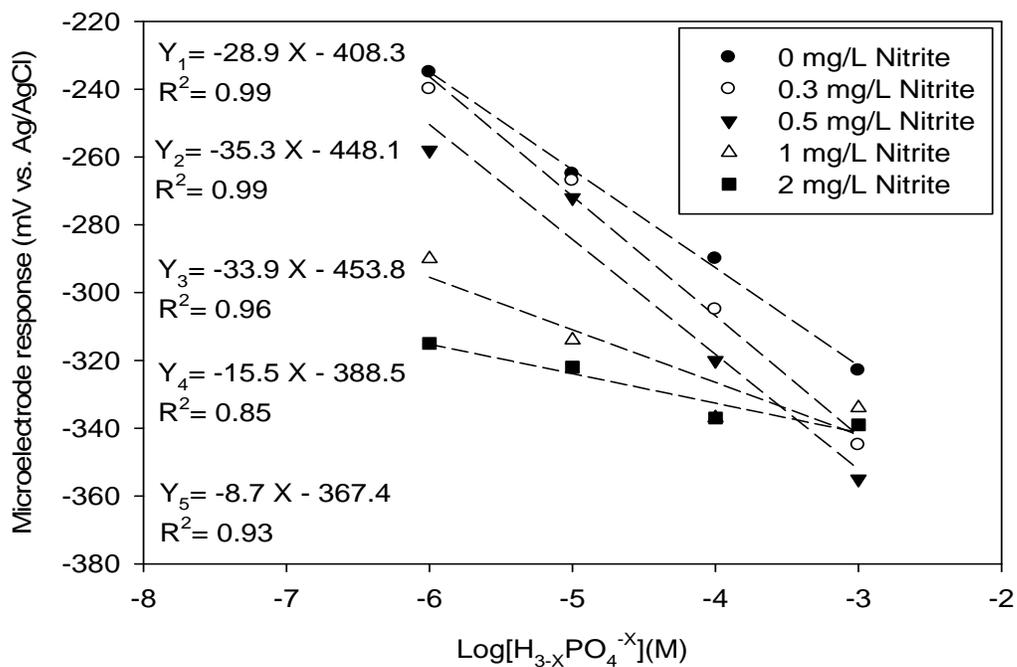**Figure 4. Sulfate ion interference**
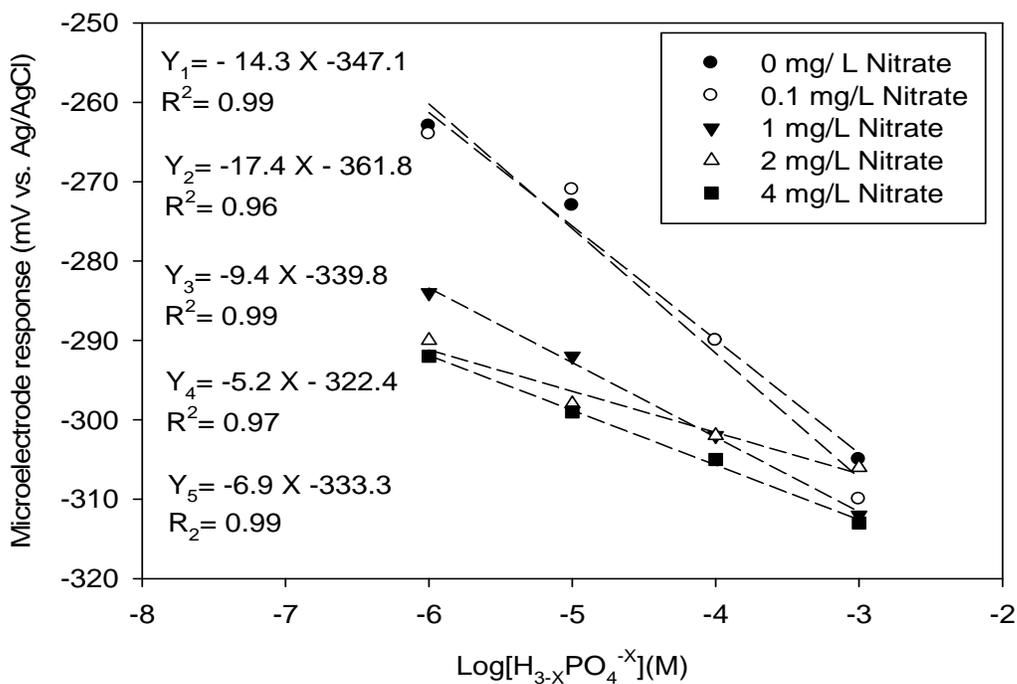
Figure 5 Nitrite ion interference

In figure 5, plots of Microelectrode response (mV vs. Ag/AgCl) against $\text{Log}[H_{3-x}PO_4^{-x}](M)$ with the following equations:

$Y_1 = -28.9\,X - 408.3$, $R^2 = 0.99$
$Y_2 = -35.3\,X - 448.1$, $R^2 = 0.99$
$Y_3 = -33.9\,X - 453.8$, $R^2 = 0.96$
$Y_4 = -15.5\,X - 388.5$, $R^2 = 0.85$
$Y_5 = -8.7\,X - 367.4$, $R^2 = 0.93$

Legend: 0 mg/L Nitrite, 0.3 mg/L Nitrite, 0.5 mg/L Nitrite, 1 mg/L Nitrite, 2 mg/L Nitrite

Figure 6 Nitrate ion interference

In figure 6, plots of Microelectrode response (mV vs. Ag/AgCl) against $\text{Log}[H_{3-x}PO_4^{-x}](M)$ with the following equations:

$Y_1 = -14.3\,X - 347.1$, $R^2 = 0.99$
$Y_2 = -17.4\,X - 361.8$, $R^2 = 0.96$
$Y_3 = -9.4\,X - 339.8$, $R^2 = 0.99$
$Y_4 = -5.2\,X - 322.4$, $R^2 = 0.97$
$Y_5 = -6.9\,X - 333.3$, $R_2 = 0.99$

Legend: 0 mg/L Nitrate, 0.1 mg/L Nitrate, 1 mg/L Nitrate, 2 mg/L Nitrate, 4 mg/L Nitrate

Dissolved Oxygen (DO) interference

Figure 6 shows signal response changes of the phosphate microsensor under different DO concentrations. Two different oxygen concentrations (0% and 10 %) were tested to monitor dissolved oxygen interference on the performance of phosphate sensor, while other test conditions such as phosphate concentration, pH, temperature, and distance between reference electrode and the phosphate microsensor were fixed. From experimental results, a 40 mV overall signal response differences was observed between 0% and 10% DO concentration. While ion interferences on phosphate sensor performance were not significant (signal shifts were relatively large at very low phosphate concentration), phosphate sensor signals shifted for all levels of phosphate solution concentration under 10% DO. These results indicated that oxygen interferes with the binding mechanism between cobalt oxide and phosphate, subsequently decreasing the sensitivity of sensors to phosphate ion [23]. In high phosphate concentration range, signal shift may cause a significant difference in phosphate concentration measurement. It is suggested that the development of calibration curves under different DO concentration as well as DO measuremenst for samples are needed for phosphate sensor applications.
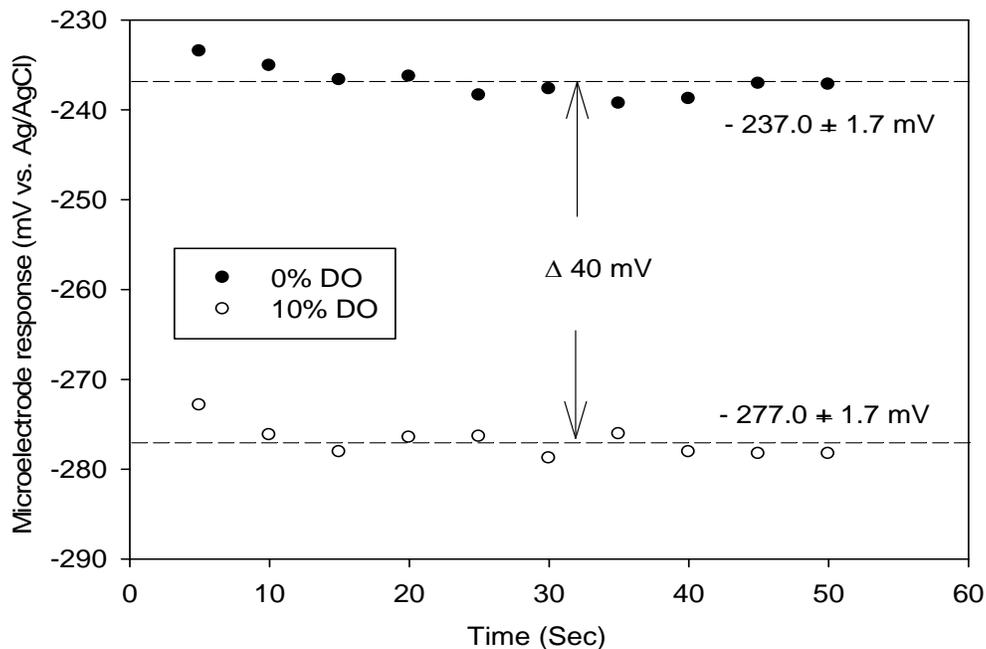
Figure 7 Dissolved oxygen interference

Sensor Applications to Environmental Samples

After calibrating the phosphate microsensor, sensors were tested with various environmental samples. First, sensors were tested with water samples. Tested water samples included wastewater treatment plant effluent and lake water samples. To validate the accuracy of the phosphate sensor, the phosphate concentration in the samples were also measured with an ion chromatography system (ICS-1000, Dionex Inc). Table 2 shows phosphate measurement comparisons. Some samples had very high ion concentration (with 541 and 241.1 mg/L sulfate concentration), which interfered phosphate measurement with the sensors. However, overall, obtained results with various water samples showed that the microsensors possess good accuracy to detect phosphate ion in sample solution, especially after DO inference was properly corrected.

Table 2. Phosphate measurement comparison

| Sample Source	Ion concentration (mg/L) (IC generated)				DO (mg/L)	Phosphate concentration (mg/L)	
	Nitrite	Bromide	Nitrate	Sulfate		Ion Chromatography	Phosphate sensor
Wastewater Plant	0	0	0	6.6	5.6	1.26	1.12*
Wastewater	0	1.15	70.12	241.1	5.5	0.1	Couldn't detect due to signal interference
Surface water	0	6.29	30.8	74.3	4.7	0.2	0.1*
Lake water	1.8	1.8	1.8	2.7	5.1	2.66	2.58*
Lake water	0	0	0	541.3	5.1	3.57	2.27*

* without data conversion for ion and oxygen interference

Phosphate sensors were also tested with lake sediment samples to estimate the applicability of phosphate sensors for SRP concentration changes in sediments. The three sediment samples were taken from the same sampling site in the Lake Erie in different months. Table 3 shows SRP and TP concentration in sediments.

Table 3. SRP and TP concentrations in test sediment samples

|	**23-Jun**	**6-Aug**	**14-Sep**
SRP (mg/g dry weight)	0.0959	0.1167	0.0956
TP (mg/g dry weight)	0.9698	0.9855	0.9456

Figure 8 shows experimental setup for phosphate sensor application to sediment samples. Before measuring SRP profile in sediment using phosphate microelectrode, preliminary experiments were conducted to monitor whether the distance between a phosphate microsensor and a reference electrode can affect SRP measurement. The preliminary test results indicated that different distances between a phosphate microsensor and a reference electrode resulted in the different responses of the sensor for sediment profiling. However, sensor signal shift was negligible whether a reference electrode was dipped in sediment samples or in bulk solution phase. In order to obtain reproducible and consistent sensor responses, the position of the reference electrode was kept secured at the same position during all the sediment SRP profiling experiments. Also, the distance between the phosphate microsensors and the reference electrode was kept constant.
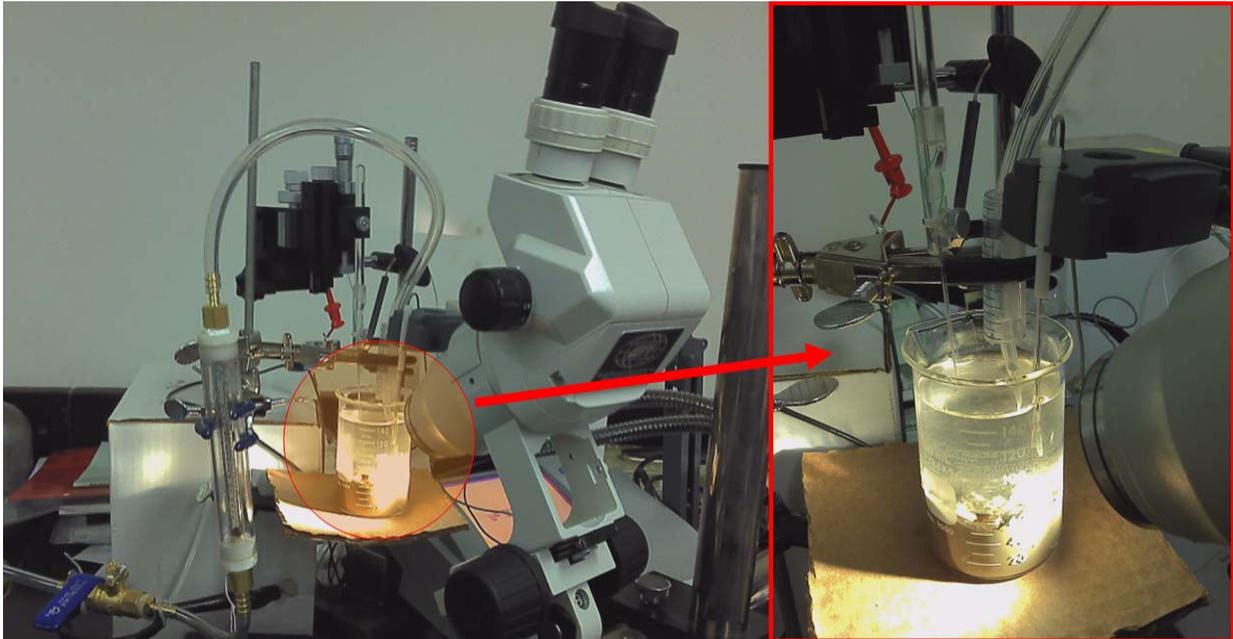
**Figure 8. Experimental setup for phosphate sensor application to sediment**

For sediment SRP profiling, a microscope was used to identify the interface between water column and sediment. Under the microscope, the microsensor was carefully positioned to 0.1 mm above the surface of the sediment using a micromanipulator. After the potential stablized above the sediment surface, the phosphate microelectrode was moved down into the sediment at 0.1 mm intervals each time and potential changes were automatically recorded. The profiling experiment collected data from the sediment surface (0 mm) to a 3.0 mm depth in the sediment sample.

Figure 9 shows the changes of potential signal in the sediment sample. First, the phosphate profile was measured without controlling dissolved oxygen (DO) level. The DO concentration in bulk solution as measured by a DO meter showed less than 1% of oxygen concentration in bulk solution. As the microelectrode penetrated into the sediment, the phosphate concentration began to increase. The surface phosphate concentration of the sediment was approximately $10^{-3.6}$ M while the phosphate concentration at the depth of 3 mm was about $10^{-1.2}$ M, nearly 250 fold of the surface concentration.
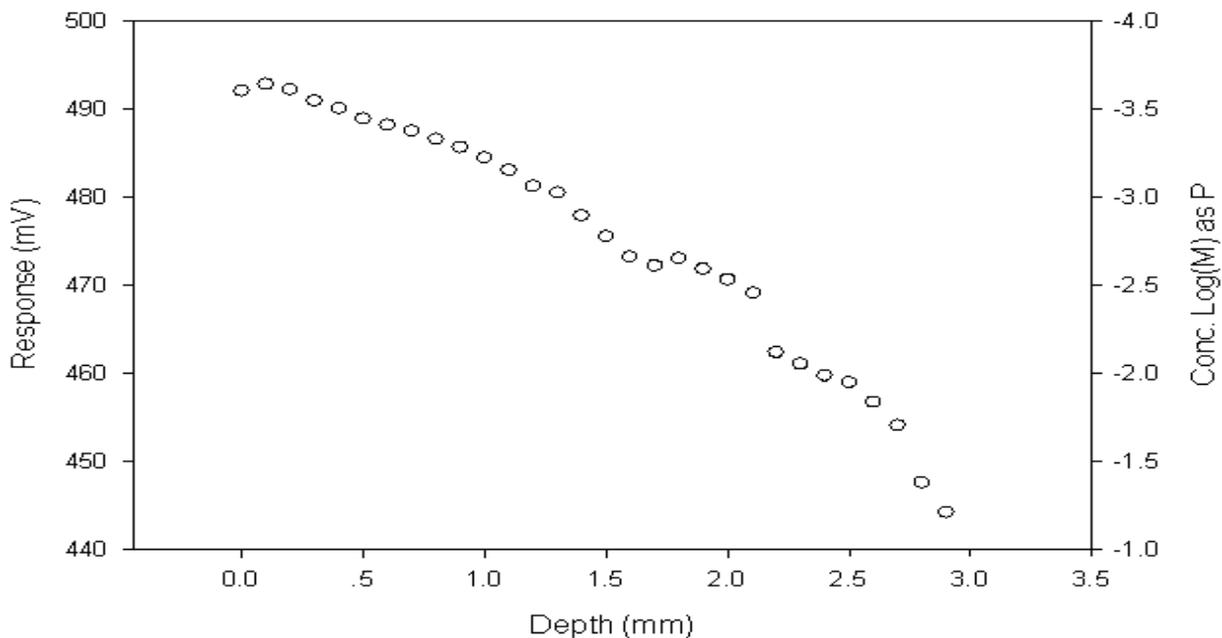
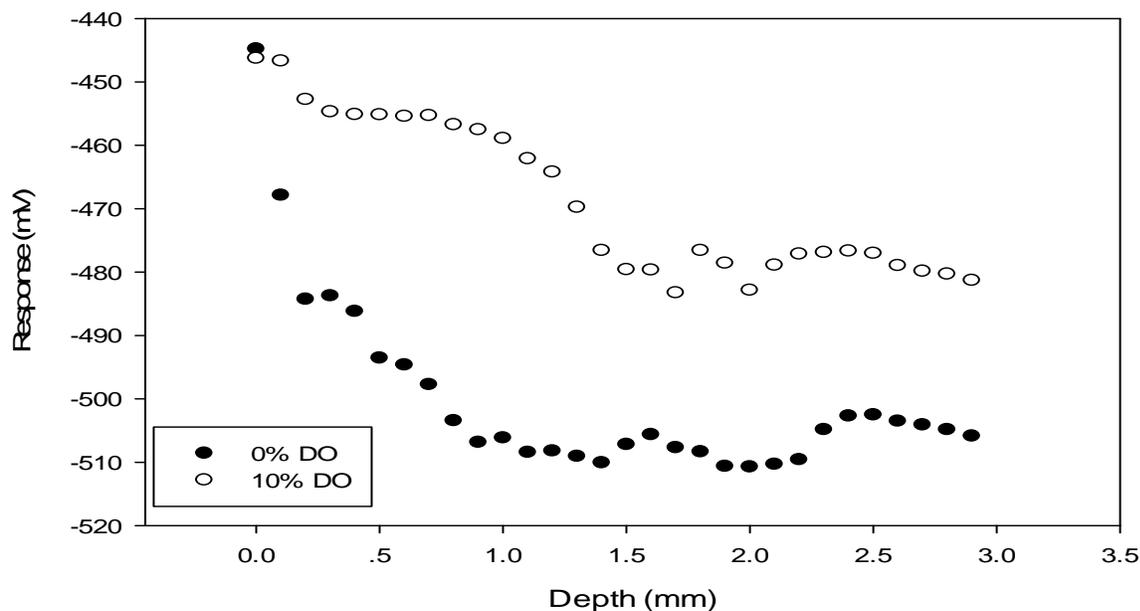**Figure 9. Sediment profiling using a phosphate microelectrode (without DO control)**

To determine the versatility and reproducibility of phosphate sensors performance for SRP profile measurement in sediment, measurements were conducted during both penetration and withdrawal of the sensors from the sample, then signal responses were compared. Figure 10 shows the measured phosphate profile in sediment. There were no significant differences between the inserting process and withdrawing process, which indicated phosphate microsensors can monitor local phosphate concentration changes in sediment samples without disturbing sediment structure.
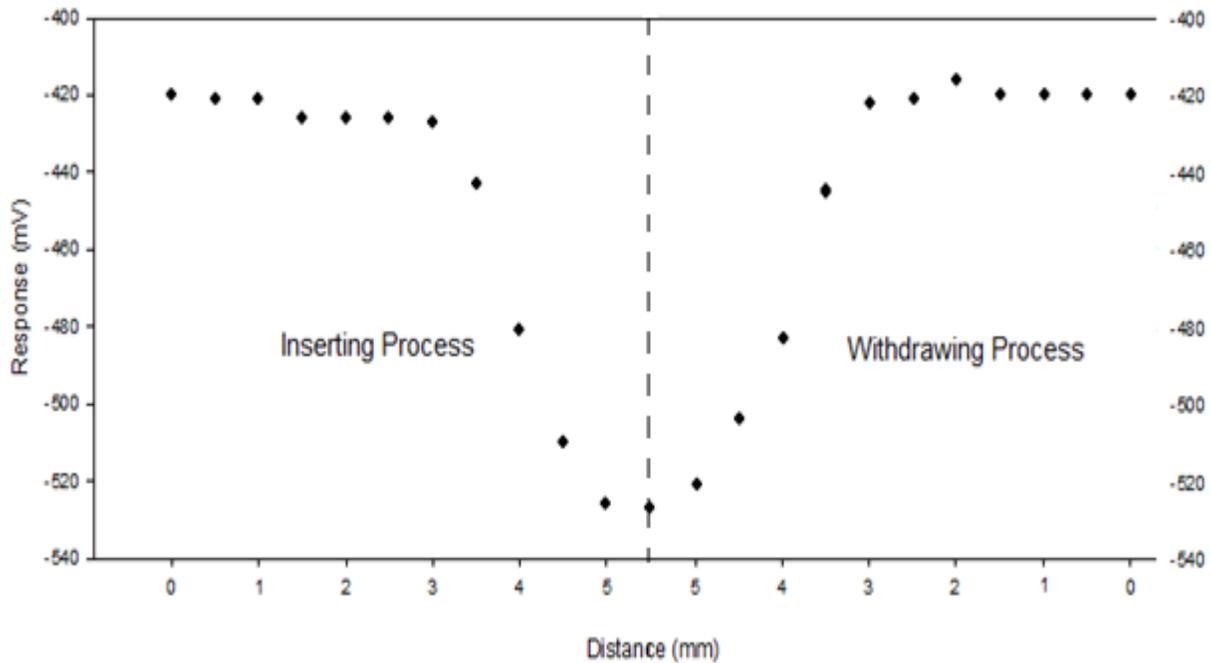
Figure 10. Phosphate profiles in sediment

Sediment profiling experiments were also conducted under controlled DO conditions (0% DO and 10% DO concentration). To create specific oxygen levels in the water column, lake water was bubbled with 10% oxygen or nitrogen gas while monitoring oxygen level with a DO electrode. The profiling procedure was the same as the profiling experiment without DO control in the previous experiment. As shown in Figure 9, the surface concentration of phosphate was similar. As depths increased, the phosphate concentration under 0% DO concentration is obviously higher than the phosphate concentration under 10% DO concentration. This difference

might occur because at higher oxygen levels, oxygen can penetrate several millimeters into the sediment surface [25]. Some biological and chemical processes are involved to reduce phosphorus under oxic condition.   Phosphorus concentration in sediment decreased [11]. Based on the obtained phosphate profile under different oxygen concentrations, it appeared that phosphate microsensors need to be accompanied by DO measurements with dissolved oxygen microsensor for precise measurement of phosphate in sediment samples.

Figure 9. Sediment profiling under 0% and 10% DO concentration

Conclusions

In this study, surface modified cobalt-based sensors were re-modified, characterized and tested to improve detection limits for phosphate. The effectiveness of surface modification on the performance of a modified cobalt-based microelectrode was fully examined for its characteristics: including detection limit, response time, selectivity, interference with ions (sulfate, nitrate, and ammonia) and dissolved oxygen (DO). After increasing phosphate sensing area and re-modifying surface, phosphate sensors showed increased detection capacity up to 1 ppb (mostly around 50-

100 ppb) concentration of phosphate ion. However, signal interferences (especially with oxygen) needed to be considered and properly addressed for sample analysis with phosphate microsensors. The response time is less than 1 min with a wide detection range of range 10^{-8} to 10^{-1} M. Overall, the successful characterization and application of re-modified microsensors showed that that the sensors can be a very useful tool to measure phosphate changes in lake water and sediment samples.

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