*Anal. Chem.* **2003,** *75,* 3915−3918

# Photonic Crystal Optrode Sensor for Detection of $Pb^{2+}$ in High Ionic Strength Environments

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**We developed an optrode sensing device that utilizes a polymerized colloidal array (PCCA) photonic crystal material. This array diffracts light in the visible spectral region due to the periodic spacing of colloidal particles. The PCCA changes diffraction wavelength due to binding of $Pb^{2+}$ to an 18-crown-6 ether molecular recognition agent. This optrode consists of a probe assembly that contains the PCCA $Pb^{2+}$ sensing film. An inexpensive, commercial diode array spectrometer and a fiber-optic reflectance probe monitors the wavelength of light back diffracted by the PCCA. Liquid inlet and outlet connections are provided to introduce the sample solution and to exchange out nonbinding ions. In low ionic strength solutions, diffraction wavelength shifts are actuated by the binding of the $Pb^{2+}$ to the crown ether to immobilize the $Pb^{2+}$ counterions. In these low ionic strength solutions, a Donnan potential forms to cause an osmotic pressure, which swells the PCCA in proportion to the number density of bound $Pb^{2+}$. This Donnan potential disappears at high ionic strengths. Thus, no response of the PCCA occurs. Our optrode design allows for the fast removal of nonbound ions from the PCCA by washing with pure water. Since the bound $Pb^{2+}$ ions have a slow off rate from the crown ether, the bound $Pb^{2+}$ PCCA diffraction transiently red shifts during washing, directly in proportion to the sample $Pb^{2+}$ concentration. This transient diffraction red-shift can be used to quantitatively determine $Pb^{2+}$ concentrations in high ionic strength solutions such as bodily fluids.**

There is a great need for sensors for metal ions in aqueous solutions. There are numerous important environmental applications such as monitoring the purity of water supplies, as well as clinical applications such as monitoring metals in bodily fluids. Our group has been developing a highly sensitive technology for sensing metal ions in aqueous solution.[1] Our approach utilizes a polymerized crystalline colloidal array (PCCA) photonic crystal which contains a molecular recognition agent.[1] These PCCA consist of a mesoscopically periodic array of colloidal particles[2] (generally fcc) polymerized into an acrylamide hydrogel.[1−3] This array diffracts light in the visible spectral region due to its ∼200-nm fcc colloidal particle lattice constant. We attach molecular recognition agents to the hydrogel, which interact with an analyte to cause the PCCA to shrink or swell. This actuates a lattice constant change that shifts the PCCA diffraction wavelength in proportion to the analyte concentration.

We have demonstrated two modes of sensing, by changing hydrogel cross-linking[4,5] or by immobilizing ions on the hydrogel.[1,6] The cross-linking motif was demonstrated for glucose sensing,[4] where a single glucose molecule bound two boronic acid molecules attached to the hydrogel to form a cross-link. These cross-links increased the elastic constant of the hydrogel, which caused it to shrink. We also demonstrated[5] that metal ions such as $Cu^{2+}$ would form cross-links between 8-hydroxyquinoline groups bound to the hydrogel.

The alternative sensing motif utilizes the formation of an ionic hydrogel where molecular recognition is used to attach charged groups to the hydrogel. We have demonstrated[1] the use of a crown ether group attached to the hydrogel to bind $Pb^{2+}$. The binding of a charged group results in the immobilization of its counterion, which results in the formation of a Donnan potential.[6,7] The Donnan potential results in an osmotic pressure, which causes the gel to swell. The resulting change in the particle array lattice constant increasingly red-shifts the diffracted light wavelength as the solution $Pb^{2+}$ concentration increases. Unfortunately, the magnitude of the shift decreases as the solution ionic strength increases and there is little sensor response[6] for solutions with NaCl concentrations of greater than 10 mM.

We recently demonstrated[8] that we could sense $<0.5\ \mu M$ $Pb^{2+}$ concentrations even at the high ionic strengths of bodily fluids by utilizing a transient response approach. The transient response method consists of incubating the IPCCA with the solution to be determined followed by washing the hydrogel with pure water. When the nonbound ions wash out of the hydrogel, the remaining bound $Pb^{2+}$ ions give rise to a Donnan potential resulting in an

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10.1021/ac034276b CCC: $25.00 
Published on Web 06/26/2003

osmotic pressure, which swells the hydrogel and red-shifts the diffraction wavelength. The magnitude of the diffraction wavelength shift depends on the number of bound ions, which is a function of the concentration of $Pb^{2+}$ in the original high ionic strength solution. The work herein utilizes this sensing motif and develops an optrode sensor to measure the magnitude of the transient response to determine $Pb^{2+}$ in high ionic strength solutions.

$Pb^{2+}$ is an important environmental toxin, which can cause disease and death at concentrations as low as 700 ppb in blood.[9] Concentrations as low as 100 ppb or 500 nM may be correlated with decreased IQ in children. Universal screening of children for lead was enunciated as a public health goal by the PHS and CDC in 1991. While this was the goal, universal lead screening was abandoned by the federal government in 1997 for economic reasons, despite the large numbers of children at risk. A rapid point-of-service method that would identify a child with an elevated lead level while the child is still present in the office would reduce the costs dramatically; universal screening would become a feasible goal.

A number of methods have been used to detect lead in various matrixes.[10] These techniques include the following: atomic absorption spectrometry, neutron activation analysis, spark source mass spectroscopy, X-ray fluorescence, proton-induced X-ray emission, inductively coupled plasma atomic emission spectroscopy, isotope dilution mass spectrometry, anodic stripping voltammetry (ASV), and differential pulse ASV.

There have been a few techniques[11] developed that allow for low-concentration detection of $Pb^{2+}$; however, these methods require expensive instrumentation, are not simple enough in their design or data interpretation, or both, and most are not designed for biological matrixes or for portability. The development of a reusable, portable, and affordable "real-time" $Pb^{2+}$ chemical detection device that is both sensitive and selective for $Pb^{2+}$ ions in complex sample matrixes would help to alleviate the problem of universal $Pb^{2+}$ screening; however, such a device cannot be found in the literature. We report here a device useful for the detection of $Pb^{2+}$ in various environments that meets the above criteria.

## EXPERIMENTAL SECTION

**$Pb^{2+}$ Sensing Hydrogel Synthesis.** IPCCA samples were made by polymerizing a solution containing 103 mg of acrylamide (Fluka), 3.95 mg of *N*′,*N*-methylenebisacrylamide (Fluka), 84 mg of 4-acryloylamidobenzo-18-crown-6 (Aldrich), 160 mg of water, and 1.96 g of a 10 wt % dispersion of 120-nm-diameter highly charged, monodisperse polystyrene colloidal particles.[2] Ion-exchange resin (Bio-Rad, AG501-X8) and three drops of diethoxyacetophenone (Aldrich) were added, and the solution was gently shaken for 5 min. The solution was centrifuged and then degassed using a vacuum oven to remove oxygen from the solution. We fabricated the $Pb^{2+}$ sensing IPCCA using methods similar to those of Holtz et al.[1] However, in the work here, we utilized a Sigmacote hydrophobic coating on the quartz plates to aid in release of the IPCCA after polymerization. In addition to using Sigmacote, the hydrogel film was also synthesized on nylon mesh cloth (Small Parts Inc. 53-$\mu$m pore size) by first placing the mesh between the quartz plates and then injecting the polymerization mixture into the cell. The cell was illuminated with UV light, after which the cell was opened to release the Nylon mesh-encapsulated IPCCA film, which was washed overnight in water.

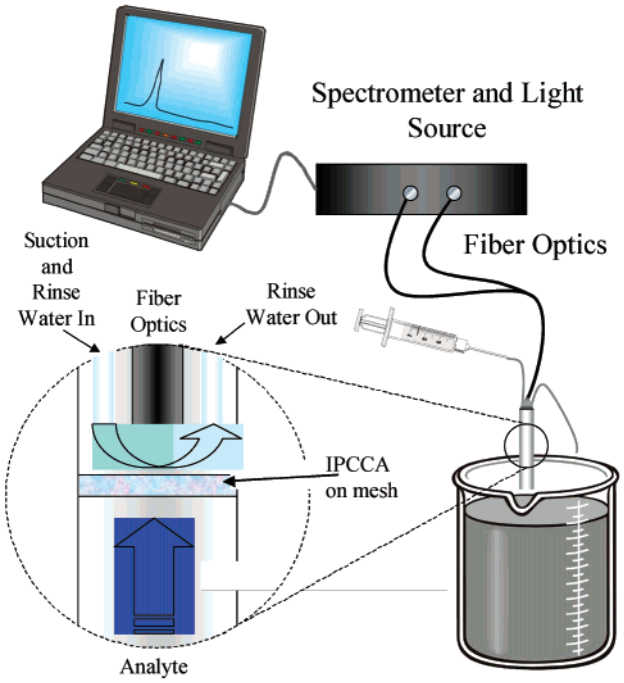


Figure 1. Optrode sensing device. The PCCA is polymerized onto nylon mesh and is sandwiched within the optrode device. The optrode is incubated with the solution to be determined. After this incubation, water is injected into the optrode device, washing the hydrogel film.

**$Pb^{2+}$ Optrode Design.** The lead optrode is shown in Figure 1. The mesh adds structural stability to the IPCCA hydrogel film, which is sandwiched at the edges. The optrode device contains a 6 around 1 fiber-optic probe connected to an Ocean Optics USB 2000 spectrometer, which measures back diffracted light from the PCCA. The device also contains a water inlet and outlet for quickly washing the PCCA film.

## RESULTS AND DISCUSSION

The optrode was incubated with $Pb^{2+}$ solutions of known concentration in Nanopure water. Figure 2 shows the IPCCA diffraction dependence as a function of $Pb^{2+}$ concentration. The diffraction monotonically shifts to longer wavelengths as the $Pb^{2+}$ concentration increases. The blue-green diffraction at 500 nm for zero $Pb^{2+}$ shifts to red at 600 nm for 1 mM $Pb^{2+}$. The inset plots the diffraction wavelength dependence on the $Pb^{2+}$ concentration. Between different $Pb^{2+}$ solutions, the sensor was washed by immersing it in a stirred reservoir of Nanopure water and by injecting Nanopure water through the washing chamber.

The response of the IPCCA to $Pb^{2+}$ results from the formation of an osmotic pressure in the hydrogel due to the immobilization of the $Pb^{2+}$ cation by the crown ether.[1,6] This forms an ionic gel.

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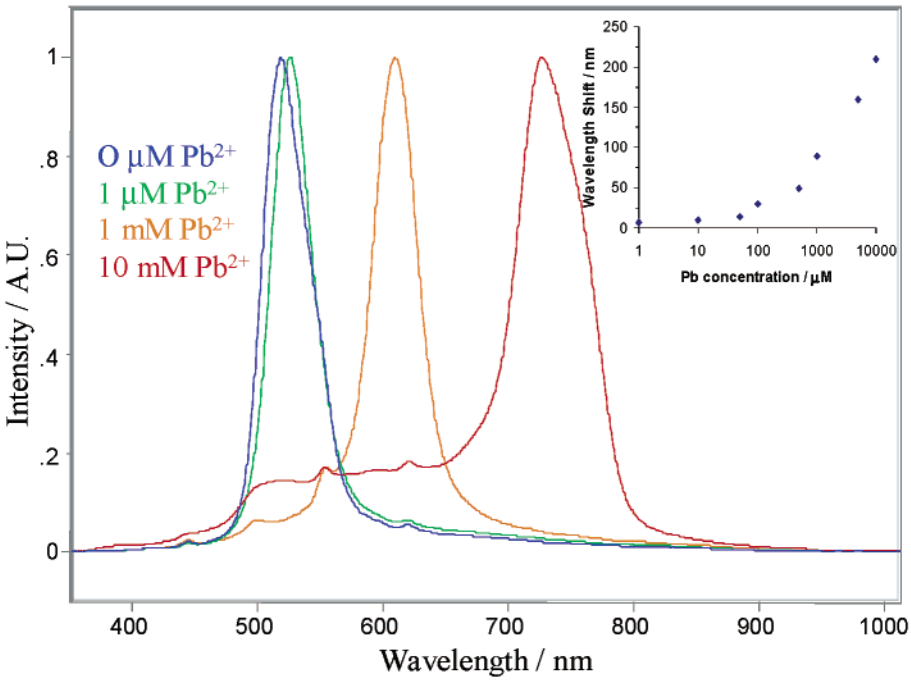


Figure 2. Optrode diffraction response to differing concentrations of $Pb^{2+}$ in Nanopure water. The inset shows the calibration curve for the diffraction wavelength of the PCCA sensor as a function of the log of the $Pb^{2+}$ concentrations.

The resulting immobilization of $Pb^{2+}$ counterion in the low ionic strength solution causes a Donnan potential, which causes an osmotic pressure, which swells the gel. High ionic strength solutions attenuate the Donnan potential, decrease the hydrogel swelling, and, consequently, decrease the sensitivity of the PCCA to $Pb^{2+}$. Our PCCA $Pb^{2+}$ sensor becomes unresponsive to $Pb^{2+}$ in solutions with ionic strength greater than that of ~10 mM NaCl.

We previously[8] demonstrated that we could determine $Pb^{2+}$ concentration by measuring the transient response that occurs upon transferring the sensor from a high ionic strength $Pb^{2+}$ solution to a pure Nanopure water solution. The IPCCA is mainly water (>85%) and the ions diffuse out, with diffusion constants similar to those in pure water. As the ionic strength decreases, the PCCA gel swells in proportion to the amount of $Pb^{2+}$ ions bound. This transient red-shift is followed by a diffraction blue-shift as the $Pb^{2+}$ diffuses out of the IPCCA.

The diffraction of the PCCA sensor in pure water red-shifts ~10 nm upon exchange by a 100 mM NaCl solution. This red-shift presumably results from an increase in the free energy of mixing of the hydrogel due to binding of $Na^+$ to the crown ethers. Replacement of the NaCl solution with pure water blue-shifts the diffraction back to the original Nanopure water value. We see no transient red-shift upon exchanging the 100 mM NaCl with pure water, presumably due to the low affinity and the fast off rate of the $Na^+$ from the crown ether.

The optrode device stored in a 100 mM NaCl solution was incubated with 100 mM NaCl containing various $Pb^{2+}$ concentrations. No diffraction changes occurred. Figure 3 shows the time dependence of the transient red-shift that occurs upon washing the PCCA with pure water. The maximum shift plotted is relative to that in pure water.

Subsequent washing of samples containing 0.5 (Figure 3A) and 10 mM (Figure 3B) $Pb^{2+}$ in 100 mM NaCl results in a transient red-shift, which has a maximum between 2 and 4 min after

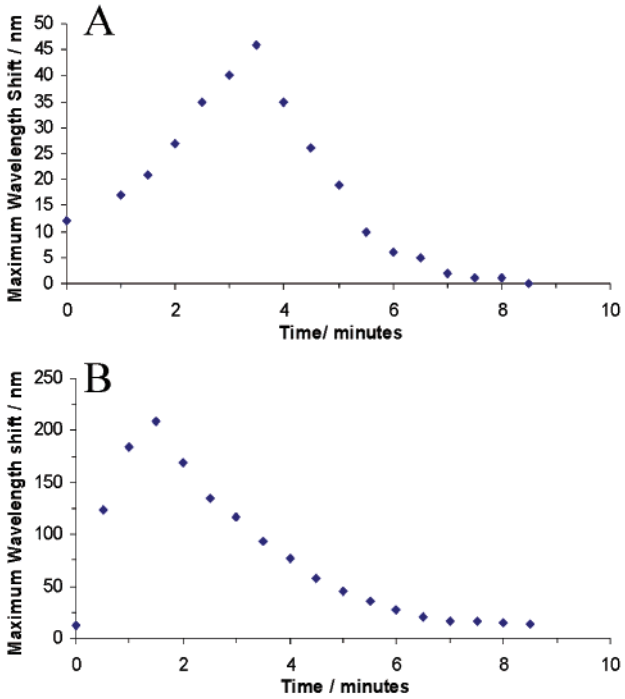


Figure 3. Optrode sensor transient response in 100 mM NaCl solution: (A) 0.5 mM $Pb^{2+}$ concentration; (B) 10 mM $Pb^{2+}$ concentration. Washing of the sensor begins at $t = 0$. After a few minutes, the maximum diffraction wavelength is reached and the sensor returns to its original diffraction wavelength in water in less than 10 min.

washing is initiated. The transient response is completed within 10 min, almost 10-fold faster than our previous transient response study[8] due to our more efficient washing procedure, where we now flow water directly through the hydrogel film. The 47-nm maximum red-shift of the 0.5 mM $Pb^{2+}$ solution is ~5-fold smaller than the ~220-nm red-shift of the 10 mM $Pb^{2+}$ solution.

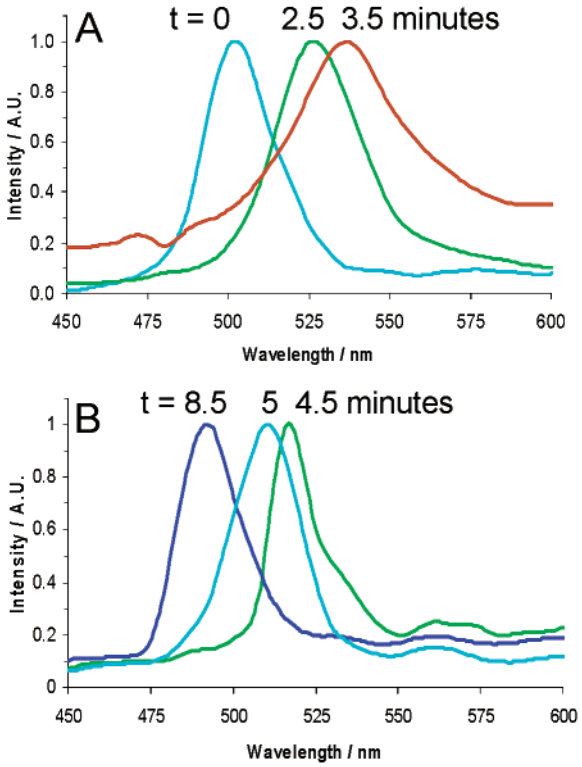


Figure 4. Optrode IPCCA transient response diffraction spectra for 0.5 mM $Pb^{2+}$ in 100 mM NaCl. The times correspond to the elapsed time after washing begins: (A) transient red-shifting of the IPCCA; (B) blue-shift of the IPCCA from the maximum transient shift demonstrating removal of $Pb^{2}$ from the IPCCA.

Figure 4 shows representative diffraction spectra for the optrode's transient response to 0.5 mM $Pb^{2+}$ in 100 mM NaCl. Figure 4A shows the transient red-shifting of the IPCCA at $t = 0$, 2.5, and 3.5 min from the start of washing as the NaCl diffuses out. Figure 4B shows the blue-shifting diffraction spectra at $t =$ 4.5, 5, and 8.5 min from the start of washing due to $Pb^{2+}$ diffusing from the IPCCA. The diffraction peak shapes are maintained with only a slight broadening observed during the diffraction red-shift, a behavior much less complex than occurred in our previous study,[8] where we observed broadening and double peaks during the transient red-shifts. Presumably, the more efficient washing results in less PCCA volume inhomogeneities during the transient response.

Figure 5 compares the maximum transient diffraction wavelength shifts for a variety of $Pb^{2+}$ concentrations in 100 mM NaCl solutions to that which would occur for these $Pb^{2+}$ concentrations in pure water. Essentially identical shifts are observed. This indicates that the NaCl ions completely diffuse out during the first 4-min washing interval. This is expected from the 125-$\mu$m-thick PCCA, since the NaCl ions should not be bound and because the diffusion constants in the hydrogel should be about that in pure water. The PCCA sensor contains a ~0.1 M crown ether concentration, which will result in roughly similar counterion concentrations within the hydrogel during washing as occurs in the 10 mM $Pb^{2+}$ pure water solutions.

The measurement errors of this optrode device depend on numerous parameters, especially the rate of water flow through and past the surface of the sensor material. There should be negligible error from the spectral measurement itself. Differential

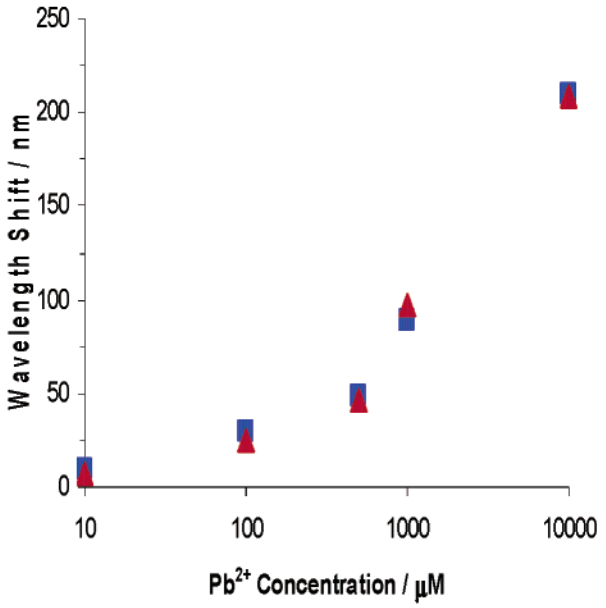


Figure 5. Maximum diffraction wavelength shifts as a function of $Pb^{2+}$ concentration; (▲) represents the maximum transient diffraction shift for $Pb^{2+}$ detection in 100 mM NaCl solution; (■) represents the maximum diffraction shift in Nanopure water.

water pressure on the PCCA sensing membrane could bias the diffraction and cause errors if the washing water pressure and flow rate varied between measurements. In this first optrode sensing paper, which examines the feasibility of sensing in high ionic strength solutions, we have not tried to control these parameters; we introduce the washing water by hand.

It appears that for our $Pb^{2+}$ sensor the rate of washing is very fast compared to the off rate of the $Pb^{2+}$ from the crown ether. This is directly responsible for the fact that the transient response is essentially identical to the equilibrium response. Thus, we expect detection limits similar to those found by Holtz et al.[1] (0.1 $\mu$M, 40 ppb) in equilibrium measurements in deionized water. Our next report will experimentally examine the limits of detection of this optrode sensor.

## CONCLUSIONS

We have developed a $Pb^{2+}$ optrode sensing device that determines $Pb^{2+}$ in high ionic strength solutions by washing out nonbound ions from the PCCA. The resulting transient red-shift maximum red-shift monotonically increases with $Pb^{2+}$ concentration. This simple system is portable and inexpensive and can be used to determine $Pb^{2+}$ in low- and high-concentration solutions. It can also be utilized for other ionic species by utilizing the appropriate chelation agents.

We are continuing to develop this sensing technology for applications such as determination of metals in the environment and for clinical chemistry applications.

## ACKNOWLEDGMENT

This work was supported by NIH Grant 2 R01 DK55348-03A1 and DOE Grant DEFG0798ER62708.

Received for review March 18, 2003. Accepted May 19, 2003.

AC034276B