Abstract We have developed intelligent polymerized crystalline colloidal array (IPCCA) chemical-sensing materials for detection of Pb\(^{2+}\) in high ionic-strength environments such as body fluids with a detection limit of <500 nmol L\(^{-1}\) Pb\(^{2+}\) (100 ppb). This IPCCA lead sensor consists of a mesoscopically periodic array of colloidal particles polymerized into an acrylamide hydrogel. The array Bragg-diffracts light in the visible spectral region because of the periodic spacing of the colloidal particles. This material also contains a crown ether chelating agent for Pb\(^{2+}\). Chelation of Pb\(^{2+}\) by the IPCCA in low-ionic-strength solutions results in a Donnan potential that swells the gel, which red-shifts the diffracted light in proportion to the Pb\(^{2+}\) concentration. At high ionic strength the Donnan potential is, unfortunately, swamped and no static response occurs for these sensors. We demonstrate, however, that we can determine Pb\(^{2+}\) at high ionic strength by incubating these IPCCA in a sample solution and then measuring their transient response on exposure to pure water. The non-complexed ions diffuse from the IPCCA faster than the bound Pb\(^{2+}\). The resulting transient IPCCA diffraction red-shift is proportional to the concentration of Pb\(^{2+}\) in the sample. These IPCCA sensors can thus be used as sensing materials in optrodes to determine Pb\(^{2+}\) in high-ionic-strength solutions such as body fluids.

Keywords Intelligent polymerized crystalline colloidal array (IPCCA) · Pb\(^{2+}\) body fluid sensor · Photonic crystal · Bragg diffraction · Hydrogel chemical sensors

Introduction

The development of novel clinical chemistry sensing methodologies is challenged technologically by the complexity of body fluids and by the relatively low concentrations of key analytes [1]. The acceptance of any new clinical chemistry methodology that fulfills the technological requirements is often limited by its cost and the requirement that the methodology be simple.

Our recent invention of intelligent polymerized crystalline colloidal array (IPCCA) chemical-sensing materials [2, 3, 4] might enable us to create new clinical chemistry methodologies that are able to fulfill the above requirements for a variety of different analytes. In the work described here we have demonstrated the applicability of IPCCA sensing materials to the detection of Pb\(^{2+}\) in body fluids. This work follows our previous demonstrations that IPCCA could be used to detect low concentrations of Pb\(^{2+}\) in low ionic-strength aqueous solutions [2, 3, 4].

Pb\(^{2+}\) is an important environmental toxin, which can cause disease and death at concentrations as low as 700 ppb in blood [5, 6]. Body concentrations as low as 100 ppb or 500 nmol L\(^{-1}\) might be correlated with reduced IQ in children. Universal screening of children for lead in blood was enunciated as a public health goal by the PHS and CDC in 1991. Current estimates from the national Center for Health Statistics state that 3.2% of American children have blood lead levels above 10 µg dL\(^{-1}\) (480 nmol L\(^{-1}\)). The rate for minority children in poverty is 20%.

Universal lead screening was abandoned by the federal government in 1997 for economic reasons, despite the large numbers of children at risk. Even when performed in large numbers the laboratory cost of analysis of a single blood specimen for lead is $8–15. The cost of drawing the blood, communicating the results, and, most importantly, finding and retrieving the child raise the price per case found to $50 or more. 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, and universal screening would once
again become a feasible goal. The impact of an inexpensive point of service quantitative serum lead test on prevention of serious lead poisoning and on the subsequent neurobehavioral casualty would be considerable.

United States (Center for Disease Control, CDC) and international (World Health Organization, WHO) guidelines both recommend that lead in body fluids should be measured with a detection limit of 10 µg dL⁻¹ or 480 nmol L⁻¹, within a measuring range of 0–60 µg dL⁻¹ or 0–2.88 µmol L⁻¹ lead, and with a precision of less than 2 µg dL⁻¹ or 10%, whichever is greater.

Several methods have been used to detect lead in biological matrices [1], including atomic absorption spectrometry, neutron activation analysis, spark-source mass spectrometry, X-ray fluorescence, proton-induced X-ray emission, inductively coupled plasma atomic-emission spectrometry, isotope dilution mass spectrometry, anodic stripping voltammetry (ASV), and differential pulse ASV. Reeder and Heineman [7] fabricated an electrochemical sensor by screen-printing techniques and coupled it with ASV to detect lead in the 10⁻⁶–10⁻⁹ mol L⁻¹ concentration range. An electrochemical analyzer coupled to screen-printed disposable sensors for field screening of trace lead was reported by Yarnitzky, Wang, and Tian [8] to detect 20–300 µg L⁻¹ lead in drinking water. Yu et al. [9] made a lead sensor by first derivatizing Pb²⁺ with sodium tetraethylborate to form tetraethyllead which was then extracted from the headspace over the sample by solid-phase microextraction gas chromatography; the limit of detection was 5–10 ppb for urine and blood samples. Finally, de la Riva et al. [10] reported a flow-injection system which utilized room temperature phosphorescent quinoline-terminated acid lead chelates immobilized on anion-exchange resin; this could be used to detect lead in seawater at the ng mL⁻¹ level.

The IPCCA materials we are developing to detect Pb²⁺ in body fluids consist of a mesoscopically periodic array of colloidal particles [11, 12, 13, 14, 15, 16, 17, 18, 19, 20] polymerized into an acrylamide hydrogel [2, 3, 4, 21, 22, 23] (Fig. 1). This array diffracts light in the visible spectral region, because of the periodic spacing of the colloidal particles. The material also contains molecular recognition, or chelating, agents for the analytes of interest [2, 3, 4]. Because the IPCCA are >85% water, analyte species are free to rapidly diffuse into the IPCCA, with diffusion constants similar to those in water. These IPCCA are characterized by rich phase transition phenomena typical of hydrogels [24, 25, 26]. The Pb²⁺ sensing IPCCA contains a crown ether which selectively binds Pb²⁺ to the hydrogel.

The volume of the IPCCA hydrogel depends on three main factors – the free energy of mixing, the elastic free energy, and electrostatic interactions such as the formation of a Donnan potential, because of the presence of immobilized ions such as Pb²⁺ attached to the crown ether [27]:

\[
\Delta G_{\text{tot}} = \Delta G_{\text{mix}} + \Delta G_{\text{elas}} + \Delta G_{\text{elec}}
\]

The Donnan potential resulting from the bound Pb²⁺ results in an osmotic pressure which causes the gel to swell against the elastic gel restoring force [2, 3, 4] The resulting change in the particle array lattice constant shifts the diffracted wavelength [11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23] (Fig. 1C). Thus, the diffracted wavelength shift is indicative of the identity and concentration of the target analyte. We have already demonstrated that we can visually detect the presence of sub-ppm Pb²⁺, and can instrumentally observe the diffraction shift from 20 ppb Pb²⁺. We have also demonstrated that we could attach a small piece of this IPCCA to the end of an optical fiber and that the device obtained worked well [3, 4] as a sensitive optrode for the remote detection of Pb²⁺.

The selectivity of this sensor depends on the selectivity of the crown ether molecular recognition group. Although K⁺ could, potentially, interfere with our lead measurements, because it binds weakly to the crown ether, our results with fetal calf serum (discussed below) indicate that interference is insignificant.

In the work described here we further characterized and optimized IPCCA for detection of Pb²⁺. As expected, these IPCCA sensors become less responsive to Pb²⁺ in high ionic strength aqueous solutions and become insensitive to Pb²⁺ at the ionic strengths of body fluids. We therefore developed a novel transient diffraction shift methodology that enables our IPCCA sensors to detect Pb²⁺ in body fluids.

Fig. 1 A. Polymerized crystalline colloidal array showing a BCC array of polystyrene colloidal particles. Also shown schematically is the hydrogel polymer polymerized around the CCA. B. Photograph of IPCCA. C. Dependence of diffraction on IPCCA volume. As the IPCCA swells, the diffraction red-shifts