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A Portable DNAzyme-Based Optical Biosensor for Highly Sensitive and Selective Detection of Lead (II) In Water Sample

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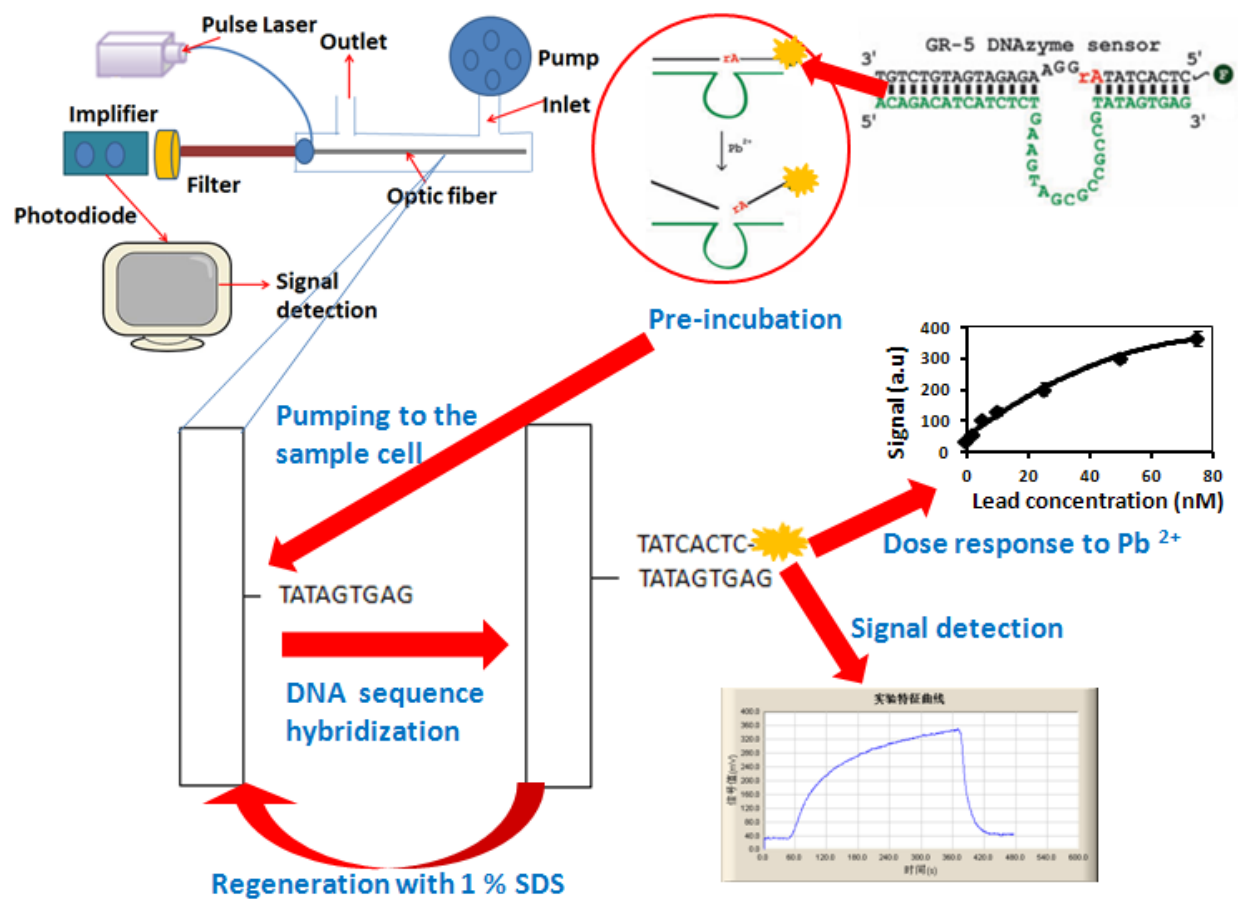
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Abstract: A portable, rapid and cost-effective DNAzyme based sensor for lead ions detection in water samples has been developed using an optical fiber sensor platform. The presence of Pb²⁺ cleaves the DNAzymes and releases the fluorescent labeled fragments, which further hybridize with the complementary strands immobilized on the optic fiber sensor surface. Subsequent fluorescent signals of the hybridized fluorescent labeled fragment provides quantitative information on the concentrations of Pb²⁺ with a dynamic range from 2 to 75 nM with a detection limit of 1.03 nM (0.21 ng mL⁻¹). The proposed sensor also shows good selectivity against other mono and divalent metal ions and thus holds great potential for the construction of general DNAzyme-based sensing platform for the monitoring of other heavy metal ions. The sensor can be regenerated with a 1 % SDS solution (pH 1.9) over 100 times without significant deterioration of the sensor performance. This portable sensor system can be potentially applied for on-site real-time inexpensive and easy-to-use monitoring of Pb²⁺ in environmental samples such as wastewater effluents or water bodies.

Keywords: Pb²⁺, DNAzyme, biosensor, optical sensor, environmental analysis.

1 Graphical Abstract



2

3 1. Introduction

Lead (Pb^{2+}) is one of the most toxic metallic pollutants that can cause neurological, reproductive, cardiovascular, and developmental disorders even at very low levels ($<100\mu\text{g/L}$ in blood) (Knecht and Sethi, 2009; Needleman 2004; Subramanian et al. 1995). Children are more vulnerable to lead exposure than adults because of higher rate of intestinal absorption and retention and of variants in iron metabolism genes that may be more susceptible to Pb absorption and accumulation (Hung et al. 2010). Thus, Pb^{2+} is regulated by the Environmental Protection Agency with a maximum content in drinking water of 72 nM (<http://www.epa.gov/safewater/contaminants/index.html>).

To minimize the health impact associated with Pb^{2+} exposure, accurate, preferably on-site and real-time detection and quantification of Pb^{2+} in the environment or in vivo is needed (Lan et al. 2010). Several methods for Pb^{2+} analysis, including classical atomic absorption/emission spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), thermal ionization mass spectrometry (TIMS), X-ray fluorescence spectrometry (XRF), anodic stripping voltammetry and reversed-phase high-performance liquid chromatography coupled with UV-vis or fluorescence detection have been developed for detection of Pb^{2+} (Casas and Sordo 2006). Although these are routinely used for metal ion analysis with satisfactory sensitivity (Ewing 1997), they require either expensive instruments or complicated operations (Wang et al. 2010). As an alternative, various chemical and biological sensors have been explored for detection of this metal ion (He et al. 2006; Kim et al. 2005). Among them, DNazymes have emerged recently as a highly promising class of recognition molecules for biosensors. DNazymes for a number of metal ions were selected out through SELEX (systematic evolution of ligands by exponential enrichment) process (Ellington and Szostak 1990; Tuerk and 1990; Breaker and Joyce 1994). These DNazymes exhibit high catalytic turnovers that allow signal amplification and have lower vulnerability to the sample background interferences (Xiao et al. 2007; Shen et al. 2008). Because of these features, DNazymes have been employed onto fluorescent, colorimetric and electrochemical sensors for a wide range of metal ions, including Pb^{2+} (Li et al. 2009; Liu et al. 2007).

The first DNzyme using Pb^{2+} as the cofactor was selected more than a decade ago Breaker and Joyce 1994). Similar to the 8–17 DNzyme reported earlier (Wang et al. 2010), GR-5 DNzyme recently reported by Lan et. al. (2010) can also catalyze the cleavage of an RNA base embedded

in the DNA substrate in the presence of Pb^{2+} (Lan et al. 2010). This GR-5 DNzyme has higher selectivity for Pb^{2+} over other competing metal ions (Lan et al. 2010).

In this study, we report the development of a new optical biosensor for rapid, highly specific and sensitive detection of Pb^{2+} based on GR-5 DNzyme. A portable, inexpensive, and easy-to-use evanescent wave fiber-optic biosensor platform was used for the detection of Pb^{2+} in aqueous solution based on this DNzyme mechanism. The biosensor's sensing time, sensitivity, specificity, resistance to background interference and reusability were evaluated.

2. Materials and Methods

2.1. Materials

All HPLC-grade oligonucleotides (DNA) were purchased from Integrated DNA Technologies (Iowa, USA). DNzyme sequence is 5'-ACAGACATCATCTCTGAAGTAGCGCCG CCGTATAGTGAG-3' and the fluorescent labeled substrate DNA sequence for this DNzyme is 5'-Cy5.5-CTCACTATrAGGAAGAGATGATGTCTGT-3'. Additionally, the aminated DNA probe sequence is 5'- NH_2 -(CH_2)₆-TATAGTGAG-3', and that of the non-complementary DNA sequence (used for control) 5'-Cy5.5-TCCCGAGA-3'. Bovine serum albumin (BSA), 3-aminopropyltriethoxysilane (APTS), and glutaraldehyde (GA) were purchased from Sigma-Aldrich (St. Louis, MO). A DNA probe stock solution was prepared with a 10mM phosphate buffered solution (PBS, pH 7.4). DNzyme, substrate DNA and non-complementary DNA oligonucleotides were dissolved in a 50mM NaHEPES (50 mM NaHEPES, 50mMNaCl and 5mMMgCl₂, pH 7.26) and kept frozen at -20°C for storage. A 1mM Pb^{2+} stock solution was prepared in ultrapure water and stored at 4°C ; its standard concentrations were prepared from the stock solution by serial dilutions in 50mM NaHEPES (50 mM NaHEPES, 50mMNaCl and 5mMMgCl₂, pH 7.26).

2.2. Instrumentation: evanescent wave all-fiber biosensing platform

The portable evanescent wave all-fiber biosensing platform was as previously described (Long et al. 2009,2010). Briefly, the laser beam from a 635-nm pulse diode laser with pigtail was directly launched into a single-mode fiber of a single multi-mode fiber coupler. The laser light then entered the multi-mode fiber with a diameter of 600 μm and numerical aperture of 0.22 from the single mode fiber. The excitation light from the laser, through the fiber connector, was coupled to a fiber probe. The incident light propagated along the length of the probe via total internal reflection. The evanescent wave generated at the surface of the probe then interacted with the surface-bound fluorescently labeled analyte complexes and caused excitation of the fluorophores. The collected fluorescence was filtered by means of a bandpass filter and detected by photodiodes through a lock-in detection. The probe was embedded in a glass flow cell with a flow channel having a nominal dimension of 60mm in length and 2mm in diameter. All reagents were delivered by a flow delivery system operated with a peristaltic pump. The controls of fluid delivery system and data acquisition and processing were automatically performed by the built-in computer.

2.3. Immobilization of DNA probes onto fiber optic sensor surface

Details of the fabrication and preparation of the fiber optical sensor were described previously (Long et al. 2008; Yildirim et al. 2012). Figure S1 depicts the steps for immobilizing a probe-DNA that complement to a partial sequence of the substrate DNA, onto the optical sensor fiber surface. The sensor fiber was pre-cleaned with a piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 3:1 (v/v)), then aminated by immersion in a 2% (v/v) APTS acetone solution for 60 min, followed by an acetone wash (three times), ultrapure water wash, and drying in an oven for 30 min at 110 °C. For immobilization of the probe-DNA, the aminated sensor was first immersed in a 5.0% (v/v) GA

solution for 1 h at 37 °C for adding aldehyde functional group, washed with water, and then immersed in 0.5 µg/ml aminated probe-DNA sequence (5'-NH₂-(CH₂)₆-TATAGTGAG-3') in PBS (pH 7.4) solution overnight at 4 °C. The sensor surface was then dipped in a 2mg/mL BSA solution for 1 h to block the remaining aldehyde sites.

2.4. Sensing Mechanism

The sensor scheme employed herein is based on a portable evanescent wave biosensing platform in which both the transmission of the excitation light and the collection and transmission of fluorescence are achieved through a single multi-mode fiber optic coupler (Long et al. 2008; Yildirim et al. 2012). When light propagates through a fiber optic on the basis of total internal reflection (TIR), a thin electromagnetic field (the “evanescent wave”) is generated, which decays exponentially with the distance from the interface with a typical penetration depth of up to several hundred nanometers (Andrade et al. 1985; Shriver-Lake et al. 1995; Marazuela and Moreno-Bondi 2002). This evanescent wave can excite fluorescence in the proximity of the sensing surface, e.g., in fluorescently labeled DNA bound to the optical sensor surface. The short range of the evanescent wave allows it to discriminate between bound and unbound fluorescent compounds, hence eliminating the normally required washing steps (Shriver-Lake et al. 1995).

Figure 1 also illustrates this enzymatic reaction mechanism for Pb²⁺ detection. It is known that GR-5 DNAzyme can catalyze the cleavage of an RNA base embedded in the DNA substrate in the presence of Pb²⁺ (Lan et al. 2010). This cleavage product is in the 5' position of the substrate and labeled with fluorescent dye (Figure 1) and it is complementary to the DNA probe sequence that was immobilized on the sensor optical fiber surface. With a pre-incubation step where a fixed concentration of DNAzyme and substrate DNA were mixed, a cleavage DNA product is generated and its concentration is proportional to the concentration of Pb²⁺ in the water sample.

For lead ion detection, the mixture was pumped to the sample cell; cleavage products hybridize with the DNA probe and the subsequent fluorescent signals of the hybridized fluorescent labeled fragment provides quantitative information on the concentrations of Pb^{2+} .

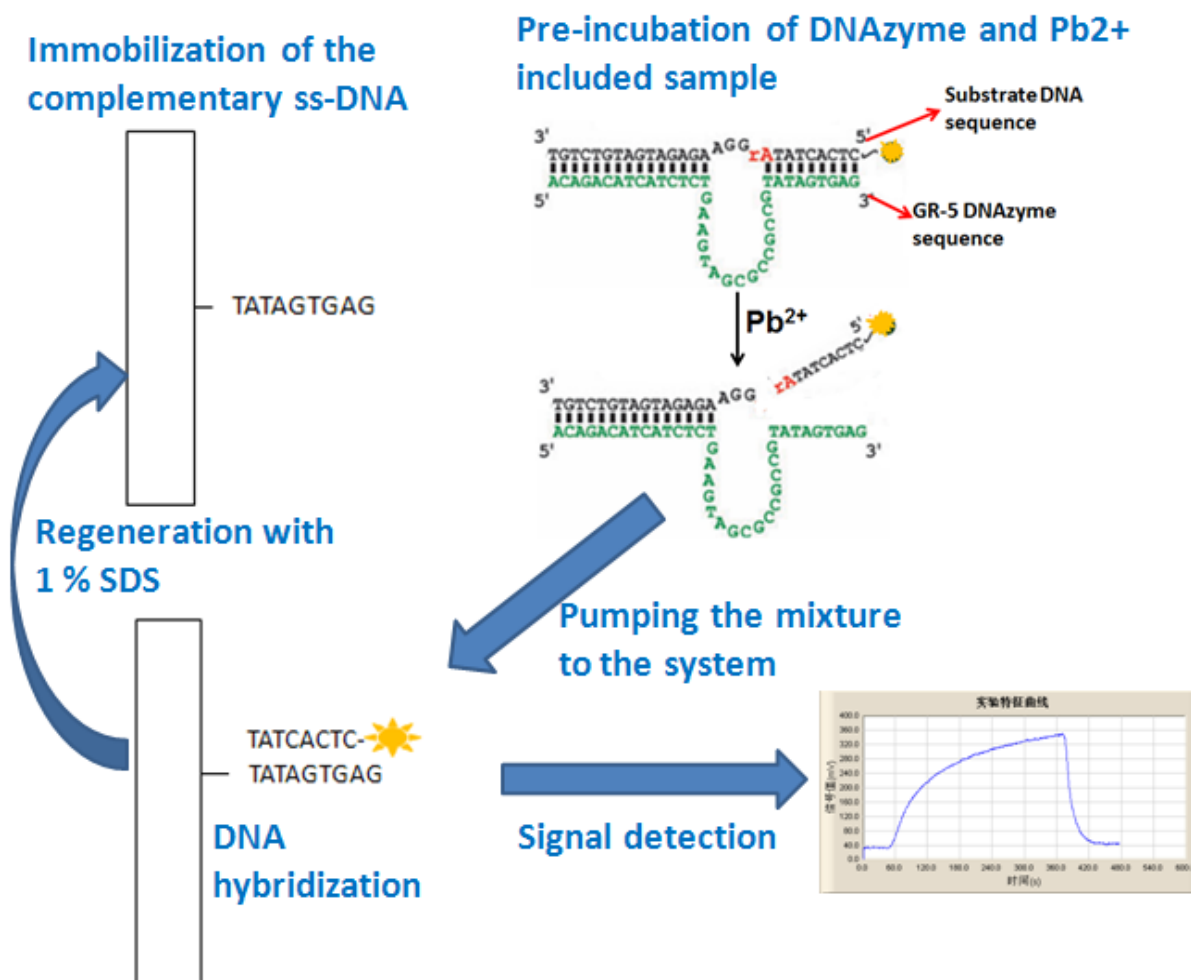
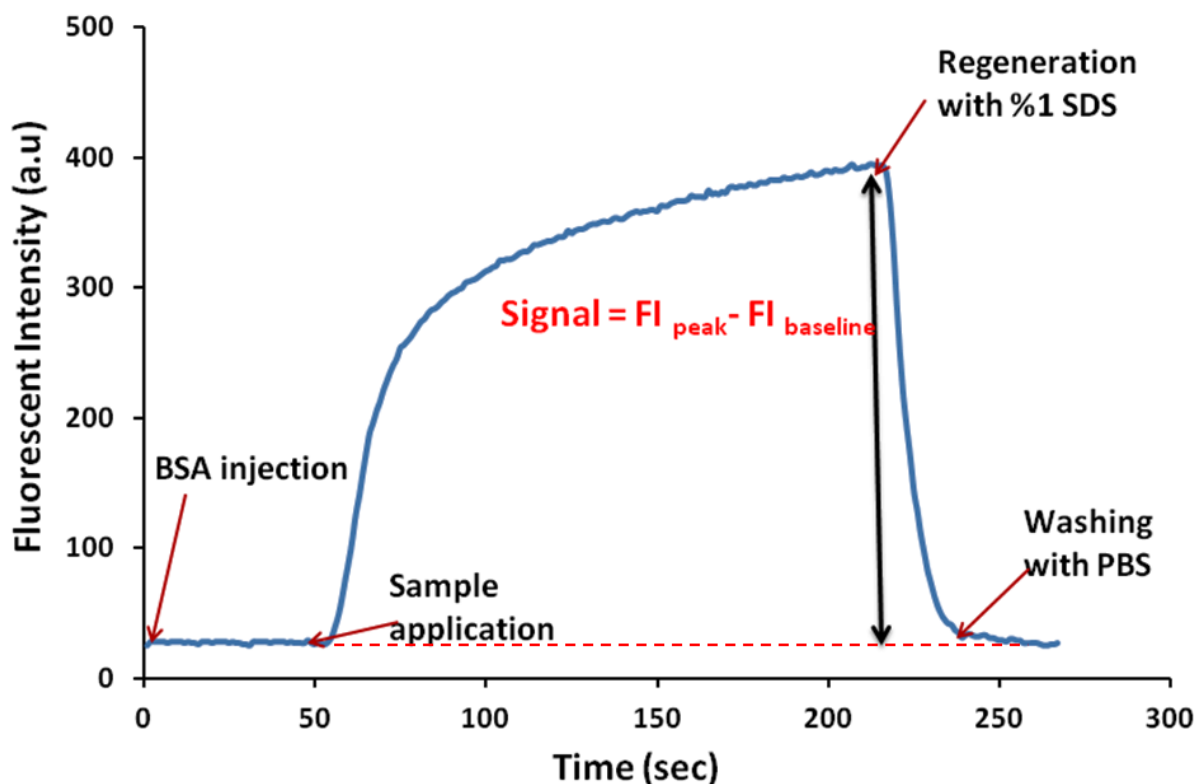


Figure 1 Schematic representation of sensing mechanism for Lead (II) ion detection using a optical fiber sensing platform and employing DNAzyme as a recognition agent. The sensing mechanism starts with pre-incubation step (enzymatic reaction) and continues with hybridization of product of the substrate DNA and probe-DNA, signal detection and regeneration steps.

- 1 Figure 2 shows the exemplary real time signals for one analysis cycle. The signal for each assay
- 2 is calculated as follow;
- 3 $\text{Signal (a.u.)} = \text{Fluorescent Intensity at the peak value} - \text{Fluorescent Intensity at the baseline}$



- 4
- 5 **Figure 2** Exemplary fluorescence intensity profile for one complete lead (II) ion detection cycle
- 6 with the DNAzyme based optical biosensor. Introduction of sample application, the
- 7 hybridization of surface immobilized probe DNA and the product of the DNAzyme reaction, and
- 8 sensor regeneration.

9 2.5. Lead ion analysis

- 10 Water samples containing different concentrations of Pb^{2+} were mixed with fluorescent labeled
- 11 GR-5 Pb^{2+} DNAzyme and substrate DNA at a fixed concentration. The mixture was pumped into
- 12 a flow cell at a rate of $300\mu\text{L}/\text{min}$ for 30 s and allowed to bind to the DNA probe for 5 minutes.

Meanwhile, the fluorescence signal was collected. The sensing surface was then regenerated with a 1% SDS solution (pH 1.9) for 60 s and washed with a PBS solution. Before every sample application 1mg/ml BSA was injected to the sample cell for blocking the non-specific binding sites in the optic fiber surface as we optimize the concentration and effect of the BSA injection in our previous works (Yildirim et al. 2012).

To evaluate potential environmental sample matrix effects on Pb^{2+} detection, spiked samples of tap water and of tertiary effluent from two wastewater treatment plants (Pinery, Colorado, USA and Loundon, Virginia, USA) were tested at concentrations of 10, 20, and 40 nM.

To assess the specificity of the sensor, its responses to such potentially interfering metal ions as Hg^{2+} , Ca^{+} , Cu^{2+} , Fe^{3+} , Mn^{2+} , Ni^{+} , Mg^{2+} , Co^{2+} and Ag^{+} at concentrations up to 40 nM were evaluated.

3. Results and Discussion

3.1. Immobilization of DNA onto sensor surface

Covalent immobilization of a DNA probe on an aminated fiber optic sensing surface was achieved using a glutaraldehyde coupling strategy (Figure S1). A six-carbon alkyl group was added as a spacer between the surface and the probe to reduce steric hindrances for DNA hybridization.

3.2. Confirmation of specific binding

To evaluate the immobilization results and to confirm that the observed fluorescence signal was from hybridization between immobilized DNA probe and its complementary sequence, two control experiments were performed and the results are shown in Figure 3. 10 nM fluorescently labeled substrate DNA (s-DNA) and non-specific DNA (ns-DNA) were delivered over the

sensing surface with and without BSA injection prior to sample pumping. 1 mg/ml BSA solution was injected to the sample cell to avoid the non-specific adsorption on the surface by blocking the binding sites as we demonstrated and optimized in our previous work (Yildirim et al. 2012). As shown in Figure 3, with non-specific DNA, certain level signal was detected without BSA injection, indicating the occurrence of non-specific DNA adsorption onto the sensor surface. However, with BSA injection, only base line fluorescent signal was observed with non-specific DNA, suggesting the effectiveness of BSA blocking of non-specific adsorption of DNA. Application of 10 nM s-DNA without BSA injection yielded a sharp increase in the fluorescent intensity that is indicative of the non-specific adsorption of DNA onto the sensor surface. This is because that DNA hybridization process is a kinetic process that exhibits continuous reaction and corresponding signal increase rather than the fast adsorption process as observed here (Yildirim et al. 2012). With BSA blocking, a characteristic sensor signal was observed as results of specific hybridization between fluorescence-labeled substrate sDNA and immobilized probe DNA.

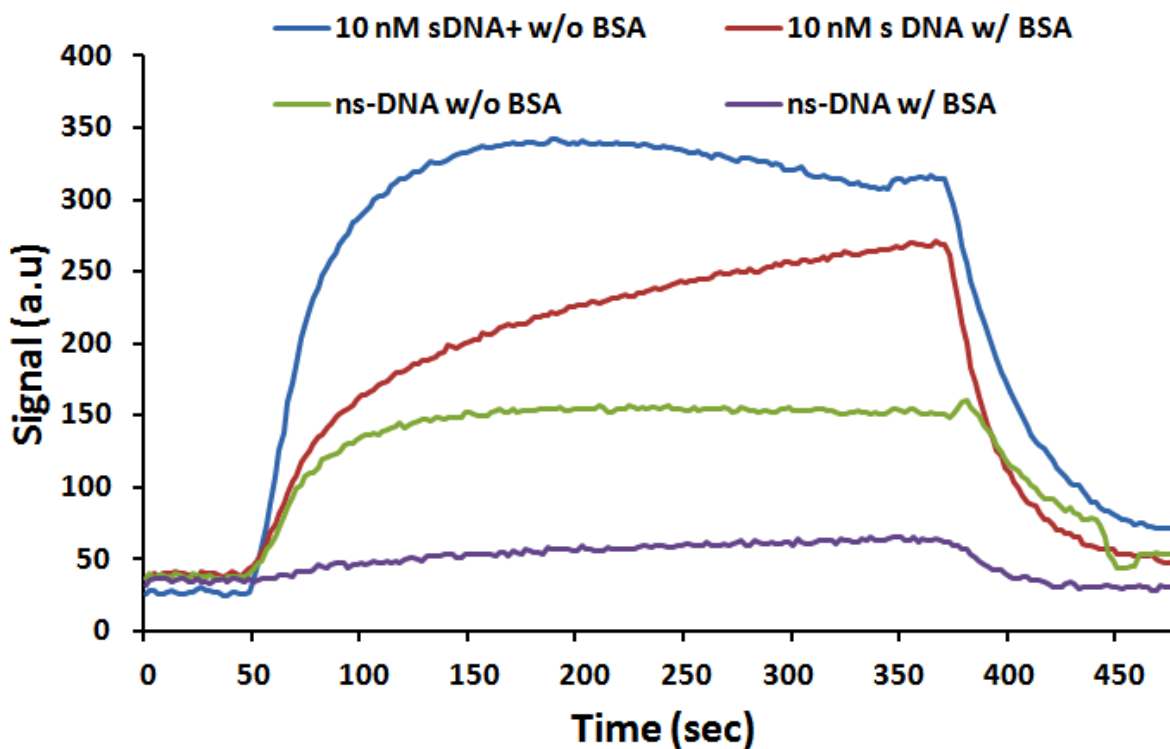
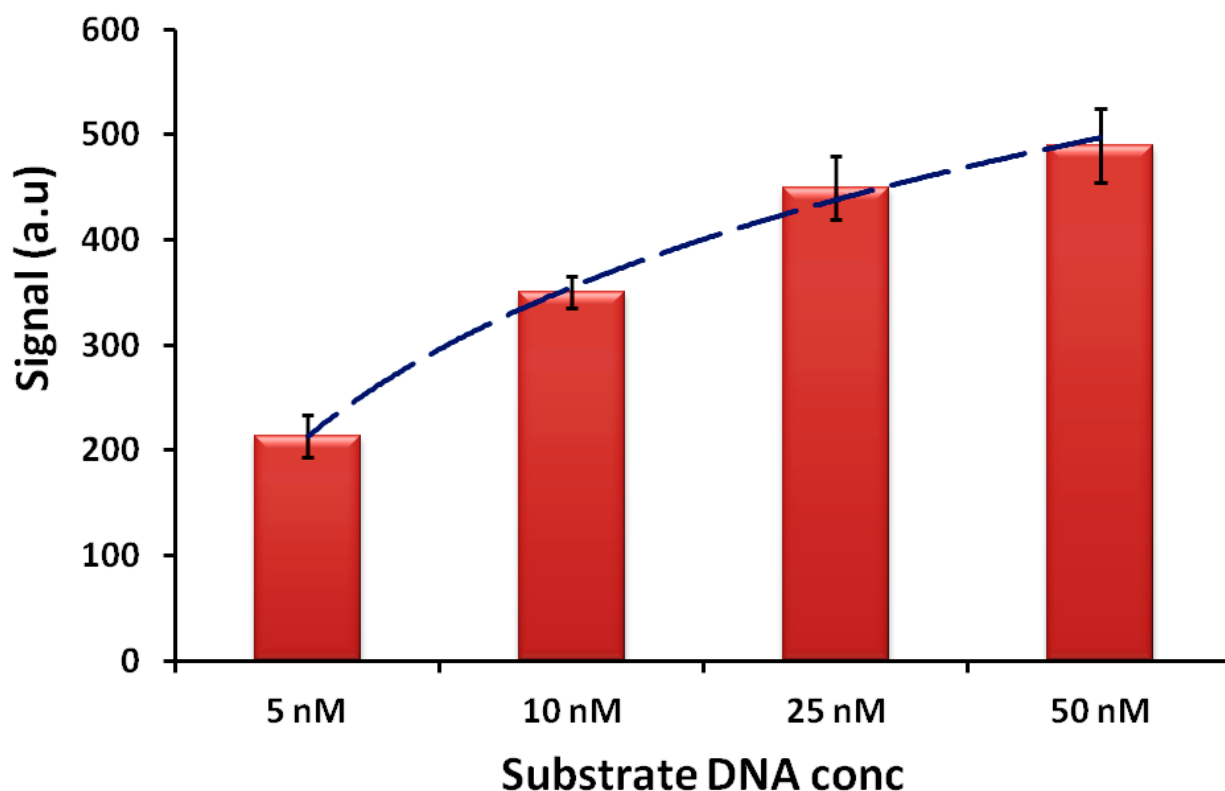


Figure 3 Assessment of the immobilization effectiveness and specific binding between probe DNA sequence (immobilized onto sensor surface) and fluorescence-labeled substrate DNA part. Here w/BSA or w/o BSA represents the experiment with or without BSA injection to block the non-specific binding sites. s-DNA is the substrate DNA that binds specifically to probe DNA and also ns-DNA is the non-specific DNA sequence as control instead of substrate DNA

3.3. Substrate DNA and GR-5 DNAzyme concentration optimization

To optimize the substrate DNA concentration used in the pre-mixing step considering both signal intensity and cost, a varying concentration of fluorescent-labeled s-DNA at 5, 10, 25 and 50 nM were applied for comparison. It is clearly seen from the Figure 4 that 25 nM is near the signal saturation limit for our biosensing platform, and also this concentration is sufficient to get

- 1 appropriate fluorescent intensity to continue next steps. Hence, fluorescent labeled substrate
 2 DNA at 25 nM was selected for the subsequent experiments.



3 **Figure 4** Experimental optimization of the fluorescent-labeled substrate DNA concentration for
 4 pre-mixing step. ΔS is the fluorescent intensity difference between peak intensity and base-line.
 5 Each data value is the average of two independent experimental results.

7
 8 For the optimization of the GR-5 DNAzyme concentration, the optimized concentration of
 9 fluorescent labeled substrate DNA (25 nM) and different amount of GR-5 DNAzyme were
 10 mixed and pumped to the sample cell, and the fluorescent signal was monitored with BSA
 11 blocking (Figure 5). Theoretically, a 1:1 ratio of GR-5 DNAzyme to substrate sDNA is required
 12 so that there is no signal in the absence of Pb^{2+} , since the fluorescent labeled substrate DNA was
 13 not free to hybridize with the probe-DNA (see sensing mechanism in Figure 1). However, the

results from Figure 5 show that a DNAzyme to substrate DNA ratio of 2:1 was required to obtain negligible signal with BSA blocking step. It is possible that there were some complementary nucleotides in the GR-5 DNAzyme and they preferred to hybridize each other rather than bind to substrate DNA sequence.

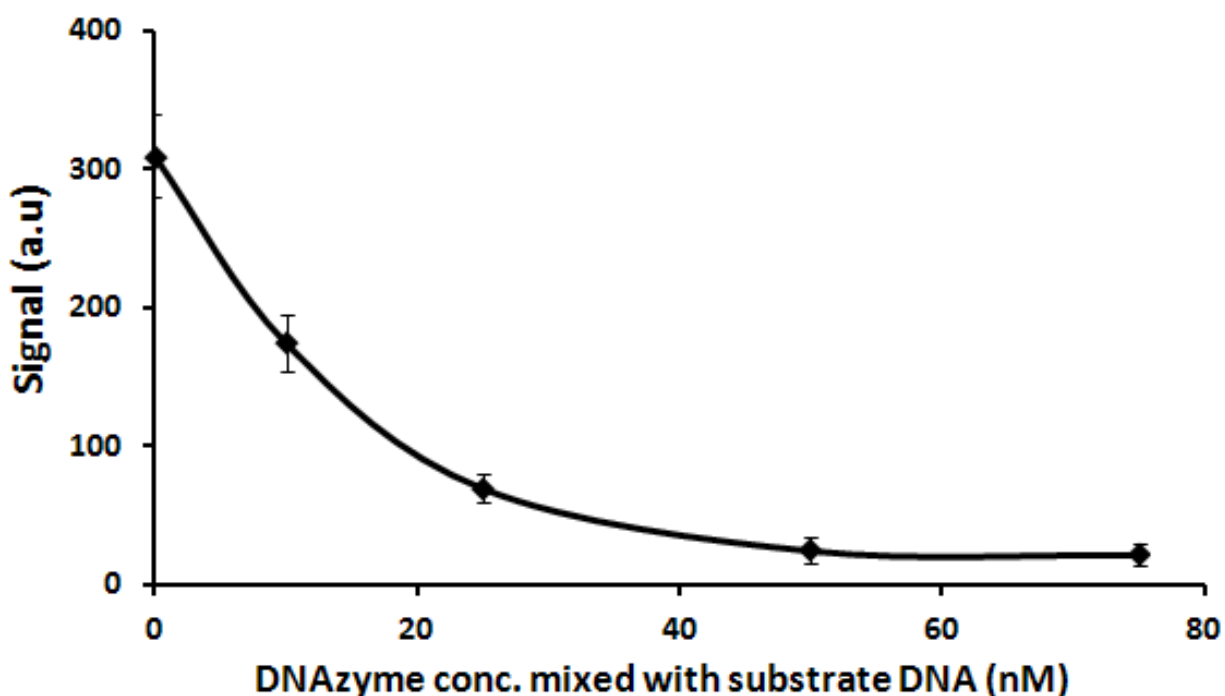


Figure 5 Experimental optimization of the DNAzyme concentration when using optimized fluorescent labeled substrate DNA (25 nM) including the BSA treatment for blocking non-specific sites. ΔS is the fluorescent intensity difference between peak intensity and base-line for each experiment. Each data value is the average of two independent experimental results.

3.4. Time optimization for pre-mixing step

To evaluate the impact of time length of the pre-mixing step on the sensor performance, 25 nM fluorescent labeled substrate DNA and 50 nM GR-5 DNAzyme were mixed and incubated for 1,

5, 10 and 15 minutes, respectively. The signal versus incubation time, as shown in Figure 6, indicated that 5 minutes is sufficient for binding between substrate DNA and GR-5 DNAzyme and therefore 5 minutes incubation time was chosen for all the subsequent analysis.

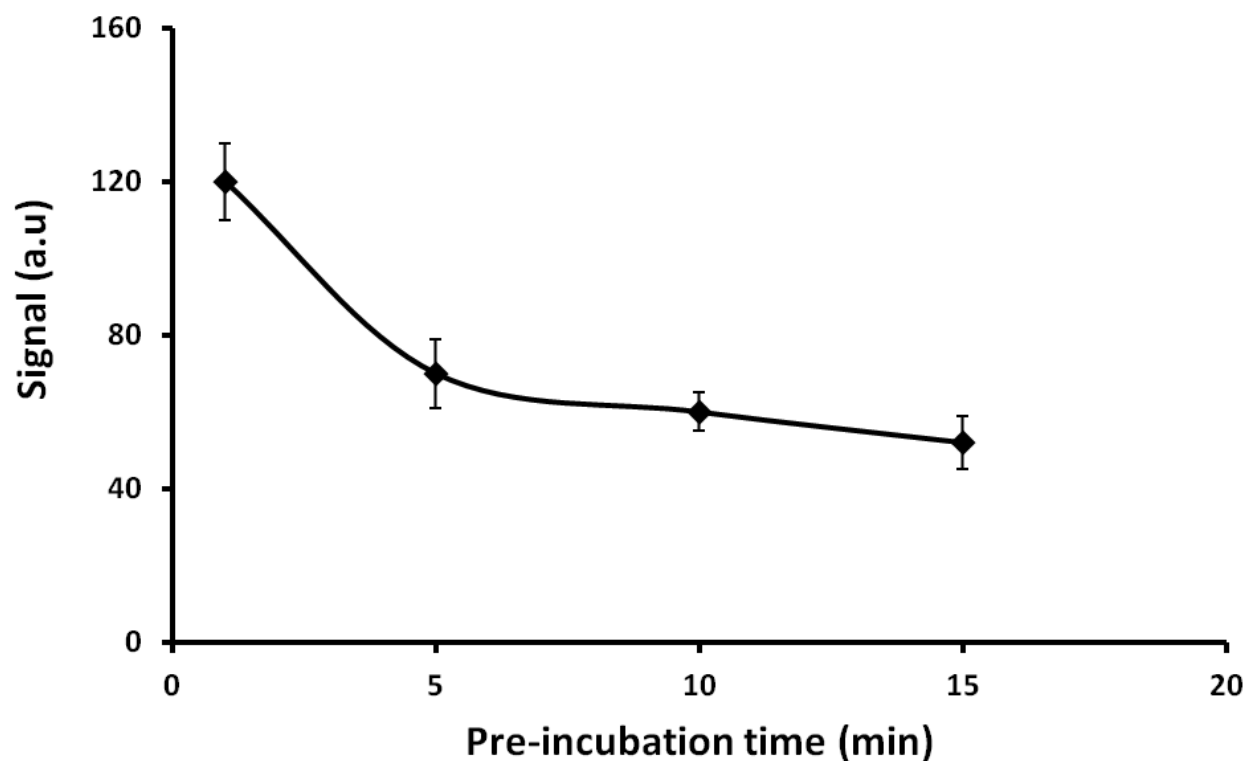


Figure 6 Experimental optimization of the pre-mixing time for DNAzyme (50 nM) and its specific substrate (25 nM). ΔS is the fluorescent intensity difference between peak intensity and base-line for each experiment. Each data value is the average of two independent experimental results.

3.5. Dose-response measurements of Pb^{2+}

Different amount of Pb^{2+} was added to the mixture that contained 25 nM of substrate DNA and 50 nM GR-5 DNAzyme and they were pumped into a flow cell immediately at a rate of 300 μ L/min for 30 s and allowed 5 minutes for the hybridization of the probe-DNA and its

1 complementary DNA (product of the GR-5 DNAzyme reaction). The sensing surface was then
2 regenerated with a 1% SDS solution (pH 1.9) for 60 s and washed with a PBS solution.

3 Figure 7 shows the temporal fluorescence signal during a typical test cycle for different amount
4 of Pb^{2+} detection using the optical sensor scheme developed herein, including the BSA treatment
5 for blocking non-specific sites. With adding Pb^{2+} to the substrate DNA and GR-5 DNAzyme
6 mixture the observed fluorescent signal was increasing depending on the Pb^{2+} concentration.
7 Since Pb^{2+} was initiating the enzymatic reaction of the GR-5 DNAzyme and the fluorescently
8 labeled product of the reaction was generated to hybridize to the probe-DNA.

9 Figure 8 shows the calibration curve for Pb^{2+} , which was normalized by expressing the signal of
10 each standard point as the ratio to that of the blank sample containing no Pb^{2+} . The error bars in
11 the figure correspond to the standard deviations of the data points in three independent
12 experiments, with the coefficient of variation of all the data points being within 3-8%.

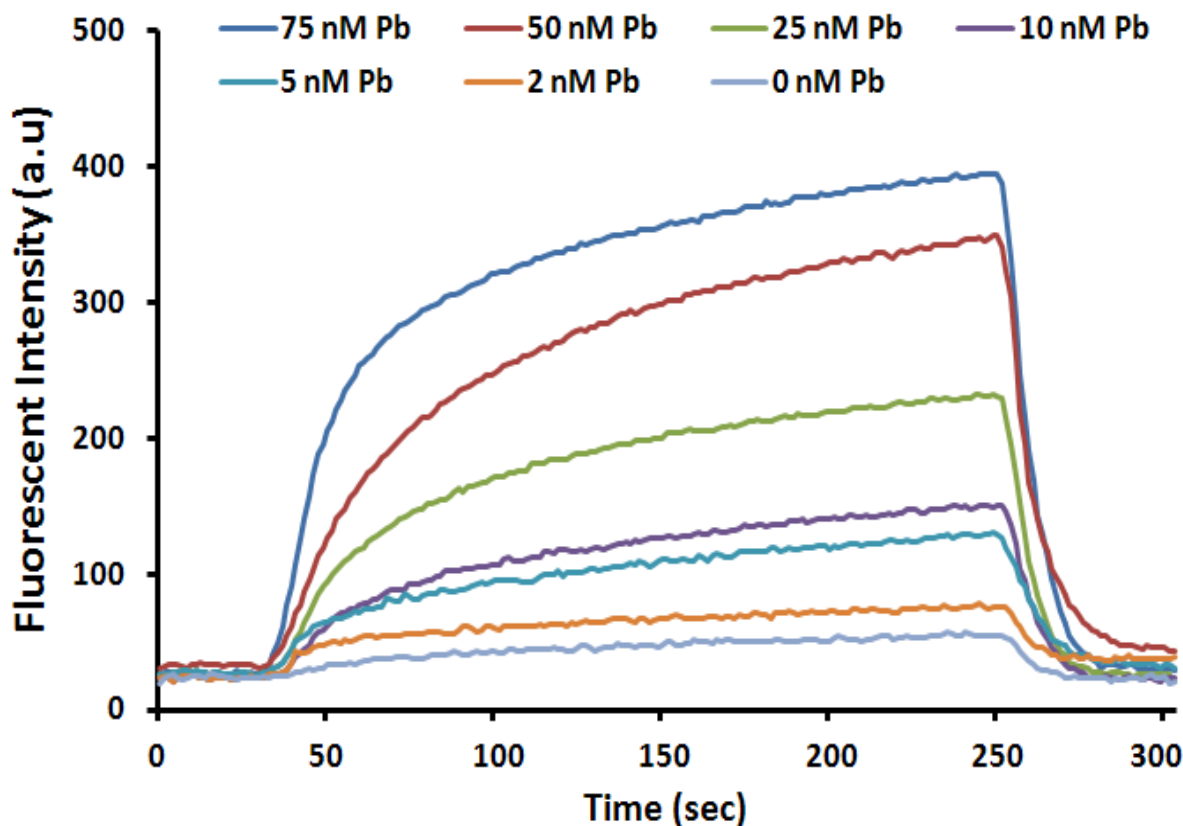


Figure 7 The fluorescence intensity responses during a typical test cycle for different amount of lead (II) ion using the optical sensor system, including the BSA treatment for blocking non-specific sites.

Based on the linear part of the dose-response curve (Figure 8) and the standard deviation of measurements, the detection limit was determined as 1.03 nM (0.21 ng mL^{-1}) by using $3\sigma/S$ calculation parameter (average standard deviation of measurements (σ) and slope of the linear range of the dose-response (S) fitting curve) (Yildirim et al. 2012; ACS Committee on Environmental Improvement 1980). The detection limit we obtained initially is comparable or lower than those reported in the literature as summarized in Table 1. In addition, compared to other sensors mentioned in Table 1, the biosensor developed here offers simpler or faster

detection (about 5 minutes detection time, and another 5 minutes for pre- incubation). And the portable platform also allows for potential on-site or real time measurements.

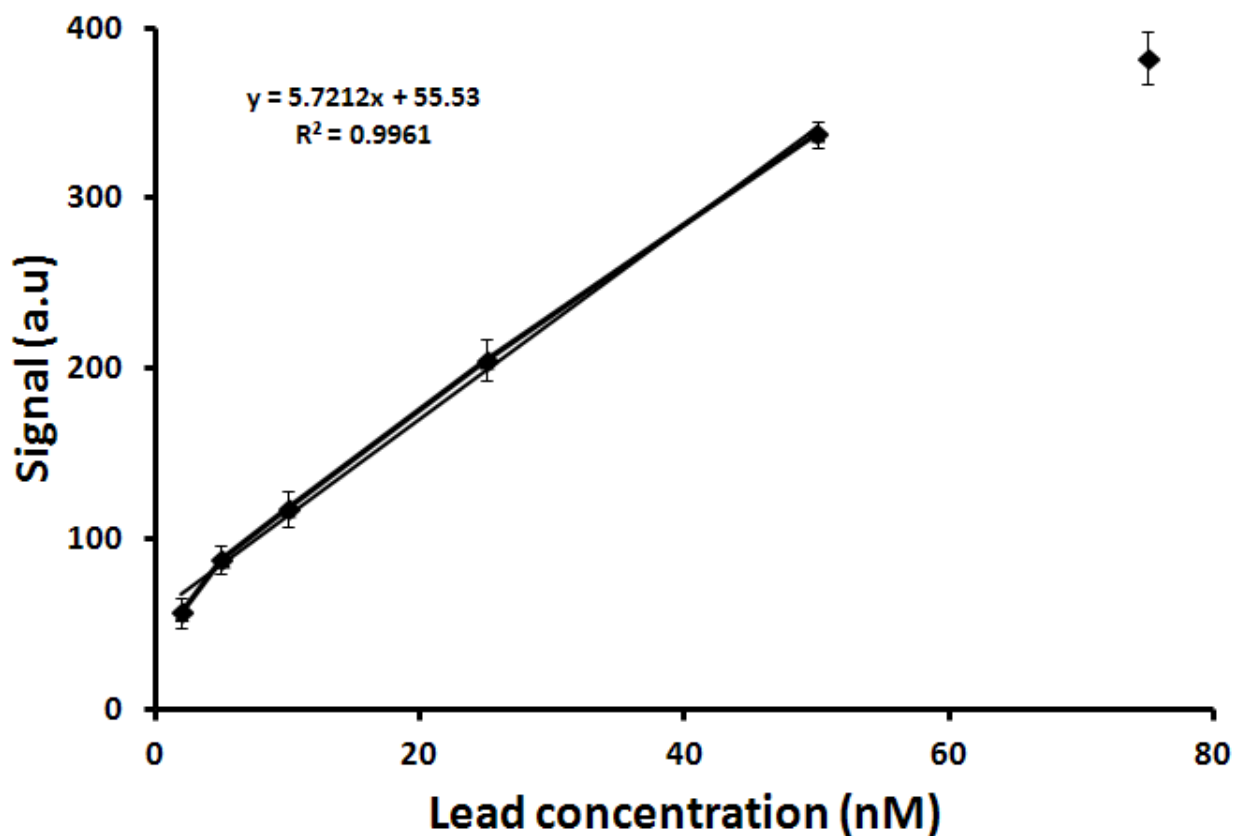


Figure 8 The calibration plot for determination of lead (II) ion concentration using the DNAzyme based fiber optic biosensor system. Signal is the fluorescent intensity difference between peak intensity and base-line. Each data value is the average of three independent experimental results.

Table 1. Comparison of the lead detection systems.

Detection method	Detection limit	Detection time	Reference
DNAzyme-based electronic detection via quantum dot	0.6 nM (0.13 ng mL ⁻¹)	60 minutes	Zhang et al. 2011
Organoclay film-based attenuated total reflectance sensor	200 ppb (0.2 µg mL ⁻¹)	10 minutes	Richardson et al. 2011
Laser-induced fluorescence detection in a laser-induced plasma	1.5 ppm (1.5 µg mL ⁻¹)	--	Laville et al. 2009
A flow-through optical fibre reflectance sensor based on immobilized gallocynine	0.06 ppm (0.06 µg mL ⁻¹)	5 minutes	Yusof and Ahmad 2003
DNAzyme sensor based on polymerase chain reaction	1 nM (0.21 ng mL ⁻¹)	15 minutes	Wang et al. 2009
Electrochemical detection via self-assembled monolayers	1.9 nM (0.4 ng mL ⁻¹)	--	Chow et al. 2005
An organically modified sol-gel membrane detection by using a fluorescence probe	8.3×10 ⁻⁸ mol/L (17.3 ng mL ⁻¹)	15 minutes	Guo et al. 2008
DNAzyme Based Biosensor on a Portable Optic Fiber Sensing Platform	1.03 nM (0.21 ng mL ⁻¹)	5 minutes	This study

3.6. Selectivity of the sensing system

To evaluate the sensor specificity and investigate potential interference from other metal ions, we evaluated the sensor's response to 40nM Hg^{2+} , Ca^{2+} , Cu^{2+} , Fe^{3+} , Mn^{2+} , Ni^{+} , Mg^{2+} , Co^{2+} and Ag^{+} . As seen in Figure 9, the sensor exhibits no significant response (<20%, as compared to a Pb^{2+} control) to these metal ions. The results showed that the developed biosensor system have high specificity toward Pb^{2+} , with much lower signal for all other metal ions tested. This high selectivity must be due to a specificity of GR-5 DNAzyme for Pb^{2+} ion.

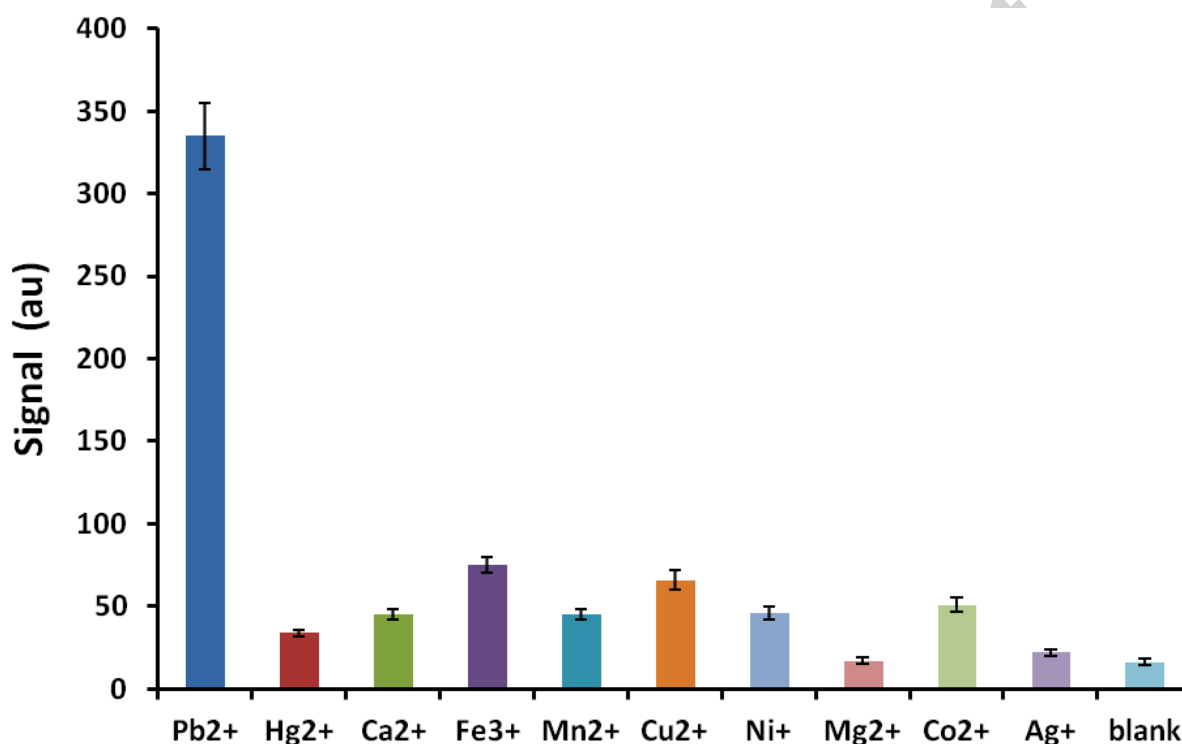


Figure 9 Comparison of sensor signals with lead (II) ion, other metal ions that present in environmental waste waters. All metal ions are at 40nM level, and each data value is the average of two independent experimental results.

3.7. Reusability and stability of the sensor

The regeneration performance of the sensing interface is an important issue for practical implementation of biosensors (Homola 2003). Therefore, the stability and reusability of the DNA probe covalently immobilized to the sensing surface was evaluated over a large number (>100) of assays. As seen in Figure 7, after each assay a complete removal of fluorescent labeled DNA, which is the product of the enzymatic reaction of GR-5 DNAzyme, was achieved using a 1% SDS solution (pH 1.9). After over 100 successive assays, less than a 5% loss of performance was observed (data not shown). We also investigated the storage stability of the proposed sensor system. After performing three daily measurements over 20 days of continuous analysis, a decrease in the average maximum signal response in the absence of analyte was less than 10% for fluorescent labeled DNA. This slight drop in fluorescence signal did not affect the DNA biosensor's specific response: all measurements were normalized with respect to the blank signal at the beginning of the daily analysis, and signal shifts in the blank and sample measurements were generally the same. After each serial determination of a competitive standard curve, a blank solution containing only GR-5 DNAzyme and its substrate DNA sequence was injected to test possible shifts from the baseline. In the figure S2 we can see summary results for this stability experiment.

3.8. Spiked environmental water samples analysis

To evaluate possible matrix effects, we tested the sensor developed herein with water samples using lab tap water and tertiary effluents from two wastewater treatment plants in USA. These environmental samples were spiked with Pb^{2+} from its stock solutions at concentrations of 10, 20, and 40 nM, followed by measuring the Pb^{2+} content as described above. The results obtained, shown in Table 2, reveal good consistencies between the actual and the measured Pb^{2+} concentrations. The recovery of all measured samples was between 88 and 98 %, and the parallel

1 tests showed that the percentage of coefficient of variation (cv %) values were between 0.07 and
 2 10.08 % (n = 3). These data confirm that the proposed sensing system is applicable for Pb²⁺
 3 detection with enough precision and accuracy even in real environmental sample matrices.

4 **Table 2.** Detection results of Pb²⁺ spiked wastewater samples.

Detection method	Detection limit	Detection time	Reference
DNAzyme-based electronic detection via quantum dot	0.6 nM (0.13 ng mL ⁻¹)	60 minutes	Zhang et al. 2011
Organoclay film-based attenuated total reflectance sensor	200 ppb (0.2 µg mL ⁻¹)	10 minutes	Richardson et al. 2011
Laser-induced fluorescence detection in a laser-induced plasma	1.5 ppm (1.5 µg mL ⁻¹)	--	Laville et al. 2009
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DNAzyme sensor based on polymerase chain reaction	1 nM (0.21 ng mL ⁻¹)	15 minutes	Wang et al. 2009
Electrochemical detection via self-assembled monolayers	1.9 nM (0.4 ng mL ⁻¹)	--	Chow et al. 2005
An organically modified sol-gel membrane detection by using a fluorescence probe	8.3×10 ⁻⁸ mol/L (17.3 ng mL ⁻¹)	15 minutes	Guo et al. 2008
DNAzyme Based Biosensor on a	1.03 nM (0.21 ng mL ⁻¹)	10 minutes (including pre-	Our work

4. Conclusion

In conclusion, we have developed a Pb^{2+} -dependent DNAzyme chemistry based evanescent wave optical biosensor for rapid and selective lead detection using a portable and easy-to-use all-fiber biosensing platform. The presence of Pb^{2+} cleaves the DNAzymes and releases the fluorescent labeled fragments, which further hybridize with the complementary strands immobilized on the optic fiber sensor surface. The sensing process can be completed in less than 10 min, with a detection limit of 1.03 nM (0.21 ng mL^{-1}). This sensor also demonstrated good selectivity against other metal ions that commonly coexist with Pb^{2+} . The performance of the biosensor evaluated in spiked wastewater samples showed good recovery, precision and accuracy, indicating that it was not susceptible to water matrix interferences even without any sample pre-treatments.

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Highlights

- A portable DNzyme based optical fiber sensor for lead detection in water samples
- DNzymes represents a highly promising class of recognition molecules for biosensors.
- A rapid, highly specific and sensitive detection of Pb²⁺ based on GR-5 DNzyme
- The detection range and limit are comparable to the levels in environmental water samples.