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ORIGINAL ARTICLE

DNAzyme Based Amplified Biosensor on Ultrasensitive Fluorescence Detection of Pb (II) Ions from Aqueous System

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Abstract A label -free DNAzyme amplified biosensor is found to be highly selective and sensitive towards fluorescent detection of Pb^{2+} ions in aqueous media. The DNAzyme complex has designed by the hybridization of the enzyme and substrate strand. In the presence of Pb^{2+}, the DNAzyme activated and cleaved the substrate strand of RNA site (rA) into two oligonucleotide fragments. Further, the free fragment was hybridized with a complementary strand on the surface of MBs. After magnetic separation, SYBER Green I was added and readily intercalate with the dsDNA to gives a bright fluorescence signal with intensity directly proportional to the concentration of Pb^{2+}ions. A detection limit of 5 nM in Pb^{2+} the detection range 0 to 500 nM was obtained. This label- free fluorescent biosensor has been successfully applied to the determination of environmental water samples. Then results open up the possibility for real-time quantitative detection of Pb^{2+} with convenient potential applications in the biological and environmental field.

Keywords Lead · Label-free DNAzyme · Biosensor · Syber green I · Fluorescence

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Introduction

Detection and removal of heavy metal ions, such as Pb^{2+}, Cd^{2+}, Cu^{2+} and Hg^{2+} has considerable attention, owing to their potential imposable in environmental and biological fields. Pb^{2+} is one of the most toxic heavy metals [1–7]. Even at very low concentration, it can affect human health and cause serious damage to the brain, nervous system, hypertension, mental retardation, reproductive system, anemia, as well as red blood cell and kidney damage [8–10]. Lead is not biodegradable and therefore remains are the ecological system and in the food chain indefinitely, exposing top levels predators to very high levels of pollution. For this reasons, safe limits or maximum contaminant level had been defined for drinking water by different organization from all over the world. The United States Environmental Protection Agency (EPA) has suggested that 15 ppb (0.07 μM) of lead is the permissible limit of drinking water, which US legislation requires (0.01 mg/L) and Bureau of Indian Standards (BIS) is 0.1 mg/L [11–14]. Therefore the development of a selective and sensitive methods for the detection of heavy metals ions is a considerable challenging.

As is well known, standard techniques for Pb^{2+} ions analysis requires sophisticated analytical technique such as, atomic adsorption spectroscopy (AAS), cold vapor atomic fluorescent spectroscopy, inductively coupled plasma, mass spectrometry and reversed –phase high performance liquid chromatography [15–21].These methods require expensive equipment, involve time consuming and laborious procedure that can be carried out only by trained professionals. In the past few years, analytical techniques based on fluorescence detection are very popular because fluorescent probes have received increasing attention due to its simplicity, sensitivity and efficiency. Recently amplification based label free methods had been reported for highly sensitive detection of

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Pb^{2+}, Hg^{2+} and Ag^{+} ions such as dynamic light scattering, rolling circle amplification and exonuclease aided recycling amplification of the biosensors are depend on the ssDNA and dsDNA [22–24]. The labeled DNAzyme based biosensor such as fluorescence [25, 26], electrochemical [27–29] and surface enhanced scattering sensor [30] are much simple and faster though it suffers from inadequate sensitive and selectivity. However amplification based label free DNAzyme biosensor technique is highly sensitive and selective for the detection of nanomole (nM) and picomole (pM) level Pb^{2+} with organic intercalating dye and gold nanoparticles [31–37].

In this work designed, by a highly selective fluorescent method of DNAzyme based amplified biosensor has been developed for detection of Pb^{2+} ions. A label free SG dye is able to intercalate with dsDNA and shows appreciable fluorescence intensity. In the presence of Pb^{2+}, the fluorescence intensity increases, when the free oligonucleotide fragment is hybridize with complementary strand on the surface of MBs. This amplified fluorescent biosensor is rapid, simple and inexpensive than other sensors and monitoring Pb^{2+} for environmental samples.

Experimental

Chemicals and Equipments

The substrate sequence 5'-ATC AGA TGA GAG AGA GA rA TCACATG-3′, GR - 5 DNAzyme 5'- TAG TCT ACT CT CT GAA GTA GCG CCG CCG TA GTG TAC -3′ and newly designed complementary strand 5′-Biotin - TC TCT CTC TCA TCT GAT −3′ were purchased from Protogen Biosciences Pvt Ltd. (Bangalore, India). The streptavadin (SA), carboxylated magnetic beads (MBs) and 10-fold concentrated organic fluorescent dye (SYBER Green I) was obtained from Invitrogen (Thermo Fisher Scientific, India). Lead nitrate, mercury(II) nitrate, calcium chloride, nickel(II) chloride, cobalt(II) chloride hexahydrate, copper(II) chloride dehydrate, cadmium (II) chloride hydrate, iron(II) chloride hexahydrate, magnesium chloride, manganese (II) chloride tetra hydrate, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDC) N-Hydroxysulfosuccinimide (sulfo-NHS) and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) were of analytical grade bought from Sigma–Aldrich. The buffer prepared from 10 mM HEPES, 10 mM NaCl and 2.5 mM $MgCl_2$ (pH = 7.4). Other standard chemicals were obtained from Sisco Research Laboratories Pvt, Ltd (India). The DNAzyme stock solution prepared by Milli-Q ultrapure water, which was used during the experiments.

All fluorescence spectra were recorded on a HORIBA JOBIN YVON Fluoromax-4 Spectro fluorometer equipped with Xenon lamp excitation sources. The excitation wavelength was set at 490 nm. The emission wavelength ranged from 490 to 530 nm, with excitation and emission slit is 10 nm. The Circular dichroism (CD) Spectra studies were carried out on a JASCO J-815 spectrometer (JASCO International Co. Ltd., Japan). All spectroscopic measurements were performed in triplicate.

Preparation of Complementary Strand DNA Coated MBs (DNA-MBs)

Preparation of SA coated magnetic beads (SA-MBs) according to the previous report [38]. One micrometer of Biotin modifies complementary strand and 1 µM of SA-MBs was reacted 30 min at room temperature. After magnetically washing three times in Tris - BSA buffer and stored at 4 °C.

Preparation of DNAzyme

To prepare the GR-5 DNAzyme, substrate DNA and GR-5 DNAzyme were mixed in a ratio of 5 µL(10 µM) and 10 µL (2 µM) in a 100 µL volume containing 10 mM HEPES (pH = 7.4). The mixture was incubated for 40 min, and the stock solution of GR-5 DNAzyme stored at 4 °C.

Fluorescence Detection of Pb^{2+}

The sensing performance of fluorescence detection of Pb^{2+} ions were carried out with 10 mM HEPES (pH = 7.4) buffer solution. To the 25 µL of GR-5 DNAzyme stock solution, the Pb^{2+} solution at various concentrations were added and then subsequently incubated at 37 °C for 40 min. Further, 50 µL of 20 nM (2.0×10^{-8} M) complementary strand on the surface of MBs were then added and gently shaken 30 min at room temperature. After magnetic separation, 6 µL of 5 µM SYBER Green I stock solution were directly added. The reaction mixture was then diluted with buffer solution and fluorescence spectra were recorded. All measurements were performed in triplicate.

Sample Analysis

To evaluate the real application of highly sensitive biosensor for detection of Pb^{2+} is environmental water samples. The samples were collected from Potheri pond (near SRM University campus). The collected samples were simply filtered to remove the insoluble substances. The water samples spiked with Pb^{2+} stock solutions at various concentrations. All the measurements was performed in triplicate. The fluorescence spectra with excitation wavelength at 490 nm indicate the presence of Pb^{2+}.

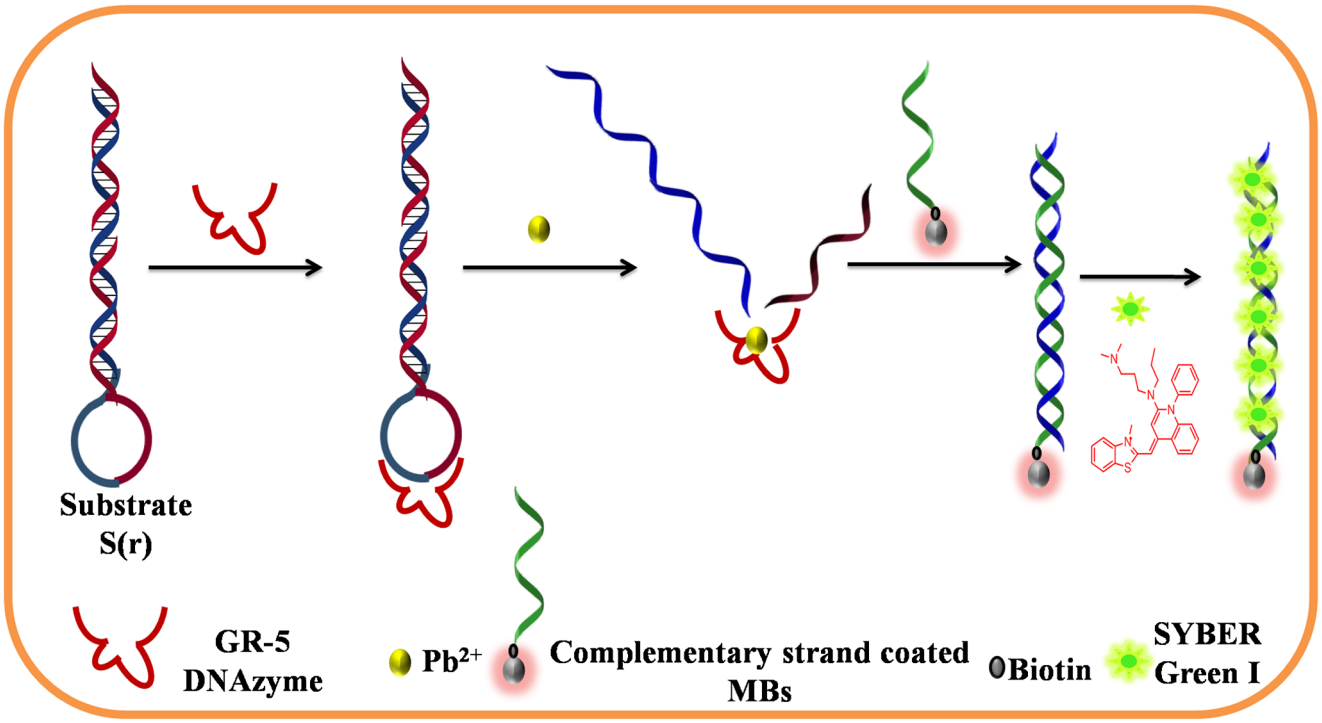
Results and Discussion

Sensing Mechanism

The detecting mechanism of the proposed biosensor was illustrated in Scheme 1. Here we developed a sensitive and selective label free fluorescent sensor for detection of Pb^{2+}ions. The sensing mechanism was introduced by GR-5 DNAzyme were developed for catalytic DNA based biosensor for its high selective towards Pb^{2+}. The formation of DNAzyme by intermolecular hybridization of substrate and enzyme strand subsequently forms a hairpin structure. According to the base pairing rule (A with T & C with G), further biotin modified complementary strand were designed. In the absence of Pb^{2+}, the hybridization between the enzyme strand and substrate strand retains the with hairpin structure. However, upon the addition of Pb^{2+}ions, the DNAzyme was cleaved the rA site into two fragments, which emerged from the cage. The cleaved substrate strand at the 5′- end of fragment is ready to bind with the modified new complementary strand on the surface of MBs to form dsDNA duplex. After magnetic separation, SYBER Green I was added to intercalate to the dsDNA, resulting in strong fluorescence intensities. In addition, some free fragment can undergo many cycles to trigger the cleavage of many hairpin substrate strands and the formation new duplex, consequently providing a significant fluorescent signal. In absence of Pb^{2+}, hybridization between the enzyme and substrate strand is stable hairpin structure, but no cleavage effect, and the fragment is not released dsDNA on the surface of MBs after magnetic separation. While SG is not intercalate with ssDNA and showing very weak fluorescent intensity compared to dsDNA. These sensing mechanisms were performed and explained by the Fig. 1, The curve (a) is the free SG and curve (b) is the GR-5 DNAzyme with addition of Pb^{2+}, but curve (a & b) is absence of fluorescence signal. In comparison to curve (c), upon the addition of DNA-MBs and SG the fluorescence intensity increases than the curve (a & b). Notably, this strategy is highly sensitive and selective for a signal amplified biosensor toward Pb^{2+}.

CD Spectroscopic Analysis of DNAzyme

To demonstrate the mechanism of this sensing system to monitor the conformational change of GR-5 DNAzyme is using CD measurements and presented Fig. 2. The CD spectra of the substrate strand (Curve a) shows a positive peak at 278 nm and negative peak at 245 nm, which is the characteristic peak of single strand DNA. The mixing of substrate and enzyme strand was formed duplex DNA with the positive peak at 278 nm and negative peak 245 nm in CD spectrum (Curve b), but the peak intensity is dramatically increased compared to single strand DNA. The CD spectra of after addition of Pb^{2+} in DNAzyme (Curve c) is explained that



Scheme 1 Schematic illustration of the label free DNAzyme biosensor for the detection of Pb^{2+} ions

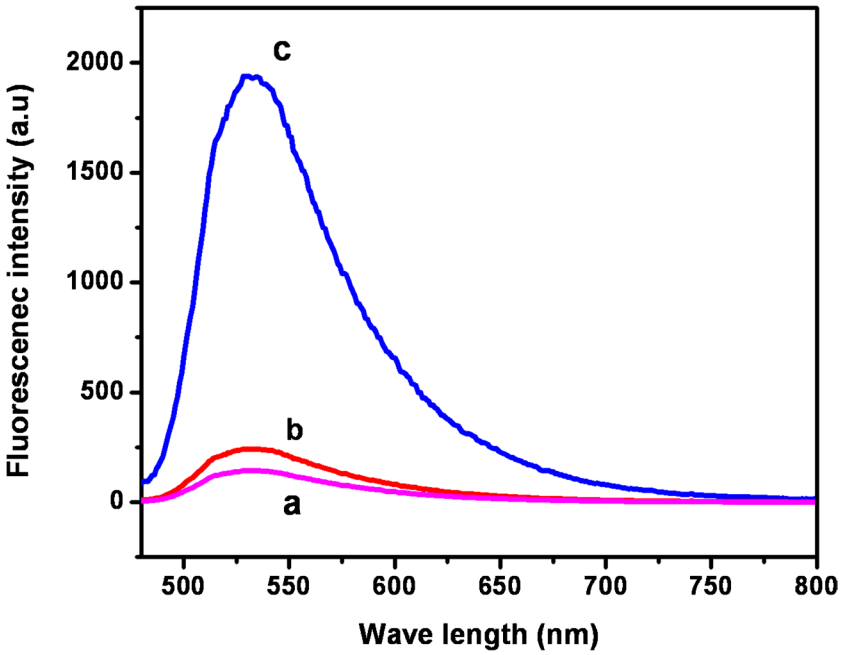


Fig. 1 Fluorescence emission spectra this sensing system **a** Free SG **b** GR- 5 DNA-zyme + Pb^{2+} **c** GR-5 DNA-zyme + Pb^{2++}complementary strand of DNA MBs + SG λ_{ex}= 490 nm and λ_{em}= 530 nm

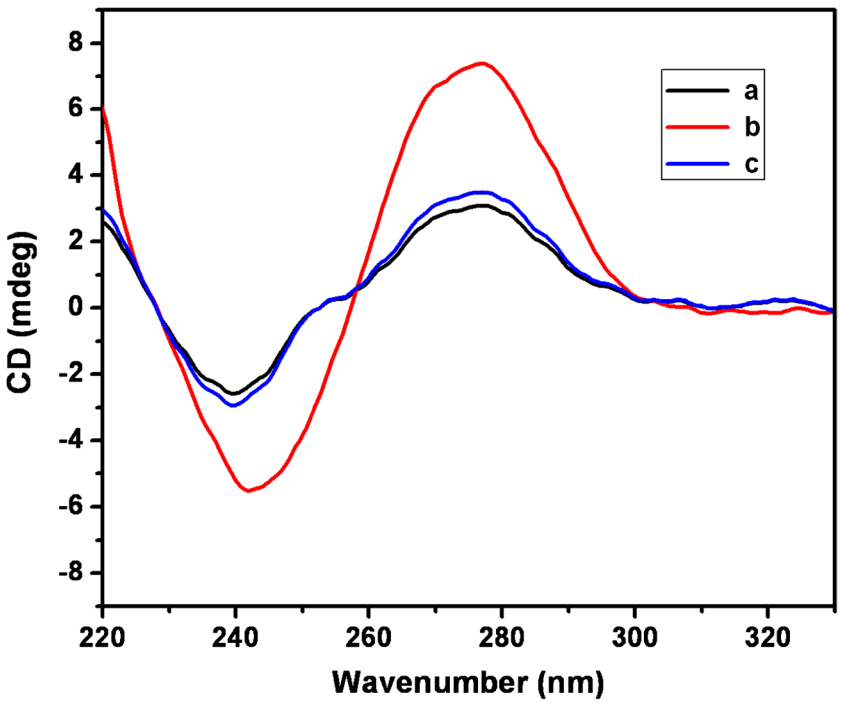


Fig. 2 CD spectra of this sensing system (a) Substrate strand (10 µM) (b) Substrate strand (10 µM) + GR-5 DNAzyme (10 µM) (c) GR-5 DNAzyme (10 µM) + Pb^{2+} (5 µM)

reduce the intensity of positive peak at 278 nm and negative peak 245 nm is almost same characteristic peak in substrate strand. This change indicates that cleavage of DNAzyme and become a single strand DNA by the addition of Pb^{2+}.

Optimization

In order to improve the sensing performance for the detection of Pb^{2+}, the required optimization conditions are complementary strand DNA concentration, SG volume, reaction time, which play a critical role in interaction of organic dye and label free DNAzyme. Figure 3 shows the effect of various concentration of complementary strand DNA hybridize with free substrate strand and fluorescence intensity can be optimized before performing detection of Pb^{2+} ions. The fluorescence intensity decreased with increasing concentration of complementary strand DNA. When the concentration of complementary strand is reached

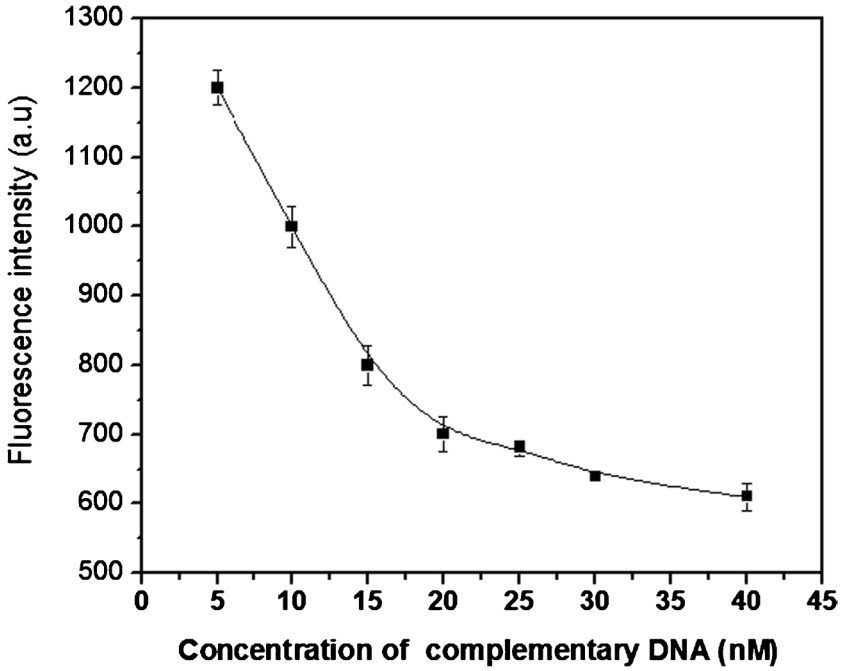


Fig. 3 The Optimization of label free DNAzyme biosensor for the detection of Pb^{2+}ions in different concentrations of complementary strand. The concentrations of substrate and enzyme strand 100 and 200 nM. Error bars represent the standard deviation in three individual experiments

up to 20 nM after increase the concentration fluorescence intensity is constant. We suggest that optimal concentration of complementary strand was selected as the 20 nM in the further study. The amount of SG was optimized by different volume is added into the after magnetic separation. Figure 4 given the effect of increase the volume of SG solution from 0 to 11 µL, fluorescence intensity increases with increasing volume of SG solution. The maximum intensity of fluorescence reaches at 6 µL and fluorescence intensity remains constant further addition of SG solution. Moreover, to study the influence reaction time for dsDNA formation. We studied the fluorescence intensity from 0 to 40 min for detection of 200 nM of Pb^{2+} ions as shown in Fig. 5. The results showed that the fluorescence intensity increased with gradually increasing reaction time, up to 20 min and then reaches a constant value over 20 min. On the basis of this result, the optimized conditions are fixed as 20 nM of complementary strand, volume of SG is 6 µL and 20 min were

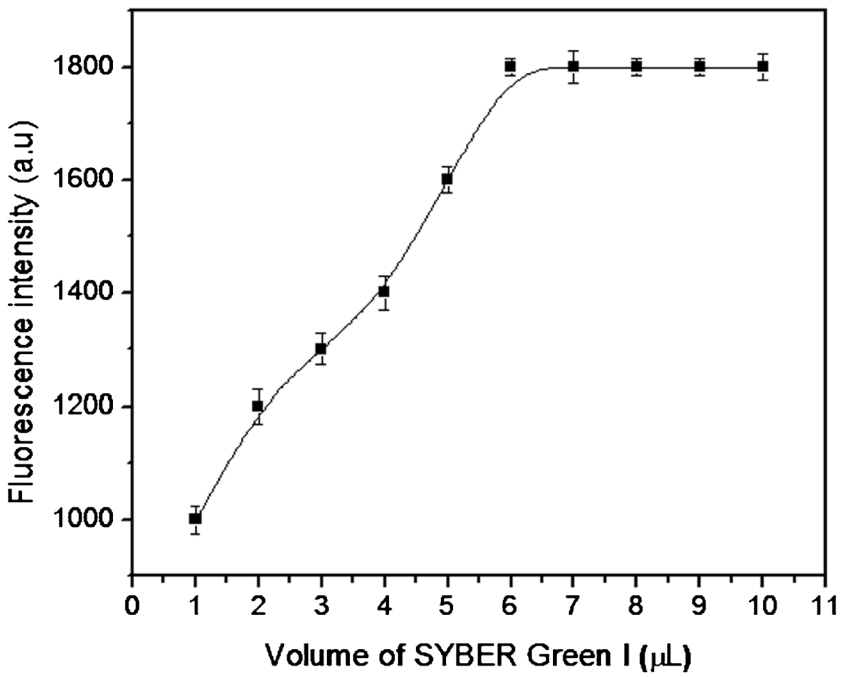


Fig. 4 The Optimization of label free DNAzyme biosensor for the detection of 100 µM Pb^{2+}ions in different volume of SG solution. The concentrations of substrate and enzyme strand 100 and 200 nM. Error bars represent the standard deviation in three individual experiments

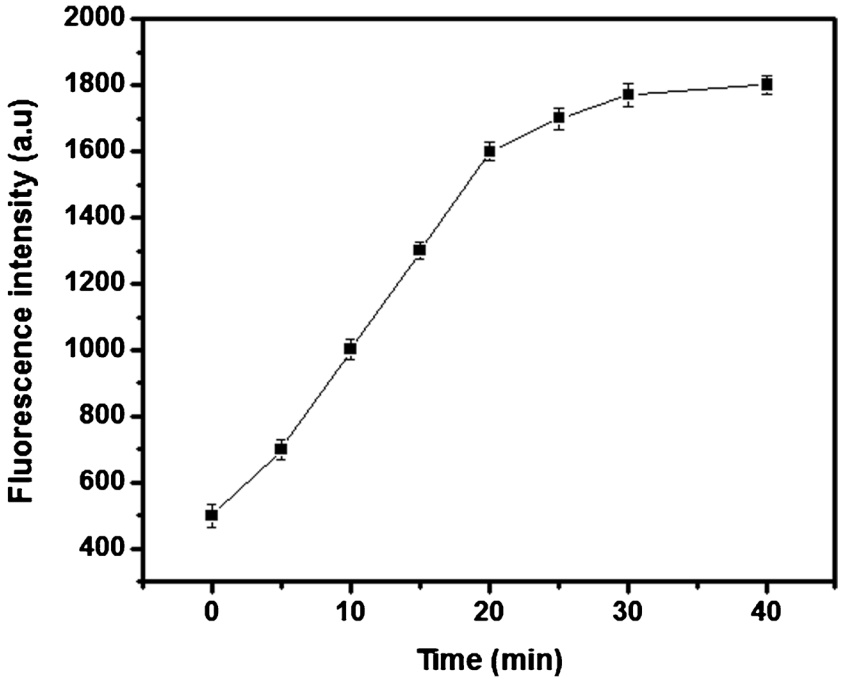


Fig. 5 The Optimization of label free DNAzyme biosensor for the detection of 100 μM Pb^{2+}ions in reaction temperature of after magnetic separation. The concentrations of substrate and enzyme strand 100 and 200 nM. Error bars represent the standard deviation in three individual experiments

chosen reaction time for dsDNA formation for further Pb^{2+} sensing experiments.

The Detection Performance of Pb^{2+}

Under the optimal conditions, the relationship between the Pb^{2+} ion concentrations and fluorescence enhancement were performed. Figure 6a displays the fluorescence spectra response to a series of concentrations of Pb^{2+} ions. The fluorescence intensity dramatically increased with the concentration of Pb^{2+} ions, ranging from 0 to 500 nM, significantly more and more substrate strand cleaved and folding to the complementary strand on the surface of MBs to the formation of dsDNA with intercalated SG dye with increasing Pb^{2+} ion concentration. Figure 6b shows the relationship between fluorescence intensity and concentrations of Pb^{2+} ions. As shown in the inset of Fig. 6b, the proposed methodology exhibited a good linear response of fluorescence intensity versus the concentration of Pb^{2+} over the range from 0 to 50 nM. The detection limit of this sensor

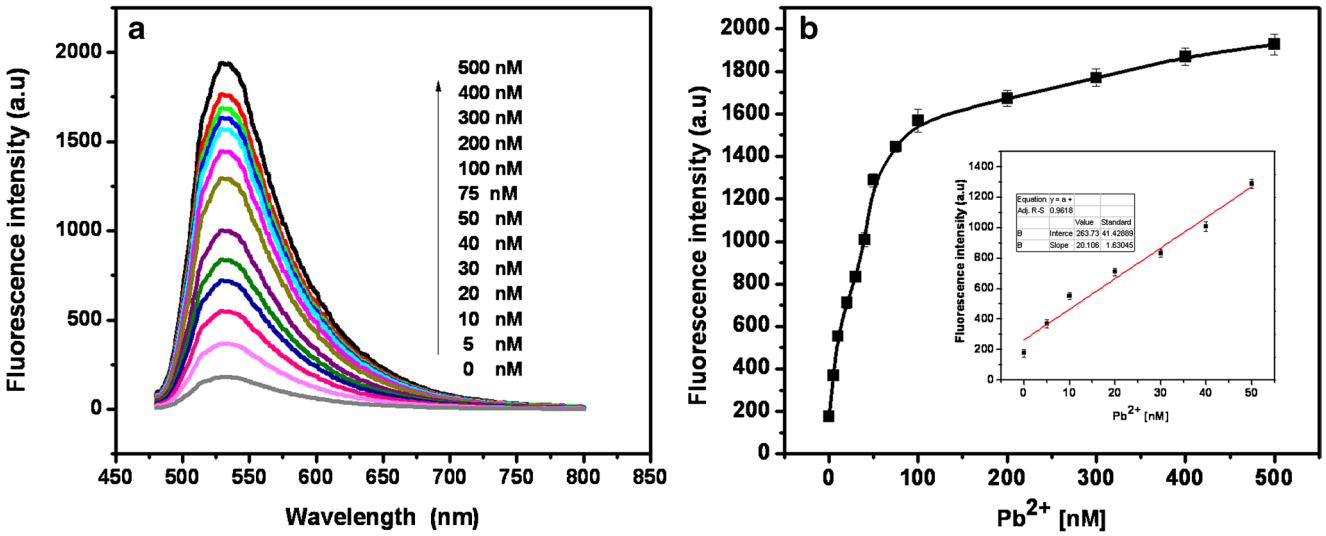


Fig. 6 **a** The fluorescence emission spectra of this sensing system upon the addition of different concentrations Pb^{2+} ions (0 to 500 nM) and then mixed SG. **b** The fluorescence intensity increases at various concentrations of Pb^{2+}. Insert shows that linear relationship between the concentration and fluorescence intensity. Error bars represent the standard deviation in three individual experiments

is 5 nM using three sigma method, which is comparable to other biosensors, where preferential fluorescence an listed in Table 1. The results indicate that the proposed Pb^{2+} sensor is sufficient for Pb^{2+} in the environment.

Selectivity of Pb^{2+}sensor

The proposed sensing systems are the selectivity performances were carried out by fluorescence spectra responses of DNAzyme to various cationic species. In the absence of metal ions a weak fluorescence was observed at 530 nm. The divalent metals such as Ca^{2+}, Fe^{2+}, Cr^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Mg^{2+}, Ba^{2+}, Sn^{2+}, Ni^{2+} and Ag^{+} is compared to Pb^{2+} were carried out fluorescence responses under the optimized conditions. However, the presence of Pb^{2+} ions can induce a fluorescence emission at 530 nm and color change from yellow to green. As shown in Fig. 7a, b, the result demonstrated that the signal intensities of other competitive metal ions were still much lower than Pb^{2+}. The selectivity towards Pb^{2+}sensing by DNAzyme in the presence of other metal ions could be attributed to several factors, such as large radius and the nitrogen affinity character of the Pb^{2+} ions. In fact, the experimental result observed that these ions shown no obviously interference for Pb^{2+} detection. Thus, DNAzyme exhibited excellent selectivity toward developed Pb^{2+}bionsensor, which makes it reasonable for practical application.

Detection of Pb^{2+} in Environmental Samples

In order to evaluate the proposed new method for the practical application, the new biosensor system was used to analyze Pb^{2+} in tap water and pond water samples. Through this detection strategy, limit of detection 5 nM for Pb^{2+} analysis is achieved. The lead stock solution at different concentrations such as 5, 10 and 20 nM was spiked in the samples and were studied. The analytical results are summarized in Table 2. As the toxic level Pb^{2+} defined by the U.S Environmental Protection Agency (EPA) in drinking water is below 10 nM. This data recovery range is above 96%, which would meet the detection requirement. It is found that the proposed sensor could accurately determine the Pb^{2+}concentraton in environmental samples.

Table 1 Comparison of the proposed method by other fluorescence detection methods for Pb^{2+}

Pb (II) ion sensor	Method	Detection limit	Environmental sample	Reference
GR-5 DNAzyme	Fluorescence	5 nM	Tap and pond water	This work
DNAzyme-Perylene complex	Fluorescence	100 pM	River water	[39]
DNAzyme-AUR	Fluorescence	0.4 nM	Soil and salt water	[40]
17E DNAzyme	Fluorescence	10 nM	Soil sample	[34]
8–17 DNAzyme	Fluorescence	20 nM	-	[41]
8–17 DNAzyme	Fluorescence	10 nM	Lake water	[42]

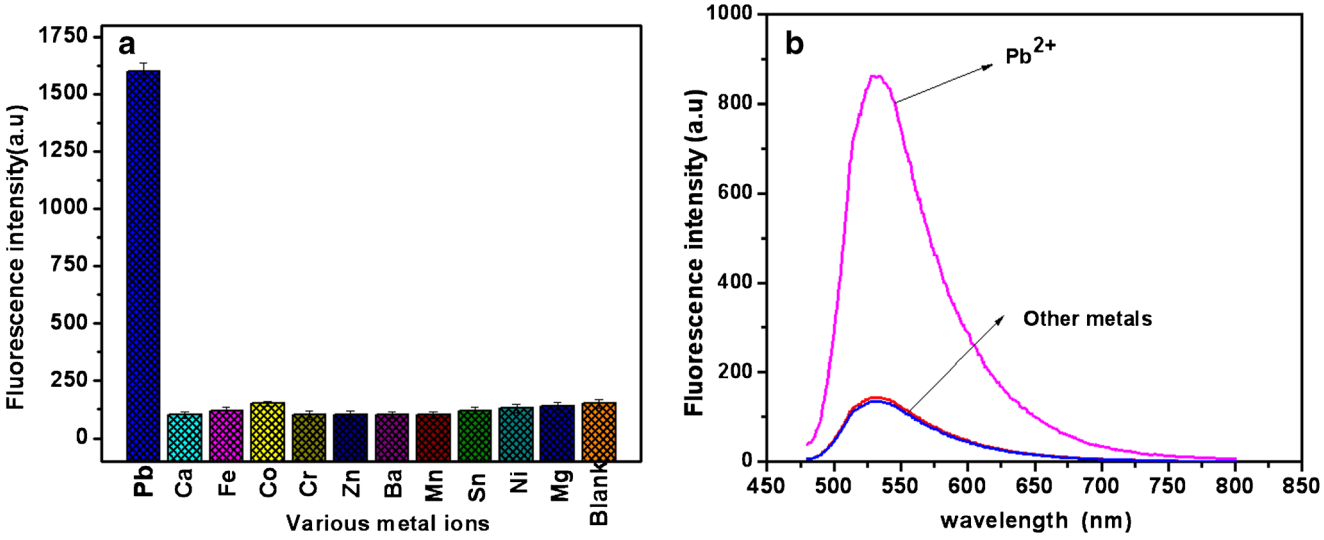


Fig. 7 **a** The selectivity analysis of DNAzyme sensor for Pb^{2+} against other competing metal ions. The concentrations of Pb^{2+} are 30 and 300 nM of other metal ions. **b** The fluorescence intensity of Pb^{2+} ions and other competing metal ions. Error bars represent the standard deviation in three individual experiments

Table 2 The amount of Pb^{2+} in environmental samples proposed by in this sensor

Sample	Spiked (nM)	Detected ± SD	Recovery (%)
Tap water	5	5.03[a] ± 0.15[b]	100.6
	10	10.26 [a] ± 0.41 [b]	102.6
	20	19.79 [a] ± 1.5 [b]	98.95
Pond water	5	5.05 [a] ± 0.13 [b]	101
	10	9.79 [a] ± 0.75 [b]	97.9
	20	19.22 [a] ± 0.38[b]	96.1

[a] Mean values of three determinations [b] Standard deviation

Conclusion

In summary, we have design a simple, highly sensitive, selective and cost effective, a label free DNAzyme based biosensor for the detection of Pb^{2+}. The organic dye SYBER Green I is able to intercalate with dsDNA, resulting excellent selectivity to Pb^{2+}. In this amplified fluorescent detection method a limit of Pb^{2+} is achieved 5 nM and over the other metal ions. We further analyzed the detection of Pb^{2+} ions in environmental water sample, and explored their potential application for determining the concentration. These results indicate that the fluorescence based biosensor could be widely used for the fast detection of toxic metal ions and wastewater treatment.

References

1. Panich S, Wilson KA, Nuttall P, Wood CK, Albrecht T, Edel EB (2004) Label–free Pb(II) whispering gallery mode sensing using self-assembled glutathione-modified gold nanoparticles on an optical micro cavity. Anal Chem 86(23):6299–6306
2. Nolan EM, Lippard SJ (2009) Small – molecule fluorescent sensors for investigating zinc metallonuerochemistry. Acc Chem Res 42(1):193–203
3. Xiang Y, Tong A, Lu Y (2009) Abasic site-containing DNAzyme and aptamer for label-free fluorescent detection of Pb^{2+} and adenosine with high sensitivity, selectivity and tunable dynamic range. J Am Chem Soc 131(42):15352–15357
4. Xiang Y, Lu Y (2014) DNA as sensors and imaging agents for metal ions. Inorg Chem 53:1925–1942
5. Li F, Yang L, Chen M, Li P, Tang B (2012) A selective amperometric sensing platform for lead based on target-induced strand release. Analyst 138:461–466
6. He QW, Miller EW, Wong AP, Chang CJ (2006) A sensitive fluorescent sensor for detecting lead in living cells. J Am Chem Soc 128:9316–9317
7. Schneider E, Clark DS (2013) Cytochrome P450 (CYP) enzymes and the development of CYP biosensors. Biosens Bioelectron 39:1–13
8. Godwin HA (2001) The biological chemistry of Lead. Curr Opin Chem Biol 5:223–227
9. Needleman H (2004) Lead poisoning. Annu Rev Med 55:209–222
10. Ratte HT (1999) Bioaccumulation and toxicity of silver compounds: review. Environ Toxicol Chem 18(1):89–108
11. BIS (1981) Tolerance limits for industrial effluents prescribed by bureau of indian standards, IS 2490(Part I) New Delhi
12. Yang X, Xu J, Tang X, Liu H, Tian D (2010) A novel electrochemical DNAzyme sensor for the amplified detection of Pb^{2+} ions. Chem Commun 46:3107–3109
13. Wuertz S, Bishop S, Wilderer PA (2003) Biofilms in wastewater treatment: an interdisciplinary approach. IWA, London
14. Teixerira CR, Andradea TFN, Oliveirac FM, Corazzac MZ, Azevedo LFM, Segatellid MG (2011) Synthesis and application of imprinted polyvinylimidazole-silica hybride copolymer for Pb^{2+} determination by flow-injection thermospray flame furnace atomic absorption spectrometry. Anal Chim Acta 703:145–151
15. Silva MVL, Frescura VL, Curtius A (2000) Determination of trace elements in water samples by ultrasonic nebulization inductively coupled plasma mass spectrometry after cloud point extraction. Spectrochimi A J Acta Part B 55:803–813
16. Elfering H, Anderson JT, Poll KG (1998) Determination of organic soils and waters by hydride generation inductively coupled plasma atomic emission spectrometry. Analyst 123:669–674
17. Wu J, Boyle EA (1997) Low blank pre-concentration technique for the determination of lead, copper and cadmium in small-volume seawater samples by isotope dilution ICPMS. Anal Chem 69:2464–2470
18. Chan MS, Huang SD (2000) Direct determination of copper in seawater using transversely heated graphite furnace atomic adsorption spectrometer with Zeeman-effect background corrector. Talanta 51:373–380
19. Santosa SJ, Tanaka S, Yamanaka K (1997) Sequential determination of trace metals in sea water by inductively coupled plasma mass spectrometry after electrochemical vaporization of their dithiocarbamate complexes in methyl isobutyl ketone. Environ Monit Assess 44:515–528
20. Rea RW, Keeler GJ (1998) Microwave digestion and analysis of foliage for total mercury by cold vapour atomic fluorescence spectroscopy. Biogeochemistry 40:115–123
21. Al-Assaf KH, Tyson JF, Uden PC (2009) Determination of four arsenic species in soil by sequential extraction and high performance liquid chromatography with post-column hydride generation and inductively coupled plasma optical emission spectrometry detection. J Anal Atom Spectrom 24:379–384
22. Miao X, Ling L, Shuai (2011) Ultraaensitive detection of lead(II) with DNAzyme and gold nanoparticles probes by using a dynamic light scattering technique. Chem Commun 47:4192–4194
23. Tang S, Tong P, Li H, Tang J, Zhang L (2013) A novel label-free electrochemical sensor for Hg^{2+} based on the catalytic formation of metal nanoparticle. Biosens Bioelectron 42:608–611
24. Xu H, Xu P, Gao S, Zhang L, Fan C, Zuo X (2013) Highly sensitive recognition of Pb^{2+} using Pb^{2+} triggered exonuclease aided DNA recycling. Biosens Bioelectron 43:520–523
25. Seeling G, Yurke B, Winfree E (2006) Catalyzed relaxation of a metastable DNA fuel. J Am Chem Soc 128:12211–12220
26. Li J, Cao Z, Lu Y (2000) Functional nucleic acid sensors. Chem Rev 109(5):1948–1998
27. Li J, Lu Y (2000) A highly sensitive and selective catalytic DNA biosensor for lead ions. J Am Chem Soc 122:10466–10467
28. Liu J, Lu Y (2012) Metal ion dependent DNAzymes and their applications as biosensors. Met Ions Life Sci 10:217
29. Zhu, X, Lin Z, Chen L, Qiu B, Chen G (2009) A sensitive and specific electro-chemiluminescnt sensor for lead based DNAzyme. Chem Commun 6050–6052
30. Gao A, Tang CX, He XW, Yin XB (2013) Electrochemilumiscent lead biosensor based on GR-5 lead-dependent DNAzyme for $Ru(phen)_3^{2+}$ intercalation and lead recognition. Analyst 138:263

31. Wu Y, Cai Z, Wu G, Rong M, Jiang Y, Yang CJ, Chen X (2014) A novel signal-on DNAzyme –based electrochemiluminescencesensor. Sensors Actuators B 191: 60–66
32. Wang Y, Irudayaraj J (2011) A SERS DNAzyme biosensor for lead ion detection. Chem Commun 47:4394–4396
33. Wang FL, Wu Z, Lu YX, Wang J, Jiang JH, Yu RQ (2010) A label free DNAzyme sensor for lead (II) detection by quantitative polymerase chain reaction. Anal. Biochem 405: 168–173
34. Zhang LB, Han BY, Li T, Wang EK (2011) Label-free DNAzyme-based fluorescing molecular switch for sensitive and selective detection of lead. Chem Commun 47:3099–3101
35. Wang J, Liu B (2008) Highly sensitive and selective detection of Hg^{2+} in aqueous solution with mercury –specific DNA and Syber Green I. Chem Commun 4759–4761
36. Chen YY, Chang HT, Shiang YC, Hung YL (2009) Colorimetric assay for lead ions based on the leaching of gold nanoparticles. Anal Chem 81:9433–9439
37. Liu JW, Lu Y (2004) Accelerated color change of gold nanoparticles assembled by DNAzymes for simple and fast colorimetric Pb^{2+} detection. J Am Chem Soc 126:12298–12305
38. Chen G, Chen J, Wu W, Fang F, Chen L, Liu Q, Wang L, Xing X, Zeng L (2013) A enzyme-free and label-free assay for copper(II) ion detection based on self-assembled DNA concatamers and Syber Green I. Analyst 138:4737–4740
39. Fu T, Zhao XH, Bai HR, Zhao ZL, Hu R, Kong RM, Zhang XB, Tan W, Yu RQ (2013) A superquenched DNAzyme-perylene complex: a convenient, universal and low-background strategy for fluorescence catalytic biosensors. Chem Commun 49:6644
40. Li CL, Liu KT, Lin YW, Chang HT (2011) Fluorescence detection of lead(II) ions through their induced catalytic activity of DNAzymes. Anal Chem 83:225–230
41. Nagaraj N, Liu J, Sterling S, Wu J, Lu Y (2009) DNAzyme catalytic beacon sensors that resist temperature-dependent variations. Chem Commun 27:4103–4105
42. Liu J, Lu Y (2003) Improving fluorescent DNAzyme biosensors by combining inter - and intramolecular quenchers. Anal Chem 75:66–6672