Scientific paper

Simultaneous Sensitive Detection of Lead(II), Mercury(II) and Silver Ions Using a New Nucleic Acid-Based Fluorescence Sensor

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Abstract

A new nucleic acid-based fluorescence sensor is reported for simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+} based on the specific catalytic activity of Pb^{2+} for a particular DNAzyme, specific regulation of Ag^{+} on "C-Ag^{+}-C" complex, and stable complex formed by Hg^{2+} and rhodamine B isothiocyanate (RBITC). Three fluorescence dyes, aminomethylcoumarin acetic acid (AMCA), 5-carboxyfluorescein (FAM), and RBITC, were modified on the probes and served as fluorescent donors. Upon DNA interaction with these metal ions and AuNP fluorescence quenching effect on the fluorescence dyes, the fluorescence recovery of RBITC and the fluorescence quenching of AMCA and FAM were monitored to detect Hg^{2+}, Pb^{2+}, and Ag^{+}, separately, without the need of using any masking reagents. This sensor exhibited high sensitivity and selectivity. The limit of detection (LOD) is 0.48 nM for Pb^{2+}, 0.23 nM for Ag^{+}, and 0.17 nM for Hg^{2+}. Finally, this sensor was successfully applied for simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+} in real sample.

Keywords: Fluorescent sensor; Au nanoparticles (AuNPs); DNAzyme; simultaneous detection; metal ions

1. Introduction

With the ever increasing pollution from modern industry, heavy metal contaminants have posed severe adverse effects on human health and ecosystems due to their high and persistent toxicities.[1,2] Therefore, it is quite necessary and urgent to rapidly and accurately detect these metal ions. Traditional methods, such as atomic absorption spectrometry (AAS),[3] inductively coupled plasma mass spectrometry (ICP-MS),[4] and anodic stripping voltammetry (ASV),[5] have high sensitivity and selectivity but require specialized instrumentation and extensive sample pretreatment processes which limit their applications for *in situ* analysis.[6,7]

In recent years, much effort has been devoted toward design of DNA-based sensors to detect heavy metal ions, especially Ag^{+}, Hg^{2+}, and Pb^{2+}, which are three of the most toxic heavy metals.[8] The detection of Pb^{2+} relies on the specific catalytic activity of Pb^{2+} for the particular DNAzyme. For Pb^{2+} detection, most detectors were based on the Pb^{2+}-dependent DNAzyme[9,10] and Pb^{2+}-stabilized G-quaduplex.[11,12] As for Hg^{2+} and Ag^{+}, the detections relies on the selective capture of Hg^{2+} by T-T mismatches to form T-Hg(II)-T base pairs,[13,14] and the exclusive recognition of Ag^{+} by C-C mismatches to form C-Ag(I)-C complex.[15,16] Accordingly, various detection techniques, such as colorimetry, [17–19] electrochemistry,[10,20,21] and fluorescence,[15,22,23] were applied to selectively detect Pb^{2+}, Ag^{+}, or Hg^{2+}. Given that metal ions usually coexist in several samples, some researches have been focused on the simultaneous detection of two or more metal ions at trace level, such as Pb^{2+} and Hg^{2+}, [24,25] as well as Hg^{2+} and Ag^{+}.[26–28]

However, with regard to the sensors designed for the simultaneous detection of three metal ions, there are only a few relevant reports. Zhang et al.[29] developed a colorimetric assay for parallel detection of Cd^{2+}, Ni^{2+}, and Co^{2+} utilizing peptide modified gold nanoparticles as a sensing element based on its unique surface plasmon resonance properties. Hien et al.[30] designed a fluorescent chemosensor based on dimethylaminocinnamaldehyde-aminothiourea and applied it for simultaneous detection of Ag^{+}, Hg^{2+}, and Pb^{2+}. Lin et al.[31] reported an unlabeled immobilized DNA-

based sensor for simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+} by electrochemical impedance spectroscopy (EIS) with $[Fe(CN)_6]^{4-/3-}$ as redox probe. However, they have some limitations including poor selectivity, insufficient sensitivity, or the need of using the masking reagent.

In this paper, we designed a DNA-based sensor to achieve a rapid, simple and simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+} based on the DNA interaction with these metal ions and AuNP fluorescence quenching effect on the fluorescence dyes. Three fluorescence dyes, aminomethylcoumarin acetic acid (AMCA), 5-carboxyfluorescein (FAM), and rhodamine B isothiocyanate (RBITC), were introduced in this assay to detect Pb^{2+}, Ag^{+}, and Hg^{2+}, respectively. Consequently, no masking reagent was needed in this method so that the detection process was simplified and speeded up. Fluorescence spectra were used at trace level due to its high sensitivity.

2. Experimental

2. 1. Materials and Instrumentation

Reagents including $AgNO_3$, $Hg(NO_3)_2$, $Pb(NO_3)_2$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Al(NO_3)_3$, $Co(NO_3)_2$, $Cu(NO_3)_2$, $Cr(NO_3)_3$, $Zn(NO_3)_2$, $Ni(NO_3)_2$, KNO_3, $NaNO_3$, $Cd(NO_3)_2$, trisodium citrate, 1% $HAuCl_4$, K_2CO_3, RBITC, High Performance Liquid Chromatography (HPLC) purified oligonucleotides (aDNA:5'-FAM-ACCCCTC-3', bDNA:5'-ATGTCACTT-3'-SH-, cDNA: 5'-AMCA-AAGTGACA TrAGGACGATCACCCCT-3'-SH-, dDNA:5'- ATCGTCTCCGAGCCGGTCGAAATGTC-3') were purchased from Shanghai Sangon Biotechnology Co., Ltd. Deionized water (18.2 MΩ cm resistivity) from a Millipore Milli-Q system was used throughout this work.

Fluorescence spectra were recorded by F-4600 fluorescence spectrophotometer (Hitachi, Japan) with the excitation and emission slit widths 5.0 nm and 10.0 nm, voltage 700 V, and excitation and emission wavelengths of 495 nm and 517 nm for FAM-ssDNA, 530 nm and 580 nm for RBITC, and 353 nm and 450 nm for AMCA, respectively.

2. 2. Preparation of Functionalized AuNPs Probe and Analytical Procedure

cDNA (5 μM) and dDNA (5 μM) were mixed uniformly, reacting for 5 min in 90 °C water bath. Then the mixture was gradually cooled to room temperature to form double-stranded "cDNA+dDNA".

RBITC solution (1 mM, 10 μL) was added into AuNPs suspension (13 nm, 1 mL); the mixture was incubated at room temperature for 2 h, and then centrifuged. The filter cake was added to double-stranded "cDNA+dDNA" solution (5 μM) and bDNA solution (5 μM), respectively, to synthesize cDNA+dDNA-AuNP and bDNA-AuNP probes. Next, the solution containing cDNA+dDNA-AuNP and bDNA-AuNP probes was mixed uniformly with the same volume of aDNA solution. All the prepared mixtures were stored at 4 °C for later use.

For Pb^{2+} sensing, Pb^{2+} solutions of different concentrations (10, 50, 100, 300, 500, 700 and 1000 nM) were prepared and added into the sensor solution prepared as described above, reacting at room temperature for 20 min. The concentration of both Ag^{+} and Hg^{2+} were 10 μM in these solutions. Afterwards, the fluorescence emission spectra were measured at excitation wavelength of 353 nm. For Ag^{+} and Hg^{2+} detection, similar procedures were followed to those described for Pb^{2+}.

For the selectivity measurement, other metal ions solution (10 μM) were added into the sensor solution, and the fluorescence spectra were monitored at excitation wavelengths of 353 nm, 495 nm, and 530 nm, respectively.

The real sample was collected from East Lake in Wuhan City and used after being filtered, and the sample was spiked with different concentrations of Pb^{2+}, Ag^{+}, and Hg^{2+} to implement the recovery test.

3. Results and Discussion

3. 1. Sensing Strategy

Fig. 1 depicts the process of simultaneous detection. Three fluorescent dyes, AMCA, FAM, and RBITC, served as fluorescent donors for detection of Pb^{2+}, Ag^{+}, and Hg^{2+}, respectively. The newly synthesized AuNPs were selected as fluorescent receptor owing to their advantages, such as: small particle size, large specific surface area, strong adsorption capacity and excellent water-solubility. RBITC was initially combined with AuNP (recorded as AuNP-RBITC), resulting in fluorescence quenching at 580 nm. AMCA was specially designed to label at one end of the substrate strand of 8-17 DNAzyme, and the other end was combined with AuNP, emitting fluorescence signal at 450 nm. FAM was combined with ACCCCTC-3' (aDNA), and this FAM-aDNA fluoresced at 520 nm. In addition, the surfaces of some AuNPs were modified with 5'-ATGTCACTT-3'-SH-(bDNA). Then when adding Pb^{2+}, Ag^{+}, and Hg^{2+} into the bulk solution, the fluorescence intensity would change due to the interaction between these metal ions and the DNA sequences labeled by fluorescent dye. Pb^{2+} cleaved the substrate strand of DNAzyme at the ribonucleic adenosine (rA) base, releasing two kinds of DNA fragments: AuNP-cDNA and AMCA-dDNA. dDNA complementarily paired with bDNA-AuNP, to shorten the distance between AMCA and the surface of AuNPs, resulting in fluorescence quenching of AMCA. Simultaneously, Ag^{+} prompted AuNP-cDNA and aDNA to form a strong double-stranded DNA via the stable "C-Ag+-C" complex, resulting in fluorescence quenching of FAM. There is also a limitation in our sensor that Ag^{+} cannot be detected in the absence of Pb^{2+}. If there is no Pb^{2+} in the system, a slight amount of Pb^{2+} should be introduced to trigger the subsequent reactions.

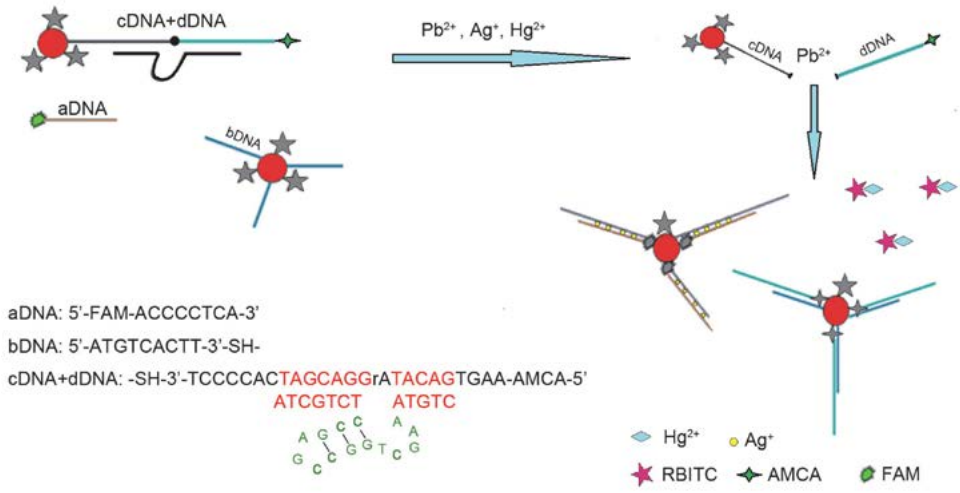

Fig. 1. Schematic of simultaneous detection of Ag^+, Hg^{2+}, and Pb^{2+} using AuNP-based fluorescent sensors.

As for Hg^{2+}, owing to the larger stability constant of the complex formed by Hg^{2+} and RBITC than that of the complex formed by AuNPs and RBITC, RBITC would displace from the surface of AuNPs and combine with Hg^{2+}, leading to fluorescence recovery of RBITC.

3. 2. Simultaneous Detection of Pb^{2+}, Ag^+, and Hg^{2+}

Fig. 2 shows the fluorescence emission spectra of the AuNP probe solution before and after adding Pb^{2+}, Ag^+, and Hg^{2+}. As illustrated in Fig. 2(A), the presence of Pb^{2+} in the AuNP-bDNA and AuNP-cDNA+dDNA-AMCA solution leads to ~95% fluorescence quenching of AMCA (compare Curves 3 with 1 or 2), due to the complementary pairing of AuNP-bDNA with the released AMCA-dDNA caused by Pb^{2+}-induced cleavage. In Fig. 2(B), fluorescence of FAM quenched ~70% with the addition of Ag^+ into FAM-aDNA and AuNP-cDNA-dDNA-AMCA (compare Curves 6 with 4 or 5) owing to the Ag^+-introduced combination of FAM-aDNA and AuNP-cDNA released after Pb^{2+}-induced cleavage. In Fig. 2(C), the significant fluorescence recovery of RBITC (compare Curves 7 and 8) proves the strong binding of Hg^{2+} and RBITC, which impelled the RBITC's departing from surface of AuNPs and caused fluorescence recovery.

In Fig.2(A), the coincidence of Curve 1 and 2 shows that fluorescence signal was almost unchanged when AuNPs-bDNA was added into AuNP-cDNA+dDNA-AMCA, indicating dDNA and bDNA would not pair in the absence of Pb^{2+}, which can guarantee the precision for Pb^{2+}. Similarly, fluorescence signal of FAM remained almost unchanged when aDNA was added into AuNP-cDNA+dDNA-AMCA (compare Curve 4 with 5). It is because cDNA part of the DNAzyme substrate strand was specially designed to be rich in an odd number of C bases arranged asymmetrically, so as to avoid the combination of "C-Ag^+-C" complex by aDNA itself in the presence of Ag^+ and improve the precision of detection of Ag^+. In order to further ensure the precision of this detection method, we analyzed the mutual impacts among the three metal ions during the detection process. As shown in Fig. 3, the detection results remained unchanged in the presence of all three metal ions or only one of these metal ions, proving

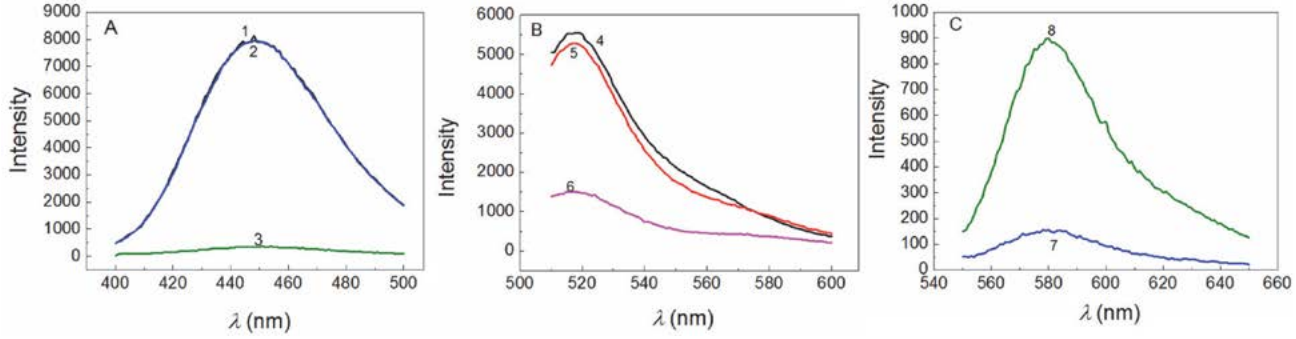

Fig. 2. Fluorescence emission spectra of the detection of (A) Pb^{2+} (λ_{ex} = 353 nm, λ_{em} = 450 nm), (B) Ag^+ (λ_{ex} = 495 nm, λ_{em} = 517 nm), (C) Hg^{2+} (λ_{ex} = 530 nm, λ_{em} = 580 nm) in the solution containing (1) AuNP-cDNA-dDNA-AMCA, (2) AuNP-cDNA-dDNA-AMCA + AuNP-bDNA, (3) AuNP-cDNA-dDNA-AMCA + AuNP-bDNA + Pb^{2+}, (4) FAM-aDNA, (5) FAM-aDNA + AuNP-cDNA, (6) FAM-aDNA + AuNP-cDNA + Ag^+, (7) AuNP-RBITC, (8) AuNP-RBITC + Hg^{2+}.

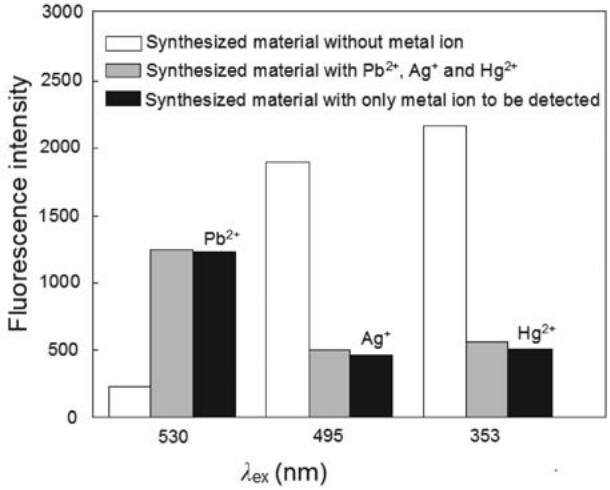

Fig. 3. Mutual impacts among the three metal ions during the detection process.

that the detection of these three metal ions was independent from each other.

Furthermore, fluorescence intensity changes rapidly in response to the addition of the metal ions. As shown in Fig. 4, the reactions reached equilibrium after about 600 s for Pb^{2+}, 200 s for Ag^{+}, and 200 s for Hg^{2+}. The results indicated that this sensor allows a rapid detection of three heavy metal ions with high stability.

3. 3. Sensitivity and Selectivity of Simultaneous Detection for Pb^{2+}, Ag^{+}, and Hg^{2+}

In order to evaluate the sensitivity of the sensor for Pb^{2+}, Ag^{+}, and Hg^{2+}, different concentrations of these metal ions were added into the sensor solution under the optimized conditions such as pH = 8.0, RBITC concentration is 1 mM and DNA concentration is 5 μM.

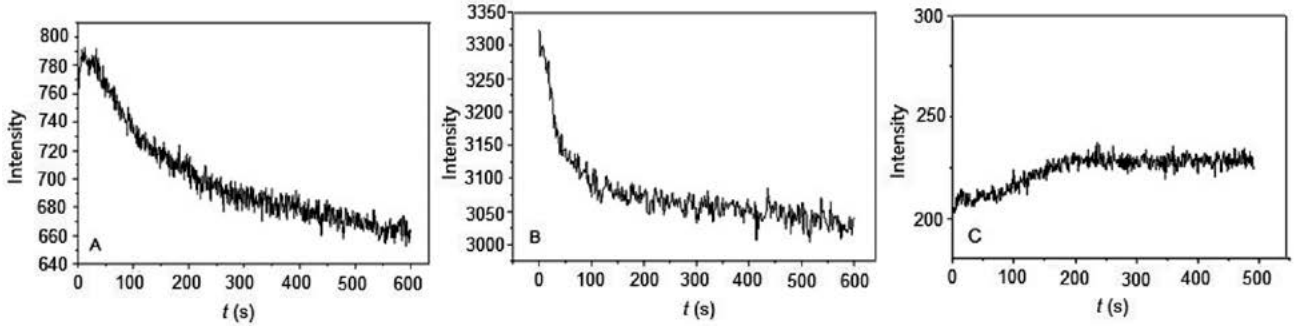

Fig. 4. Fluorescence intensity changes vs. time after adding (A) Pb^{2+}, (B) Ag^{+}, and (C) Hg^{2+}.

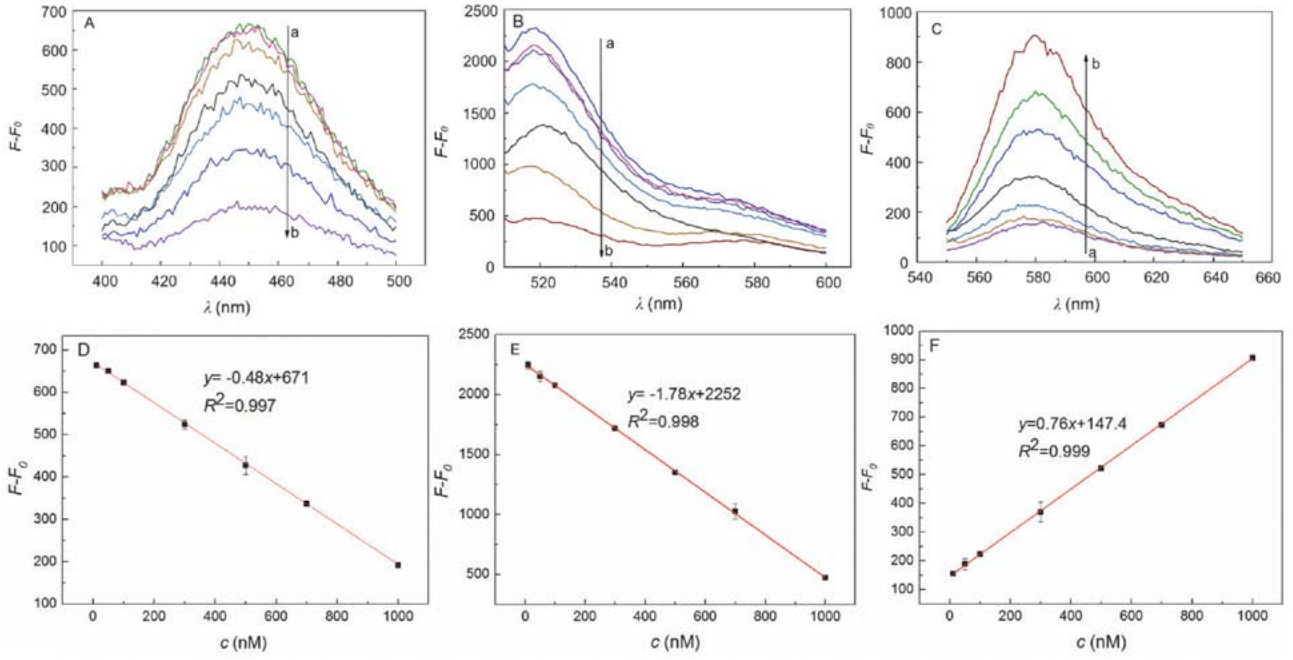

Figure 5. Fluorescence emission spectra of (A) Pb^{2+} (λ_{ex} = 353 nm, λ_{em} = 450 nm), (B) Ag^{+} (λ_{ex} = 495 nm, λ_{em} = 517 nm), and (C) Hg^{2+} (λ_{ex} = 530 nm, λ_{em} = 580 nm), and standard curves of (D) Pb^{2+}, (E) Ag^{+}, and (F) Hg^{2+} in the concentration of (a~b) 10, 50, 100, 300, 500, 700, 1000 nM. *F* is measured fluorescence intensity, F_0 is background fluorescence intensity.

As shown in Fig. 5, the fluorescence spectra changed regularly. The intensity of AMCA (λ_{ex} = 353 nm, λ_{em} = 450 nm) and FAM (λ_{ex} = 495 nm, λ_{em} = 517 nm) gradually decreased with increased concentration of Pb^{2+} and Ag^{+}, respectively; while the intensity of RBITC (λ_{ex} = 530 nm, λ_{em} = 580 nm) increased with the increased concentration of Hg^{2+}, which proved the fluorescence quenching caused by Pb^{2+} and Ag^{+}, and the fluorescence recovery caused by Hg^{2+}. The LOD (limit of detection) of this assay was 0.48 nM, 0.23 nM and 0.17 nM for Pb^{2+}, Ag^{+}, and Hg^{2+}, respectively. The linear range was 10 nM~1000 nM for the three ions.

The selectivity of the sensing system was also explored: the fluorescence spectra were monitored upon adding other metal ions (such as 10 μM Mg^{2+}, Ca^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+}, Cu^{2+}) to the sensing system in the presence of 1 μM Ag^{+}, Hg^{2+}, and Pb^{2+}. As shown in Fig. 5, Only Pb^{2+} , Ag^{+}, and Hg^{2+} caused considerable changes in the intensity of fluorescent signals while other ions have little effect on this system. These results illustrated that the sensor was specifically responding to the three metal ions.

The comparison of the proposed sensor with other methods for simultaneous determination of three metal ions is listed in Table 1. As it can be seen, the proposed sensor has a comparable and even higher sensitivity to the previous reports, and no masking reagent was used in this method, indicating that this sensor was an appropriate platform for the determination of these metal ions.

3. 4. Detection of Pb^{2+}, Hg^{2+}, and Ag^{+} in Real Samples

This sensor's application in real samples (water of the East Lake) were investigated by Standard Recovery Test. the sample of the East Lake water was filtered through a 0.45 μm cellulose acetate filter membrane, and then the water sample (10 μL) was added to the prepared sensor. Afterwards, the standard solution of three metal ions was added to reach the final concentration of 10 nM. The fluorescence intensities were detected at 450 nm, 580 nm and 517 nm. The fluorescent quenching responding to Pb^{2+} and Ag^{+}, and recovery responding to Hg^{2+} were also observed in East Lake water (as indicated in Fig. 7), and Table 2 shows that the satisfactory recoveries were obtained for the real samples. The average recoveries are

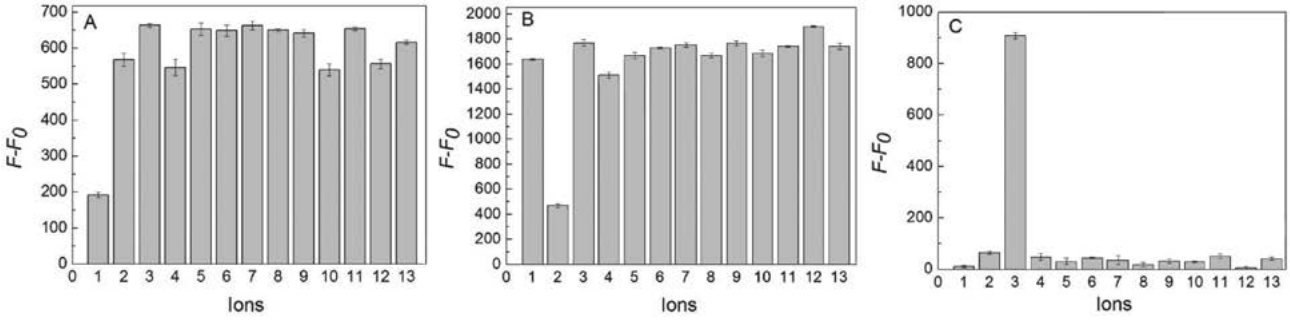

Figure 6. Selective detection of three metal ions at (A) λ_{ex} = 353 nm, λ_{em} = 450 nm; (B) λ_{ex} = 495 nm, λ_{em} = 517 nm; (C) λ_{ex} = 530 nm, λ_{em} = 580 nm. 1-13: Pb^{2+}, Ag^{+}, Hg^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+}, Cu^{2+}. The concentrations of Pb^{2+}, Ag^{+}, Hg^{2+} to be measured were 1 μM, and the concentrations of other metal ions were 10 μM. *F* is measured fluorescence intensity, F_0 is background fluorescence intensity.

Table 1. The comparison of our sensor with other methods for simultaneous determination of three metal ions.

Method	Ions detected and its LOD	Using masking reagent	Ref.
peptide modified gold nanoparticles probe	Cd^{2+} (0.05 mM) Ni^{2+} (0.3 mM) Co^{2+} (2 mM)	Y	[29]
fluorescent chemosensor based on dimethylaminocinnamaldehyde-aminothiourea	Ag^{+} (1.0 ppb) Hg^{2+} (2.8 ppb) Cu^{2+} (0.8 ppb)	Y	[30]
DNA-based sensor	Ag^{+} (10 nM) Hg^{2+} (0.1 nM) Pb^{2+} (10 pM)	Y	[31]
DNA-based sensor	Ag^{+} (0.23 nM) Hg^{2+} (0.17 nM) Pb^{2+} (0.48 nM)	N	Our method

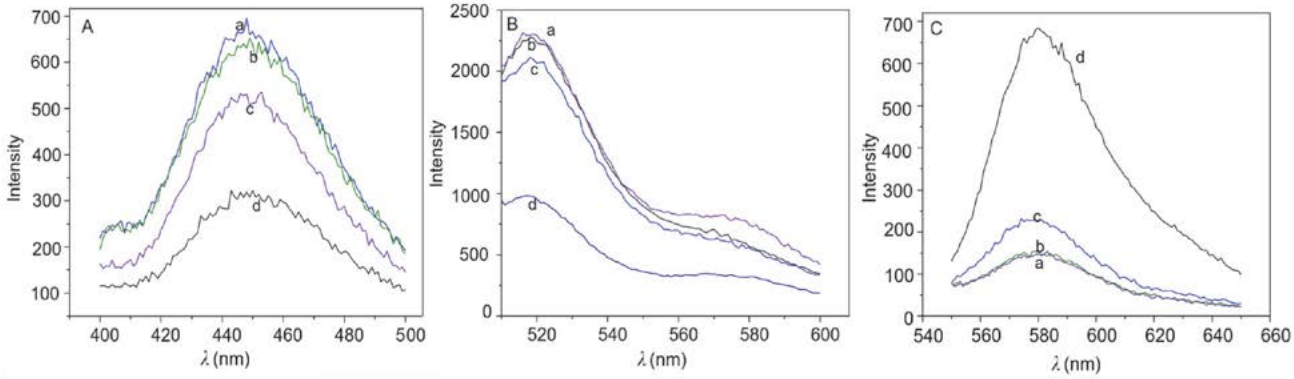

Figure 7. Fluorescence curves of (A) Pb^{2+} (with added standard concentration of (a) 0, (b) 50 nM, (c) 300 nM, and (d) 700 nM), (B) Ag^{+} (with added standard concentration of (a) 0, (b) 10 nM (c) 100 nM, and (d) 700 nM), and (C) Hg^{2+} (with added standard concentration of (a) 0, (b) 10 nM (c) 100 nM, and (d) 700 nM) in East Lake water.

Table 2. Standard Recovery Test for detection of the metal ions in East Lake water.

Metal ions	Sample	Measured concentration in test sample /nM	Added standard concentrations /nM	Measured concentration after adding standard concentrations / nM	Recovery /%
	1	9.79	50	67.71	115.8
Pb^{2+}	2	9.79	300	331.04	107.1
	3	9.79	700	726.46	102.4
	1	1.12	10	12.36	112.4
Ag^{+}	2	1.12	100	105.62	104.5
	3	1.12	700	719.72	102.7
	1	1.45	10	11.32	98.7
Hg^{2+}	2	1.45	100	105.39	103.9
	3	1.45	700	707.76	100.9

108.43% for Pb^{2+}, 106.53% for Ag^{+} and 101.17% for Hg^{2+}. These results confirmed that the proposed sensor can be successfully used to detect Pb^{2+}, Ag^{+}, and Hg^{2+} in real samples.

4. Conclusions

In this paper, we have described a successful design of a new and simple fluorescent sensor for simultaneous detection of Pb^{2+}, Ag^{+}, and Hg^{2+} based on the specific catalytic activity of Pb^{2+} for a particular DNAzyme, specific regulation of Ag^{+} on "C-Ag^{+}-C" complex, stable complex formed by Hg^{2+} and RBITC, and AuNP fluorescence quenching effect on fluorescence dyes. Fluorescence quenching of AMCA and FAM, and the fluorescence recovery of RBITC indicated the presence of Pb^{2+}, Ag^{+}, and Hg^{2+}, and the intensity changed corresponding to concentration of these ions. The detection limits of three metal ions were 0.48 nM for Pb^{2+}, 0.23 nM for Ag^{+} and 0.17 nM for Hg^{2+}.It has been proven that this sensor is characterized by good stability, high sensitivity and selectivity, fast detection speed, and easy operation, and has successfully produced satisfactory detection results in real samples. These attributions suggest that our approach provides a well suitable method to simultaneous detection of a variety of heavy metal ions in environmental monitoring.

5. Acknowledgements

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Povzetek

Poročamo o novem fluorescenčnem senzorju na osnovi nukleinskih kislin za simultano detekcijo Pb^{2+}, Ag^{+} in Hg^{2+} ionov. Osnovan je na specifični katalitski aktivnosti Pb^{2+} ionov za določen DNA encim; na specifični regulaciji Ag^{+} na »C-Ag^{+}-C« kompleksu; na stabilnem kompleksu, ki ga tvorita Hg^{2+} in rodamin B izotiocianat (RBITC). Tri fluorescenčna barvila: aminometilkumarin ocetna kislina (AMCA), 5-karboksifluorescein (FAM) in RBITC, smo dodali raztopinam in so služila kot donorji fluorescence. Zaradi interakcije DNA s temi kovinskimi ioni in efekta dušenja fluorescence, ki so ga imeli AuNP delci na fluorescenčna barvila, smo lahko spremljali povečanje fluorescence RBITC za detekcijo Hg^{2+} ter dušenje fluorescence pri AMCA in FAM za ločeno detekcijo Pb^{2+} in Ag^{+}, ne da bi bilo treba uporabljati maskirne reagente. Senzor je pokazal visoko občutljivost in selektivnost. Meja zaznave (LOD) je 0,48 nM za Pb^{2+}, 0,23 nM za Ag^{+} in 0,17 nM za Hg^{2+}. Na koncu smo senzor uspešno uporabili za hkratno detekcijo Pb^{2+}, Ag^{+} in Hg^{2+} ionov v realnem vzorcu.