

Poly(thymine)-templated Fluorescent Copper Nanoparticles for Ultrasensitive Label-free Detection of Pb²⁺ Ion

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Polythymine (poly T)-templated copper nanoparticles (CuNPs) were demonstrated as novel and sensitive fluorescence probes for the detection of Pb²⁺ based on the fluorescence quenching effect. The as-prepared CuNPs displayed strong fluorescence emission. However, the fluorescence of CuNPs was readily quenched in the presence of Pb²⁺. These changes in fluorescence intensity of CuNPs allowed for the analysis of Pb²⁺ with rapidity (<10 min), simplicity (label-free), high sensitivity (LOD 0.4 nM), high selectivity (no interference from other metal ions) and at low-cost (without any labels and sophisticated operation). We validated the practicality of using CuNPs for the determination of Pb²⁺ in environmental samples through analyses of tap water samples.

Keywords Polythymine, copper nanoparticles, label-free, fluorescence detection, lead ions

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Introduction

It is well-known that lead (Pb²⁺) is a highly toxic pollutant, existing widely in the environment. Accumulation of Pb²⁺ in the human body can cause serious harm to human health, including decreased intelligence, kidney disease, and impaired growth.^{1,2} Therefore, the development of simple, rapid, sensitive, and selective strategies for detecting Pb²⁺ is of great significance for biochemistry, environmental monitoring and biomedicine.

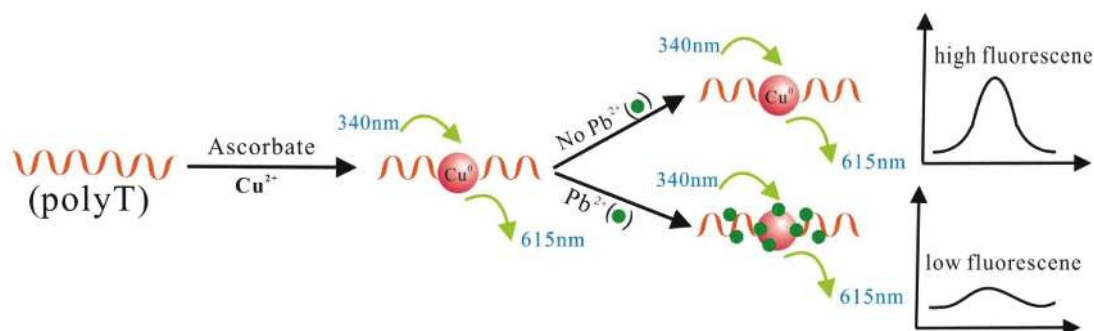
Conventional methods for the detection of Pb²⁺ include atomic absorption spectrometry,³ atomic fluorescence spectrometry,⁴ and inductively coupled plasma mass spectrometry.⁵ These assays, however, are not only laborious and time-consuming but also require sophisticated instruments and operation by a skilled technician. Many new strategies have been developed in recent years for rapid detection of Pb²⁺. Examples are fluorophores,^{6,7} electrochemical approaches,⁸ nanoparticles,^{9,10} quantum dots,¹¹ Pb²⁺-dependent DNazymes,¹²⁻¹⁴ G-quadruplex DNAs.¹⁵⁻¹⁸ Despite the importance of these techniques, their adoption has been limited due to the need for complicated processing steps, high enzyme cost or instability of biomolecules. Thus, the design of alternative strategies for the sensitive and selective detection of Pb²⁺ with simple and rapid operation and low cost is still in great demand.

Fluorescent noble metal nanoparticles (*e.g.* Au, Ag) have attracted much attention due to their strong fluorescence and discrete electronic transitions. Especially, oligonucleotide-templated fluorescent nanoparticles have attracted special research interest because of their easy synthesis, tunable

fluorescence emission, high photostability, good biocompatibility and water solubility. For example, dsDNA can act as an efficient template for the formation of fluorescent copper nanoparticles, which have been successfully applied for proteins,¹⁹ DNA mutation identification,²⁰ small molecules²¹⁻²³ and enzyme assays.^{24,25} Very recently, Qing *et al.* reported that polythymine (poly T) can also be employed as a promising template for copper nanoparticles (CuNPs) synthesis.^{26,27} The poly T-templated CuNPs show high fluorescence. In addition, the size and fluorescence of the CuNPs can be regulated by the length of poly T. More importantly, the poly T-templated CuNPs are synthesized within several minutes, which is much faster and more convenient than the synthesis produced by other fluorescent metal nanoparticles, such as dsDNA-templated CuNPs (>1 h for efficient hybridization),²² silver nanoclusters (24 h in the dark at 4°C),²⁸ and gold nanoclusters (2 days).²⁹ These provide an alternative approach of poly T-templated CuNPs as a novel competent biosensing platform for chemical/biological analysis.

Herein, we developed a simple, rapid, robust and sensitive fluorescence biosensing strategy for detecting Pb²⁺ using poly T-templated CuNPs as fluorescent probes. It was found that the presence of the Pb²⁺ can quench the fluorescence of the CuNPs effectively. Our approach toward sensing Pb²⁺ was based on the fluorescence decrease that occurs as a result of the quenching effect in fluorescent CuNPs induced by Pb²⁺. The presented method for Pb²⁺ was not only sensitive (detection limit 0.4 nM) and selective, but also rapid (<10 min), simple (label-free), and low cost.

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Scheme 1 Schematic illustration of the Pb^{2+} sensing strategy based on poly T-template CuNPs.

Experimental

Materials and chemicals

The Oligonucleotides poly T (T30) (5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT-3') was synthesized by Sangon Biotechnology (Shanghai) Co., Ltd. and purified by HPLC. The stock solutions of poly T DNA were prepared by dissolving them in sterile deionized water. Supplies of 3-(*N*-morpholino)-propane sulfonic acid (MOPS), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{Pb}(\text{Ac})_2$ were commercially obtained from Dingguo Biotechnology Co., Ltd. (Beijing, China). We purchased (+)-sodium ascorbate from Sigma-Aldrich (Germany). The MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6) was used for the formation of fluorescent CuNPs. All reagents were of analytical grade and solutions were prepared using ultrapure water (electric resistance $>18.3 \text{ M}\Omega$).

Apparatus

Fluorescence measurements were carried out on F-7000 fluorescence spectrometer (Hitachi Ltd., Japan). The optical path length of the quartz cuvette was 1.0 cm. The excitation wavelength was 340 nm and the emission spectra were recorded from 360 to 660 nm with both excitation and emission slits of 5 nm.

Synthesis of fluorescent copper nanoparticles

DNA-templated CuNPs were synthesized according to previous reports. Briefly, 2 mM sodium ascorbate was added to a 5- μM DNA solution (diluted in 10 mM MOPS buffer, 150 mM NaCl, pH 7.6). After blending completely, 0.5 mM CuSO_4 was added to this mixture and incubated under a gentle stirring at room temperature in the dark to form fluorescent CuNPs.

Fluorescence detection of Pb^{2+}

Pb^{2+} of different concentration was added to the ssDNA-CuNPs solution. After 5 min of incubation, the fluorescence spectrum was recorded at room temperature. To evaluate the selectivity of our sensing system, we investigated the influence of other metal ions on the fluorescence of CuNPs. All the fluorescent assays were performed in a similar manner.

Results and Discussion

Design of the detection scheme

According to Qing's report, poly T ssDNA can act as an efficient template for CuNPs synthesis and the formed CuNPs have excellent fluorescence. Based on poly T-template CuNPs,

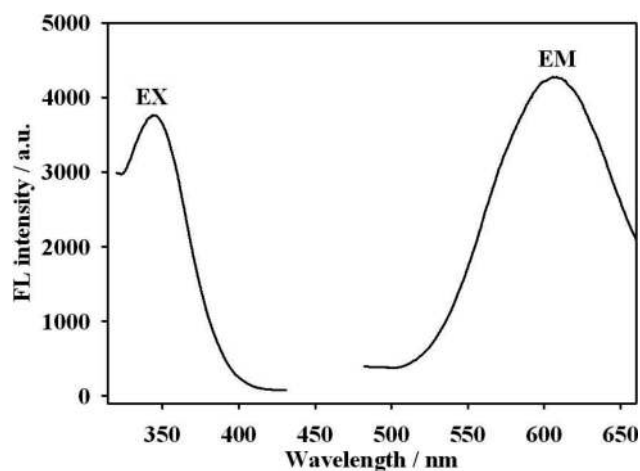


Fig. 1 The maximum excitation and emission spectra of the highly fluorescent poly T-template CuNPs (ex: 340 nm, em: 615 nm).

a simple, rapid, sensitive and low-cost fluorescence method for Pb^{2+} assay was proposed in this work. The developed strategy is shown in Scheme 1. Poly T strand (T30) was chosen as the template for Cu^{2+} reduction and CuNPs formation. The poly T-template CuNPs exhibited excellent fluorescence performance acting as sensitive signal report probes. However, in the presence of the Pb^{2+} , the fluorescence of the CuNPs was greatly quenched. The mechanism is still not very clear at present. Presumably, such a quenching effect might be attributed to metallophilic interaction. For growing fluorescent CuNPs on ssDNA, Cu^{2+} was reduced to Cu^0 by ascorbate. Then, the formed Cu^0 clustered on ssDNA. However, it is well known that this reduction process is accompanied by the formation of a Cu^+ intermediate. The surface of the poly(thymine)-stabilized CuNPs was surrounded by many Cu^+ in the presence of ascorbate as a reducing agent and stabilizing ligand. The Pb^{2+} could react with Cu^+ at the surface of the copper nanoparticles via the $5d^{10}(\text{Pb}^{2+})-3d^{10}(\text{Cu}^+)$ metallophilic interactions to destroy the structure of the CuNPs. As a result, a fairly fluorescence signal was observed.

Fluorescence characterization

In order to investigate the optic properties of the as prepared CuNPs, we monitored the absorption and fluorescence spectra of CuNPs. As shown in Fig. 1, the formed CuNPs exhibited strong fluorescence emission at 615 nm with excitation at 340 nm. The fluorescence intensity at the maximum emission

wavelength was used to evaluate the effect of Pb^{2+} on the fluorescence of the CuNPs. As shown in Fig. 2, the as prepared CuNPs displayed high fluorescent in the absence of Pb^{2+} (curve a). However, the fluorescence intensity decreased dramatically when 500 nM Pb^{2+} was added into the poly T-template CuNPs analytical system within 5 min (curve b). These observations conveyed that the selectivity quenching reaction on CuNPs gave a specific response to the Pb^{2+} target, demonstrating that the developed method holds promise for Pb^{2+} detection.

Analytical performance of the sensor

To further investigate the ability of the developed strategy for quantitative detection of Pb^{2+} , different concentrations of Pb^{2+} were added to the CuNPs. As presented in Fig. 3A, the fluorescence intensity of the CuNPs at 615 nm decreased dramatically with increasing Pb^{2+} concentration in the range from 0 to 500 nM. A quasilinear correlation between F_0/F (F_0 and F are the fluorescence intensities of the CuNPs in the absence and presence of Pb^{2+} , respectively) and the concentration of Pb^{2+} was obtained in the range from 1 nM to 500 nM with a linear correlation coefficient of 0.998, as shown in Fig. 3B. The detection limit was 0.4 nM in terms of the 3σ rule, which was lower than that of the other fluorescent biosensors (Table 1).^{17,21,30-32}

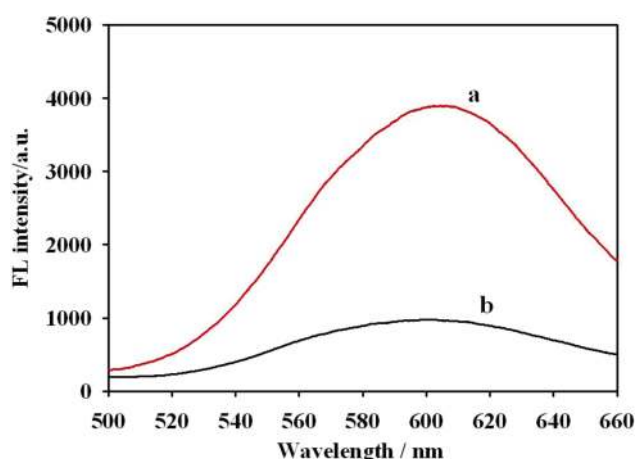


Fig. 2 Fluorescence spectra of CuNPs without (a) and with (b) Pb^{2+} .

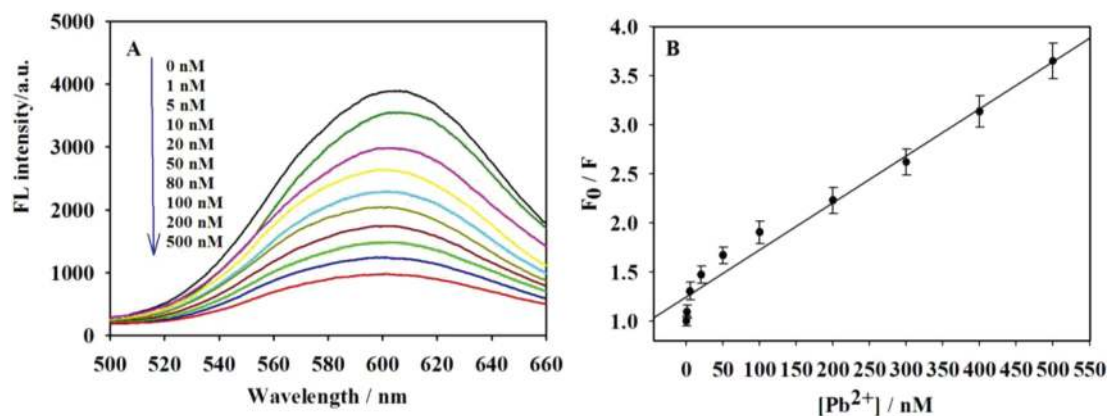


Fig. 3 (A) Fluorescence emission spectra representing the quenching effect of different concentrations of Pb^{2+} toward CuNPs. (B) Plot of fluorescence quenching effect (F_0/F) of the CuNPs versus Pb^{2+} concentration.

Optimization of the reaction condition

Following the design strategy, the performance of the developed method for Pb^{2+} assay is strongly influenced by the fluorescence probes of poly T-template CuNPs. Different synthesis conditions and the response time of CuNPs to the Pb^{2+} were investigated in our studies, and it revealed that of most importance were the concentrations of Cu^{2+} , sodium ascorbate and poly T DNA for the synthesis of CuNPs.

The CuNPs are formed by the reduction of Cu^{2+} to Cu^0 . Then, the latter is clustered on ssDNA to produce producing fluorescent CuNPs. A high concentration of Cu^{2+} is then expected to yield an intensive fluorescence signal. Figure 4A depicts the effect of Cu^{2+} concentration on the fluorescence of CuNPs. It was observed that the fluorescence peaks increased substantially when the concentration of Cu^{2+} changes from 0.1 – 0.5 mM. However, the fluorescence signal exhibits a gradual decrease as the Cu^{2+} concentration increased to more than 0.5 mM. This phenomenon was possibly attributed to the fact that a high concentration of Cu^{2+} might degrade the DNA template via oxygen-based radicals after being activated by a reducing agent. As a result, the optimal concentration of Cu^{2+} for synthesis was selected as 0.5 mM in subsequent studies.

The concentration of sodium ascorbate also had great effect on the fluorescence signal intensity of the CuNPs. It is clear from Fig. 4B that the fluorescence intensity of the CuNPs increased rapidly with increasing sodium ascorbate concentration and became saturated over 2 mM. Thus, 2 mM was selected as

Table 1 Comparison of our proposed method with some previously reported Pb^{2+} fluorescence sensors

Tool	Linear range	Detection limit/nM	Ref.
G-quadruplex and protoporphyrin IX	5.0 nM – 1.0 μM	1.0	17
dsDNA-templated CuNPs	5.0 – 100 nM	5.0	21
Glutathione-capped QDs	0 – 200 nM	20	30
Fluorophore and quencher labeled hairpin-structured DNAzyme	2.0 – 20 μM	3.0	31
AuNPs on graphene	50 – 1000 nM	10	32
poly T-templated CuNPs	1.0 – 500 nM	0.4	This work

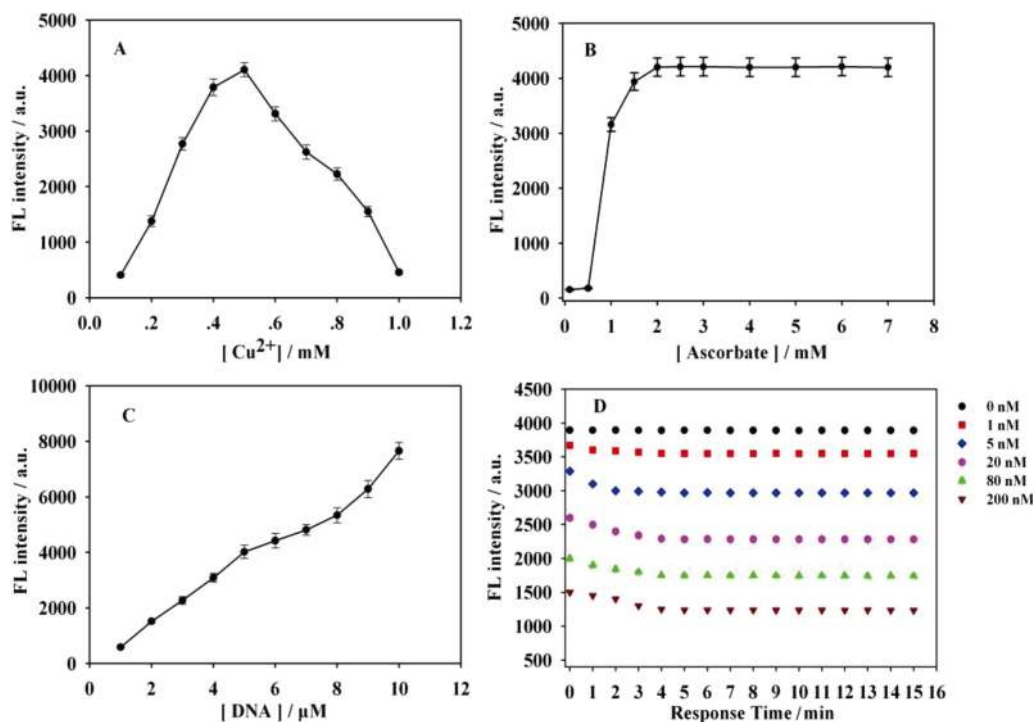


Fig. 4 Effects of Cu^{2+} concentration (A), sodium ascorbate concentration (B) and T30 concentration (C) on the formation of fluorescent CuNPs and the response time of CuNPs to different concentrations of Pb^{2+} (D).

the optimum concentration for sodium ascorbate.

Poly T was used as the template for the formation of CuNPs. Thus, the fluorescence intensity of the CuNPs was dependent upon the amount of the poly T. The greater the volume of poly T, the greater the fluorescence signal. So, a higher concentration of poly T was expected to generate an enhanced fluorescence signal. As shown in Fig. 4C, the fluorescence intensity of the CuNPs increases gradually with an increasing concentration of poly T, which was in good agreement with the expected result. We selected 5 μM poly T in the study because the corresponding fluorescence intensity can satisfy the detection requirements. In addition, the sensitivity will be lower at a higher poly T concentration, which would require a relatively high target concentration to get the same quenching effect in fluorescent CuNPs. The quenching kinetics of the CuNPs depending on different concentrations of Pb^{2+} was also investigated. As shown in Fig. 4D, the quenching effect occurred immediately once the Pb^{2+} was added to the CuNPs solution, and the change of the fluorescence reached a constant value in 5 min. Such a fast response of this method makes it suitable for analytical applications.

Selectivity study

The selectivity of the CuNPs-based detection system was also studied by using other interfering metal ions such as Zn^{2+} , Mg^{2+} , Mn^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Ni^{2+} and Cd^{2+} , as shown in Fig. 5. All the detections were carried out under identical conditions except the concentration of Pb^{2+} and interfering metal ions were 500 nM and 5 μM , respectively. It was revealed that the non-specific metal ions had no obvious effect on the fluorescence of the CuNPs and almost no fluorescence signal changes were obtained. Therefore, the quenching effect on the fluorescent of the CuNPs was specific to the Pb^{2+} , implying excellent selectivity of this strategy for Pb^{2+} detection.

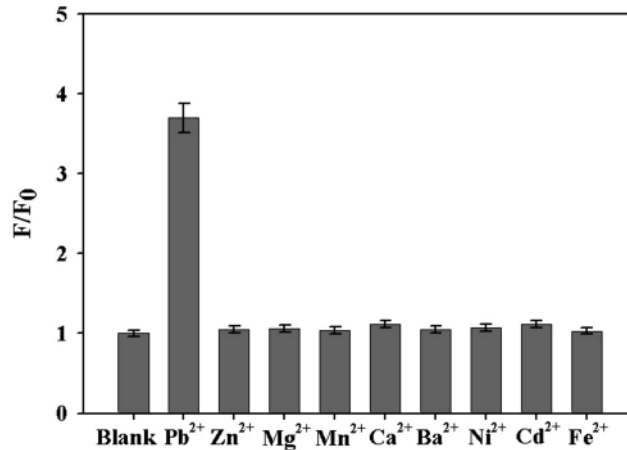


Fig. 5 The effect of different metal ions on the fluorescence emission of CuNPs (Pb^{2+} , 500 nM; other metal ions, 5 μM).

Applications

To test the practical application of the proposed method in environment analysis, detection of Pb^{2+} in tap water was performed. Different concentrations of Pb^{2+} were added to the real samples collected from tap water inputs. The results are listed in Table 2. The recovery of Pb^{2+} in the tap water was between 96.7 - 99.1% indicating that the poly T-templated CuNPs have potential for Pb^{2+} detection in actual environmental samples.

Table 2 Determination of Pb²⁺ in tap water sample

Sample	Pb ²⁺ added/nM	Pb ²⁺ found/nM	Recovery, %
Tap water 1	50.0	48.5	97.0
Tap water 2	80.0	79.0	98.7
Tab water 3	100.0	96.7	96.7
Tab water 4	150.0	148.7	99.1

Conclusions

A simple and convenient strategy was developed for the sensitive detection of Pb²⁺ based on the selective quenching effect of Pb²⁺ on the fluorescence of CuNPs. The T-templated CuNPs are easily synthesized (5 min), allowing for a very simple and rapid analysis for Pb²⁺. In addition, the as-proposed detection protocol provides high sensitivity with a low detection limit of 0.4 nM and also excellent selectivity, with no interference from other metal ions. Furthermore, this method is cost-effective without the need for any chemical modification or specific nucleic acid sequence design of DNA, such as Pb²⁺-dependent DNAzymes and G-quadruplex DNAs. In view of these advantages, the novel T-templated fluorescent CuNPs hold promise for applications in bioimaging, biosensing and biomedicine.

Acknowledgements

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