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Colorimetric Detection of Lead Ion Based on Gold Nanoparticles and Lead-Stabilized G-Quartet Formation

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Abstract

In this report, we present a method for the detection of Pb^{2+} based on the different adsorption capacity on the surface of gold nanoparticles (AuNPs) between ssDNA (single-stranded DNA) and G-quartet. In the absence of Pb^{2+}, the DNA oligonucleotides probe, which is guanine-rich ssDNA, can be adsorbed on the surface of AuNPs protecting them from aggregation. After adding Pb^{2+}, the DNA oligonucleotides probe can specifically form compact G-quartet, which can induce the aggregation of unmodified AuNPs, especially after adding NaCl aqueous solution. Consequently, the color turns from red to blue. Pb^{2+} can be detected by colorimetric response of AuNPs; its detection limit can reach 5 μM only observed by naked eyes. Most metal ions have no interferences, and the interference of Cu^{2+} can be effectively eliminated by adding cysteine. It provides a simple and effective colorimetric sensor for on-site and real time detection of Pb^{2+}.

Keywords

Gold Nanoparticles, G-Quartet, Pb^{2+}, Colorimetric Detection

1. Introduction

Lead ion, one of the most toxic heavy metal ions, can have serious effects on the environment and human health. Because lead is non-degradable, accumulation of high level of lead in children can cause irreversible brain damage, retard mental and physical developments. For adults, high levels of lead can cause irritability, poor muscle coordination, and nerve damage to the sense organs [1]. These traditional analysis methods, including

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atomic absorption spectrometry (AAS), inductive coupled plasma mass spectrometry (ICP-MS) and inductive coupled plasma atomic emission spectrometry (ICP-AES) and so on, have been widely used for the detection of Pb^{2+} [2] [3], but they often require expensive sophisticated instrumentation and complicated sample preparation processes. It is critical to have sensors that can provide rapid on-site detection of Pb^{2+}.

Colorimetric sensor has drawn more and more attention because of its simplicity and cost-effective [3]. Many Pb^{2+} colorimetric assays were developed based on AuNPs probes modified by specifically combining molecules of with Pb^{2+} specific agents, such as DNAzyme [4]-[8] and molecular containing sulfydryl group, carboxyl group and nitrogen [9]-[14]. Resent years, the combination of AuNPs and DNA has generated stimuli-responsive nanoscale materials with tunable properties as well as colorimetric sensors for a wide range of targets. AuNPs are usually chosen to be the colorimetric reporter, because they have distance-dependent localized surface plasmon resonance properties, which rival or even exceed the most intense organic dyes [15]. DNA molecules are often chosen as recognition element because of its highly stable and specific binding properties. It can keep their binding ability and catalytic activity after many cycles of denaturation and renaturation. Moreover, some DNA has metal ion binding properties [16] [17], which make them a promising candidate for establishing sensors, especially for metal ion sensors. DNAzyme is often chosen as molecular recognition elements for Pb^{2+} detection by transforming the cleaving event into colorimetric, electrochemical [18], fluorescent signals [19], because of its high sensitivity and specificity. But DNAzyme has high synthesis cost and low stability. As a substitute, Pb^{2+} induced allosteric G-quartet oligonucleotide is being used as Pb^{2+} recognition elements. Some G-quartet based lead biosensors have been developed by coupling with different kinds of signal outputs [20]-[23]. But, colorimetric sensor for Pb^{2+} based on AuNPs and lead-stabilized G-quartet formation [24] relatively has few studies.

It has been reported that ssDNA and dsDNA have different adsorption capacity with AuNPs [25]-[27]. ssDNA expose nitrogenous base which has high affinity to gold, making themselves adsorb on AuNPs and prevents AuNPs from aggregation, but dsDNA cannot do so because the electrostatic repulsion between their phosphate backbones and negatively charges AuNPs. Interestingly, formation of rigid G-quartet structure not only reduces the exposure of nitrogenous base but also increases surface charge density, thus disfavoring adsorption on AuNPs. We just make use of this property to design our assay. As shown in **Figure 1**, DNA probes stay in random-coil status without adding Pb^{2+}, which can absorb on the surface of AuNPs protecting them from aggregation. When Pb^{2+} was added into the system, the formation of Pb^{2+} stabilized G-quartet resulting in obvious color change of unmodified AuNPs especially after adding enough salt.

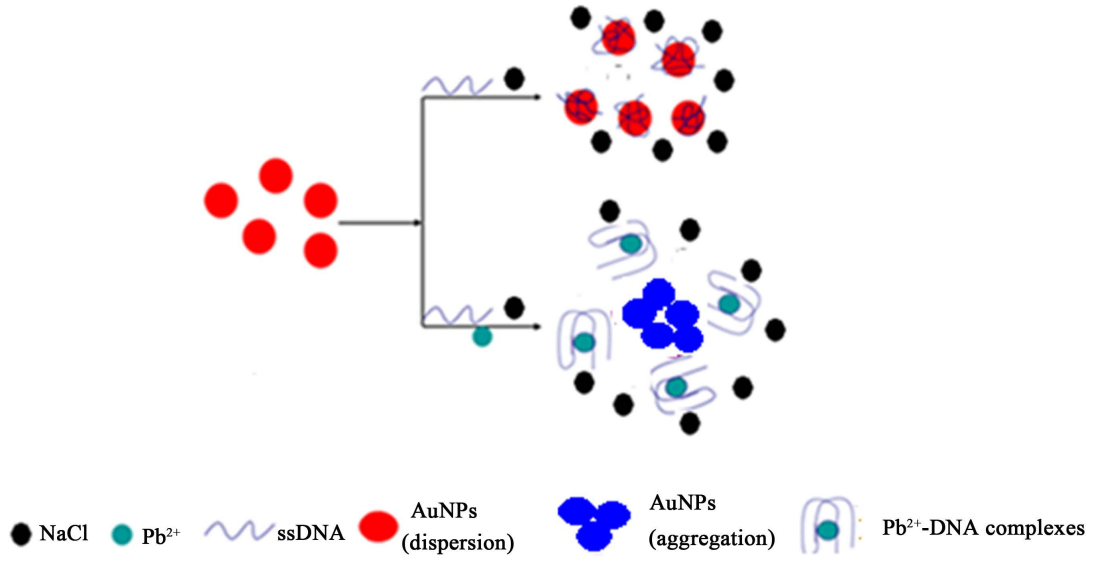


Figure 1. Scheme representation of the colorimetric detection of lead (II) based on formation of lead (II)-stabilized G-quartet and unmodified AuNPs. In the presence of Pb^{2+}, Pb^{2+} would bind to the ssDNA, and AuNPs are not stabilized by ssDNA; thus, they are readily aggregated by salt (solution displaying blue colors); in the absence of Pb^{2+}, AuNPs are stabilized by the aptamer (ssDNA), showing high resistance to salt-induced aggregation (solution staying in red).

2. Experimental Section

2.1. Reagents

Gold nanoparticles of ca.10 nm in diameter (with diameter of 10 nm) were purchased from Sigma-Aldrich (Dalian China). The base sequences of guanine-rich ssDNA as follows: 5'-GGGTAGGGCGGGTTGGG-3' (HPLC grade) were purchased obtained from TaKaRa Biotechnology Co., Ltd. (Dalian China). The DNA stock solutions were prepared by dissolving oligonucleotides in 10 mM Tris-HCl buffer (pH = 7.4), then heating to 85˚C for 5 min and cooled slowly to room temperature. The concentration of DNA stock solution was calculated according to the absorbance at 260 nm. These oligonucleotide solutions were stored at 4˚C before use within one week. All other reagents are analytical grade and used without further purification. Ultrapure water was used throughout all experiments.

2.2. Instrumentation

The UV-absorption spectra were recorded at room temperature on a Shimadzu UV-2550 spectrophotometer equipped with a circular water bath to keep temperature constant. Transmission electron microscopy (TEM) measurements were made on a Hitachi 7500 transmission electron microscope. The photographs were taken with a canon Power Shot A640 digital camera.

2.3. Colorimetric Detection of Pb^{2+}

First, 10 μL 200 nM ssDNA probe was mixed with 5 μL lead nitrate solution with an appropriate concentration. Second; 200 μL 6nM AuNPs was added to the solution; and allowed to react for 5 min at room temperature. Then, 50 μL 200 nM sodium chloride solution was added to produce color change. Finally, 350 μL of ultrapure water was added in the mixture for UV-vis measurement. The concentration of Pb^{2+} was quantified by the absorption ratio (A_{630}/A_{520}).

2.4. The Selectivity of the Colorimetric Sensors

In control experiments, lead (II) ions were simply displaced substitute or mixed by other metal ions/other metal ions with L-cysteine (*i.e.*, Na^{+}, K^{+}, Ca^{2+}, Co^{2+}, Mn^{2+}, Cu^{2+}, Cr^{3+}, Zn^{2+}or Cd^{2+} in this work.)

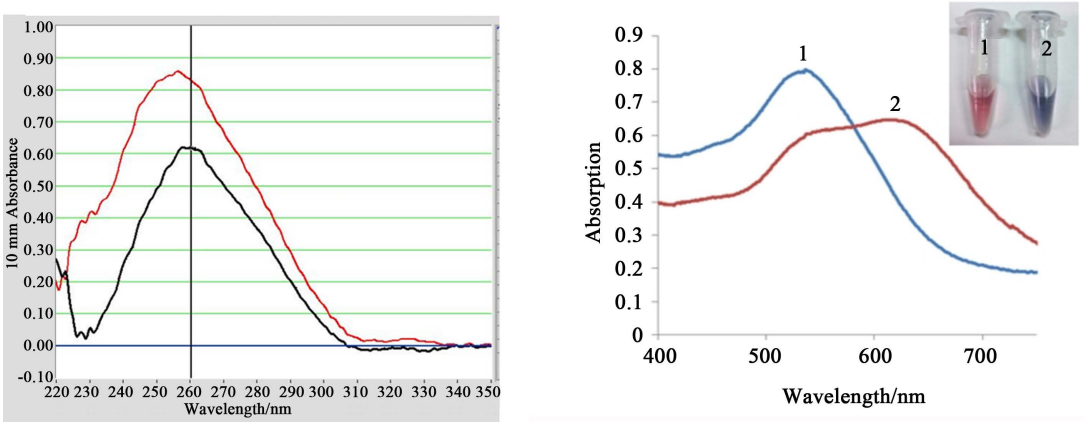
3. Results and Discussion

3.1. Sensing Mechanism

AuNPs which used in this experiment were stabilized by adsorbed negative citrate ion, have a surface plasma resonance absorption peak at about 520 nm, and appear pink. But when adding high concentration of salt will screen the charge on surface of AuNPs, resulting in the aggregation of AuNPs, and then the AuNPs appear blue-gray in color. Upon addition of guanine-rich ssDNA in AuNPs, they could be adsorbed on the surface of AuNPs. DNA phosphate backbone existed on the outside of AuNPs, thus the electrostatic repulsion enhanced the stability of AuNPs and kept them remained red under high-salt condition. However, when adding appropriate Pb^{2+} solution, the conformation of guanine-rich ssDNA changed from random coil structure to rigid G-quartet structure (**Figure 1**). The rigid G-quartet structure cannot adsorbed on AuNPs to prevent them from aggregation, resulting in an obviously color change from red to blue under high-salt condition.

3.2. UV-Visible Absorption Spectra and TEM Images of AuNPs

To know the interaction of DNA with Pb^{2+}, UV-vis absorption spectra of DNA in the absence and presence of lead (II) was investigated. As shown in **Figure 2(a)**. We can see that the peak at 260 nm has an obvious increase in intensity, which indicated more bases were exposed owing to the release of free DNA and formation of G-quartet. The color of gold colloid is very sensitive to the degree of aggregation of AuNPs in suspension. **Figure 2(b)** shows UV-vis absorption spectra and corresponding photographs of AuNPs in the absence and presence 10 μM Pb^{2+}. As can be observed in **Figure 2(b)**, AuNPs could be protected by ssDNA, they remained red color even after high concentration salt. AuNPs and ssDNA system have a surface plasma resonance absorption peak at about 530 nm. After adding lead (II) in this system, the color changed to blue, the surface plasma resonance absorption peak shifted to the long wavelength.



(a) (b)

Figure 2. (a) UV-vis spectra of DNA in the absence (black) and presence (red) of 10 μM Pb^{2+} (b) UV-vis absorption spectra and corresponding photographs of AuNPs + DNA system in the absence (1) and presence (2) 10 μM Pb^{2+} in the same experimental condition.

To know the microstructure of AuNPs and ssDNA system in the absence and presence of lead (II), the TEM experiments were performed (**Figure 3**). Original AuNPs were monodispersed (**Figure 3(a)**). Because ssDNA can expose nitrogenous base which have high affinity to AuNPs, making themselves adsorb onto the surface of AuNPs through Au-N bond. Therefore, the phosphate backbone of ssDNA existed on the outside of AuNPs, thus the electrostatic repulsion between their phosphate backbones enhance the stability of AuNPs, which prevent AuNPs from aggregation (**Figure 3(b)**). While in the prsence of Pb^{2+}, Pb^{2+} can make ssDNA form the stable G-quartet formation, which cannot adsorb onto AuNPs. Therefore, AuNPs are unstable and tend to aggregate easily (**Figure 3(c)**).

3.3. Optimization of Assay Condition

The performance of this assay was optimized by investigating the effect of various parameters. First, DNA and AuNP concentration can influence the detection of lead (II). When the DNA concentration and AuNP concentration are too high, it is unsuitable for detection of small amounts of metal ions because too large a background is present. The experimental results showed that the optimum DNA concentration and AuNP concentration were separately 200 nM and 8 nM. Second, since aggregated AuNPs tend to precipitate easily, 5 min were chosen as the detection time of this assay based on high sensitive and time-saving by experimental verification. Third, the effect of final concentration of NaCl on aggregation of AuNPs and DNA system was studied, we found that when the final concentration of NaCl was greater than 400 mM, the color of AuNPs and DNA system changed from red to blue in the absence of lead ions, thus increased the interference of background. To avoid this situation, 200 mM NaCl was chosen based on higher sensitivity and lower background.

3.4. Sensitivity and Selectivity

To detect the concentration of Pb^{2+} using AuNPs and guanine-rich ssDNA, the UV-vis spectra of AuNPs with different concentration of Pb^{2+} in the range of 0 - 40 μM under optimized conditions were recorded **Figure 4(a)**. As can be seen in **Figure 4(a)** and inset (a), the maximum absorption peak moved to longer wavelength and the color changed gradually from red to blue with the concentration of lead ions increased. The A_{630}/A_{520} were calculated based on the data shown in **Figure 4(a)**, and a good linear relationship (correlation coefficient R = 0.9948) between A_{630}/A_{520} and concentration of Pb^{2+} was observed in the range of 100 nM - 10 μM (**Figure 4(b)**). The detection limit was 5 μM when the ratio of signal to noise 3(S/N = 3).

Under the optimum conditions, we investigated the specificity of our analytical approach toward Pb^{2+} against other metal ions. As shown in **Figure 5(a)**, the UV-vis adsorption spectra of the assay for different metal ions was recorded. In the presence of Pb^{2+}, a significant shift of maximum absorption wavelength was observed, whereas no obvious change of the adsorption spectra when other metal ions were added in the double concentration of Pb^{2+},except for Cu^{2+}. We can get the same conclusion from the color change and the value of A_{630}/A_{520},

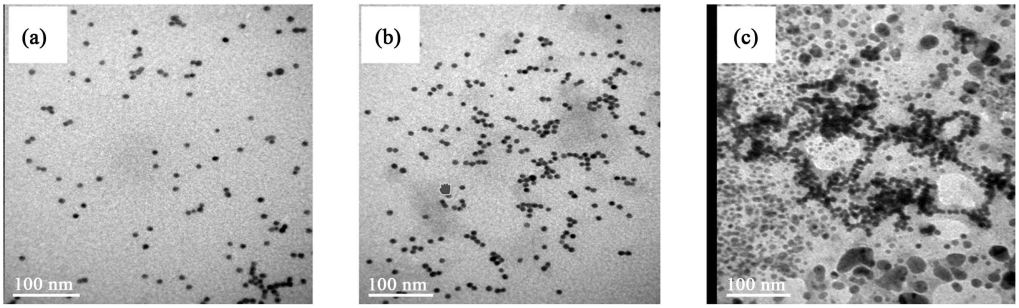
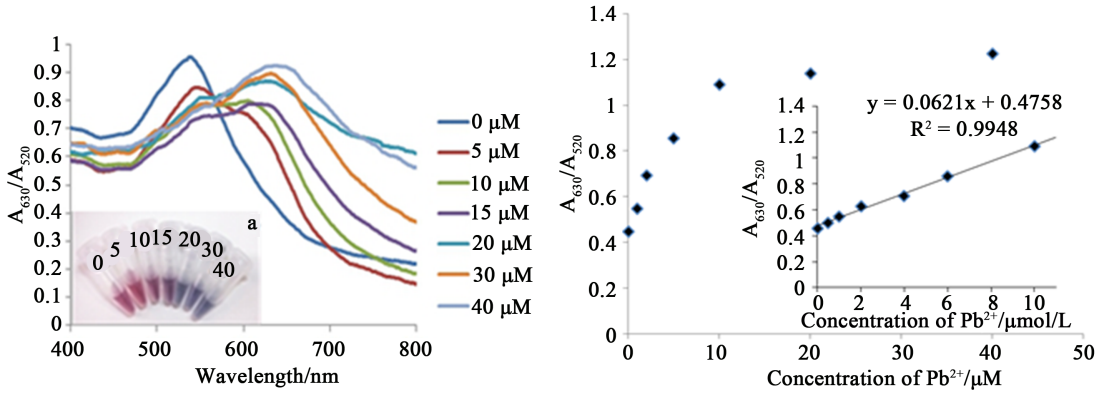
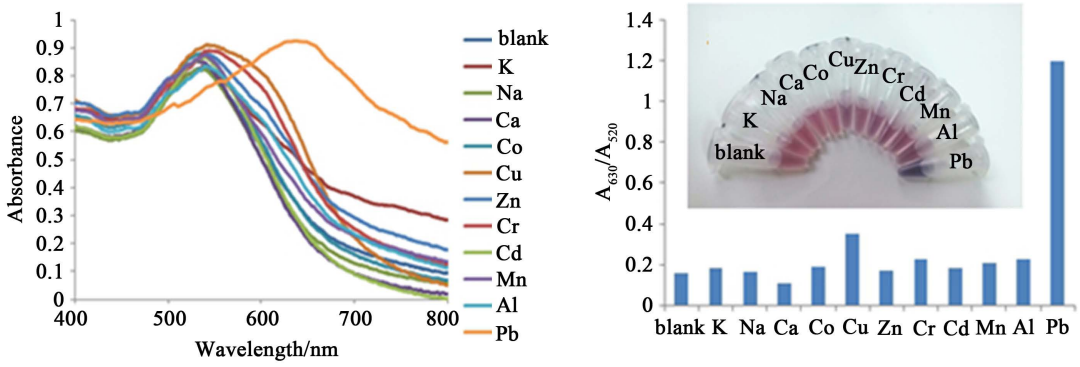


Figure 3. TEM images of AuNPs (~10 nm) (a); ssDNA + AuNPs + NaCl (b); and ssDNA + Pb^{2+} (1×10^{-3} mol·L^{-1}) + AuNPs + NaCl (c). The scale bar represents 100 nm. The magnification times of TEM is 600,000.



(a) (b)

Figure 4.(a) UV-vis spectra and photograph (inset a) of AuNPs-DNA system in the presence of different concentration of Pb^{2+} (0 - 40 μM) and 200 mM NaCl. The incubation time is 5 mins; (b) Plot of absorbance ratio of A_{630}/A_{520} versus Pb^{2+} concentration.



(a) (b)

Figure 5. Selectivity of Pb^{2+} ions colorimetric assay (a) UV-vis adsorption spectra of the assay for different metal ions; (b) The value of (A_{630}/A_{520}) of AuNPs and guanine-rich ssDNA system upon adding different metal ions. The final concentration of Pb^{2+} ions was 40 μM and the concentrations of the other metal ions were 80 μM.

as shown in **Figure 5(b)**. In order to exclude the interference, cysteine was used for masking Cu^{2+} [28] [29], after adding cysteine, the selectivity of our approach for Pb^{2+} was achieved. The result shows that only Pb^{2+} ions could significantly increase the A650/530 ratio by a factor of 10-fold or more relative to the other metal ions.

4. Conclusion

In summary, a simple, cost-effective, and comparatively fast detection method for Pb^{2+} has been developed,

based on unmodified AuNPs and lead-stabilized G-quartet formation. The detection of limit of this assay is 5 μM only observed by naked eyes, which is higher than the safety limit 72 nM approved by the US EPA. Compared with the previous standard method, this proposed sensor is simple, low-cost and does not require costly instrument. As a result, we still need a much greater effort to improve it. But, this assay doesn't require sophisticated instrument and modification on the surface of AuNPs, thus it's suitable for on-site and real-time detection for Pb^{2+}. We believe this simple, rapid and selective sensor will make its promising for monitoring lead in practical applications.

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