Simple Colorimetric Assay for Detection of Hg^{2+} Based on Gold Nanoparticles

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ABSTRACT

A simple and rapid colorimetric assay for detection of mercury ions (Hg^{2+}) has been developed in recent years as the traditional instrumentation methods are tedious, complicated and expensive. The developed assay was based on the aggregation of citrate-capped gold nanoparticles (AuNPs) in the presence of Hg^{2+}. The surface of AuNPs was modified with thymine, which interacted mostly with Hg^{2+}, to induce the aggregation of AuNPs in the presence of Hg^{2+}. The aggregation was easily distinguished with the naked eyes. The color of AuNPs solution was changed from wine-red to purple corresponding to the Hg^{2+} concentration. The sensitivity and selectivity of the developed assay were investigated by UV-Vis spectrophotometer. The results suggested that thymine modified AuNPs of the developed assay was selective for Hg^{2+}.

Keywords: Mercury ions, Gold nanoparticles, Colorimetric sensor

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Introduction

The environmental pollution of heavy metals can threat seriously to both public health and biodiversity. Mercury ion (Hg^{2+}) is one of the highest toxic heavy metals which can cause many health complications including carcinogenic effects, damaging of organs functions and central nervous system disorders (Clifton, 2007; He et al.,2011). Consequently, detection of Hg^{2+} is necessary for preventive as well as the treatment. The analytical techniques based on instrument such as atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) have been applied (Chen et al., 2015; Ganbold et al., 2010). However, all instruments require skillful labors, high cost, complex sample preparation steps even though this method is standard method for heavy metals detection (Ahmed *et al*., 2003). Consequently, many methods were developed for Hg^{2+}detection including colorimetric assay (Chen *et al*., 2016).

Among colorimetric assay, gold nanoparticles (AuNPs) based sensors are effective cost, highly sensitive and chemically stable method for Hg^{2+}detection (Couture *et al*., 2013; Szunerits *et al*., 2014; He *et al*., 2008). The color changes of nanoparticles are related to the surface plasmon resonance (SPR) phenomenon. The size of nanoparticles can induce the color of its solution from the aggregation of AuNPs. For the AuNPs based sensing assay, several type of ligands were modified on the AuNPs surface to generate the aggregated AuNPs. In the midst of many ligands, T-rich (thymine-rich) oligonucleotides was applied in mostly because of the highly sensitive ligand for Hg^{2+}detection whereas the high cost and preparation steps had to be considered (Liu *et al*., 2010)

Thus, thymine was the sensitive compound for Hg^{2+}ion and it was selected to develop AuNPs based sensing assay. This assay was developed based on the mechanisms of T-Hg^{2+}-T coordination chemistry without complicated preparation steps (Chen *et al*., 2011). The sensitivity and selectivity of this assay were tested by UV-Vis Spectrophotometer**.** The absorption spectrum peak of AuNPs solution was changed from 530 nm (separated AuNPs form) to 590 nm (aggregated AuNPs form). The color of Hg^{2+}solution was observed within 5 minutes by naked eye. The results indicated that this assay was highly selective for Hg^{2+} ion.

Objective of the study

To develop the colorimetric assay for Hg^{2+} detection based on AuNPs and thymine ligand.

Materials and Methods

Chemicals and Reagents

Gold nanoparticles (with the diameter of 10 nm), thymine powder and tris-HCl powder (Trizma@base) were bought from Sigma-Aldrich, Co., Ltd. Copper (II) sulfate ($CuSo_4$), iron (III) chloride ($FeCl_3$), lead (II) chloride ($PbCl_2$), manganese chloride ($MnCl_2$) and zinc chloride ($ZnCl_2$) were bought from Bio Basic Inc.Ltd. Cobalt (II) chloride ($CoCl_2$) and nickel (II) sulfate ($NiSO_4$) were bought from Fisher Scientific, Co., Ltd. Mercury (II) nitrate ($Hg(NO_3)_2$ and aluminium chloride ($AlCl_3$) were bought from LOBA-Chemie, Co., Ltd. The stock solutions of all chemicals were prepared by dissolving in deionized water.

Colorimetric detection

UV-Vis Spectrophotometer (Thermo Fisher Scientific) was used for the determination of the absorption spectra in the range of 400-700 nm. The light intensity at 530 nm and 590 nm were related to the dispersion and aggregation process of AuNPs particle, respectively. Therefore, aggregation and dispersion were investigated by the absorbance ratio of light intensity between 590 nm and 530 nm. A higher ratio value was indicated the aggregation of AuNPs with blue color, while a lower ratio value was indicated the dispersion of AuNPs with red color.

Experimental Protocols

Sensitivity and Selectivity

Firstly 0.5 M of thymine was dissolved in 1M NaOH and gentle heated for 1-2 mins. 0.1M tris-HCl buffer was made by dissolving 0.6 g of tris-HCl powder with 50 ml of water and pH adjustment was done by 5M of HCl in order to get pH-7 of the solution. The stock solution of Hg^{2+} (2000 ppm) was prepared by dissolving Hg $(NO_3)_2$ in 0.1M of tris-HCl and later serial dilution of Hg^{2+} at different concentration (20 ppm, 10 ppm, 5 ppm, 1 ppm and 0.5 ppm). To assess the selectivity tests, the following metals (Al^{3+}, Cu^{2+}, Co^{2+}, Ir^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) were dissolved in 0.1M of tris-HCl at the concentration of 100 ppm.

To study the sensitivity and selectivity of the test, AuNPs (10 µl) was mixed with tris-HCl buffer (5 µl) and then 0.5 M of thymine solution (2.5 µl) was added. In the case of Hg^{2+} presence, the color of the tested solution would be changed from red to purple color as a result of AuNPs aggregation. After color was developed, the samples were measured by UV-Vis spectrophotometer in order to check for the aggregation of gold.

Results

Principle of Assay

In this study, the sensing mechanism for Hg^{2+} detection is explained in the Fig. 1. The citrate ion is used to store the aggregation of AuNPs particle. When thymine is added to the AuNPs solution, the citrate ion is removed and replaced by thymine compound. According to the previous research, thymine cannot attach to each other as T-T mismatch (thymine-thymine mismatch) and specific attach to the Hg^{2+} ion rather than other metal ions. In case of Hg^{2+} absence, AuNPs particle continually separate from each other. However, in the case of Hg^{2+} presence, it has strong affinity to attract between two thymines to form the T-Hg^{2+}-T complex and can stable like Watson-Crick base pairs (Tanaka *et al*., 2006). At the time of attraction between two bonds, the attached ligands will pull the nanoparticles and form the aggregation. Consquently, the color changes may be seen from red to purple color. This sensing strategy is selective binding for Hg^{2+} rather than other metals.

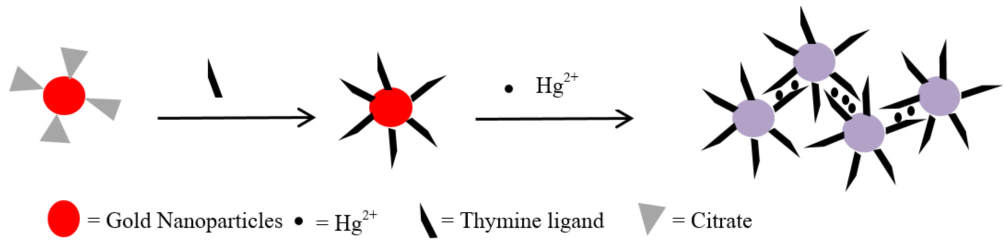

Figure 1 The sensing mechanisms of the aggregation of AuNPs for detection of Hg^{2+}

Detection of Hg^{2+}

For the sensing mechanisms, thymine ligand was exchanged with citrate ions on the surface of AuNPs and detected the Hg^{2+} base on the theory of T-Hg^{2+}-T coordination mechanism. The absorbance ratio (A_{590nm}/A_{530nm}) was investigated to check the aggregation and dispersion of AuNPs with and without the Hg^{2+} in solution (see in Fig.2). However, the absorbance ratio of this test was reduced after 50 minutes, as shown in Fig.3. After the absorbance spectra were measured to confirm the aggregation and dispersion of AuNPs, the sensitivity tests were performed with different concentrations of Hg^{2+}. The tests were steadily increased until 5 ppm but from 10 ppm it was greatly increased (see in Fig.4). The selectivity tests were also performed at the absorbance ratio of (A_{590nm}/A_{530nm}), and the ratio of Hg^{2+} was the highest among all the metals even in the low concentration of Hg^{2+}, as shown in Fig.5.

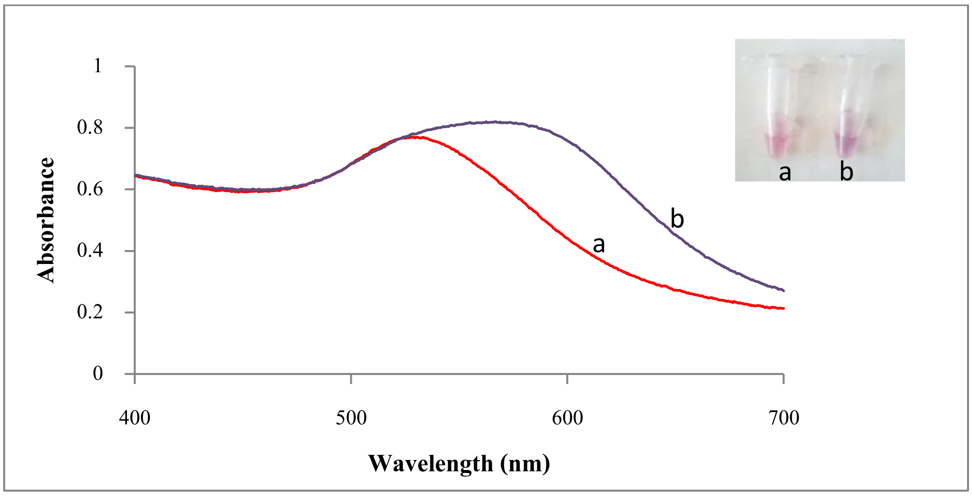

Figure 2 UV-vis absorption spectra of colorimetric response (a) absence of Hg^{2+} and (b) presence of 20 ppm Hg^{2+} (insert image: the color changes by naked eyes determination).

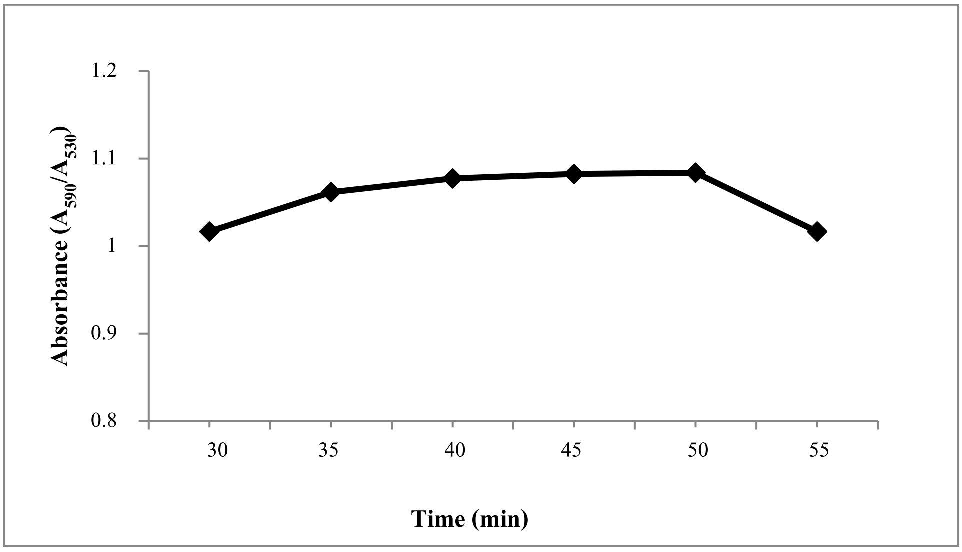

Figure 3 The absorbance ratio (A_{590nm}/A_{530nm}) of AuNPs solution for 20 ppm of Hg^{2+} after adding thymine.

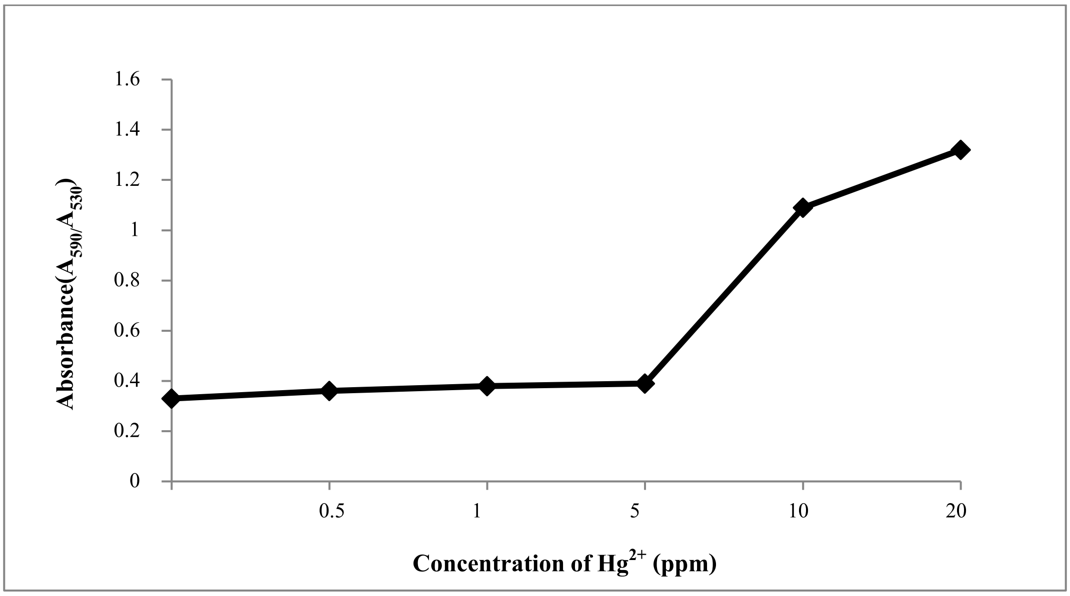

Figure 4 Colorimetric responses (A_{590nm}/A_{530nm}) along with the concentration of Hg^{2+} from 0-20 ppm.

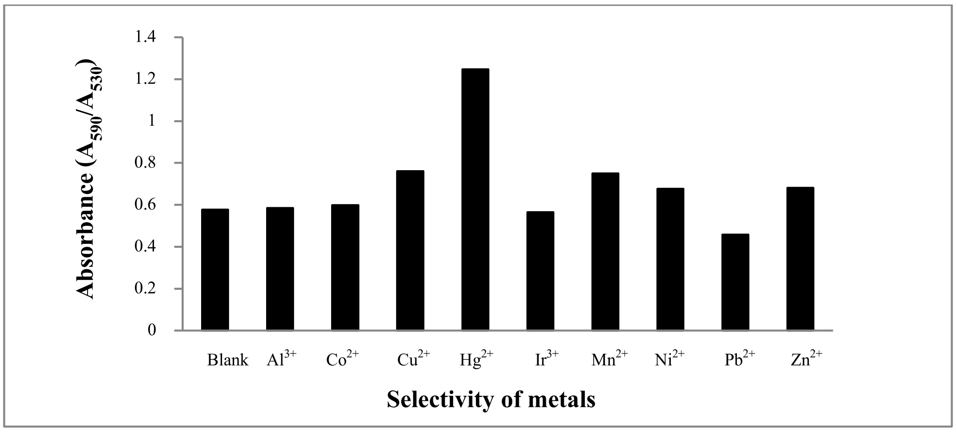

Figure 5 The absorbance ratio (A_{590nm}/A_{530nm}) for selective detection of Hg^{2+} at 10 ppm and other metals (Al^{3+}, Co^{2+}, Cu^{2+}, Ir^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) at 100 ppm.

Discussion

The sensing mechanism of Hg^{2+}detection based on thymine-attached AuNPs particle was related to the T-Hg^{2+}-T coordination strategy (Chen *et al.*, 2011). This coordination induced the aggregation of AuNPs in the case of Hg^{2+} presence that was perceived by changing of its absorption spectrum. This phenomenon was termed as surface plasmon resonance (Couture *et al.*, 2013). Consequently, the absorption spectrum of the thymine-attached AuNPs solution was changed from 530 nm to 590 nm after adding Hg^{2+} ion. The color of this solution was changed from red to blue color that was investigated by naked eyes (see in Fig.2). The ratio between two wavelengths (A_{590nm}/A_{530nm}) was observed for the aggregation of AuNPs. The effect of time to induce AuNPs aggregation is presented in Fig. 3. This aggregated AuNPs should be investigated within 30 minutes of the experiment because the ratio of A_{590nm}/A_{530nm} would be changed. Therefore, this Hg^{2+}test was measured within 30 minutes after the reaction started. The sensitivity of this test was performed under different concentrations of Hg^{2+} ion (see in Fig. 4). This assay presented the sensing signal at the low concentration of Hg^{2+} ion at 0.5 ppm. The selectivity of Hg^{2+} ion was studied with other metals under the same conditions with Hg^{2+} solution (see in Fig. 5). Hg^{2+} ion with low concentration at 10 ppm demonstrated higher ratio of A_{590nm}/A_{530nm} than other metals with high concentration at 100 ppm. The results confirmed that this assay was selectively for detection of Hg^{2+}. Therefore, this assay has the high potential for Hg^{2+} quantification and qualification.

Conclusions

The colorimetric assay for Hg^{2+} detection based on the interaction between thymine and Hg^{2+}ion was developed. This reaction corresponded to the T-Hg^{2+}-T coordination strategy that induced the aggregation of AuNPs. The advantages of this method are time saving, cost effective, simplicity and high selectivity for Hg^{2+} ion. Moreover, the color of this reaction can be easily observed by naked eye after adding the Hg^{2+}contaminated solution. This assay can provide the opportunities to apply for on-site application such as Hg^{2+}contaminated water problem. Thus, the research will be continued on Hg^{2+}contaminated water samples.

Acknowledgement

This research project is supported by Mahidol University.

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