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# A simple and selective colorimetric mercury (II) sensing system based on chitosan stabilized gold nanoparticles and 2,6-pyridinedicarboxylic acid

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ABSTRACT

The development of simple and cost-effective methods for the detection and treatment of $Hg^{2+}$ in the environment is an important area of research due to the serious health risk that $Hg^{2+}$ poses to humans. Colorimetric sensing based on the induced aggregation of nanoparticles is of great interest since it offers a low cost, simple, and relatively rapid procedure, making it perfect for on-site analysis. Herein we report the development of a simple colorimetric sensor for the selective detection and estimation of mercury ions in water, based on chitosan stabilized gold nanoparticles (AuNPs) and 2,6-pyridinedicarboxylic acid (PDA). In the presence of $Hg^{2+}$, PDA induces the aggregation of AuNPs, causing the solution to change colors varying from red to blue, depending on the concentration of $Hg^{2+}$. The formation of aggregated AuNPs in the presence of $Hg^{2+}$ was confirmed using transmission electron microscopy (TEM) and UV–Vis spectroscopy. The method exhibits linearity in the range of 300 nM to 5 μM and shows excellent selectivity towards $Hg^{2+}$ among seventeen different metal ions and was successfully applied for the detection of $Hg^{2+}$ in spiked river water samples. The developed technique is simple and superior to the existing techniques in that it allows detection of $Hg^{2+}$ using the naked eye and simple and rapid colorimetric analysis, which eliminates the need for sophisticated instruments and sample preparation methods.

## 1. Introduction

In recent years, a great amount of attention has been focused on the detection and treatment of mercury ($Hg^{2+}$) in the environment, due to the serious health risk that it poses to humans and animals [1–4]. Exposure to mercury, even at very low levels, can cause damage to the brain, heart, lungs, kidney, and immune system, and can result in symptoms such as memory loss, vision problems, deafness, and lack of coordination [3]. Mercury contamination to environment comes from various sources, such as volcanic eruptions, the gold industry, solid waste disposal, and the combustion of fossil fuels [1–2]. Although mercury is highly evaporative, it is also very stable in water, making it incredibly hazardous to the ecosystem [4]. Thus, development of a mercury detection system with high sensitivity and rapid response time is needed greatly.

Mercury can be detected by many techniques such as atomic absorption spectroscopy, fluorescence spectroscopy, inductively coupled plasma-mass spectrometry, electrochemical sensing, etc. [5]. However, these approaches require expensive facilities and time-consuming processes. Studies on various mercury sensing techniques that address these issues have received much attention in the past few years [6–9]. In this context, colorimetric sensing techniques, based on the induced aggregation of nanoparticles have attracted special interest. It has been shown that even a small change in the size, shape or dielectric constant of nanoparticles can lead to tunable optoelectric properties, which in turn can be used for sensing applications [9–11].

AuNPs have been widely studied in field of biosensing and medical devices due to their size-dependent surface plasmonic properties [12–29]. Among them, a number of studies based on AuNPs have been reported for the detection of metal ions. Most of these studies utilized DNA functionalized AuNPs for the detection [13,15,20,22–27]. However, most DNA gold nanoparticle assays rely very sensitively on accurate control of detection conditions such as temperature. In addition, DNAs are quite fragile and expensive limiting the application of this technique for day-to-day use. The other type of colorimetric assay for $Hg^{2+}$ detection is based on acid capped gold nanoparticles [10]. Acid capped gold nanoparticles are simple but lack specificity for $Hg^{2+}$ because other kinds of metal ions can readily interact with acid capped gold nanoparticles causing aggregation and hence affects the selectivity of the method. Tan et al. reported that the addition of 2,6-pyridinedicarboxylic acid (PDA) improves the selectivity for $Hg^{2+}$ and provides a mask for metal ions such as cadmium and lead [30]. Taking advantage of the above finding, here we report a simple, selective and efficient method for $Hg^{2+}$ detection based on chitosan, AuNPs, and PDA. The choice of chitosan as a stabilizer for AuNPs was made due to its eco-friendly nature and cost-effectiveness. The binding of $Hg^{2+}$ by AuNPs was studied by monitoring the changes in the surface plasmon resonance (SPR) absorption spectra.

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It was found that metals other than $Hg^{2+}$ don't show any detectable change in the SPR spectra. The results obtained indicate that the method has potential to form a viable colorimetric chemical sensor for $Hg^{2+}$ as it provides naked eye detection and doesn't require any expensive chemicals or sophisticated instruments for the analysis.

## 2. Material and methods

### 2.1. Chemicals and instrumentation

The chemicals used in this study were as follows: chitosan (low molecular weight, ~50,000 g/mol, 85% deacetylated), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), and mercury (II) chloride (metals basis, 99.999%). All above chemicals were purchased from Alfa Aesar and 2,6-pridinedicarboxylic acid (99%) was purchased from ACROS organics. All reagents were used as received without further purification. Deionized (DI) water was used in the preparation of the solutions.

For synthesis of the AuNPs, centrifugation was performed using a Legend Micro 17/17R centrifuge (ThermoScientific). The transmission electron microscopy (TEM) images of the AuNPs were taken using an FEI Tecnai G2 TF20 transmission electron microscope under 200 kV accelerating voltage. For TEM, the sample was prepared by placing a drop of the colloidal solution on a Formvar/carbon-coated copper grid and allowing the solvent to evaporate at room temperature. All the absorption spectra were recorded on LAMBDA 950 UV/Vis/NIR Spectrophotometer (PerkinElmer) with 1 nm resolution at room temperature. The wavelength range from 800 nm to 400 nm was used, and the path length of the UV–Vis cuvette was 1 cm. The ratio of the absorbance values at 620 nm and 525 nm ($Ex_{620\ nm}/Ex_{525\ nm}$) in the UV–Vis spectrum were used to quantify the analysis and determine the optimal sensing condition. A lower ratio value indicates the dominance of separated AuNPs in the solution (red color), and a higher ratio is associated with more aggregated AuNPs in the solution (blue color).

### 2.2. Synthesis of AuNPs

The AuNPs were synthesized using a wet chemical reduction method with chitosan [31]. In a typical reaction, 2 mL of a 1% (w/v%) chitosan solution was placed into a glass reaction vessel and heated to boiling point using a hotplate. 1 mL of $HAuCl_4$ solution (10 mM) was added quickly to this solution and the reaction mixture was again boiled under vigorous magnetic stirring. The gradual formation of a red colored solution after 5 min indicated the formation of AuNPs. The reaction was allowed to continue for another 10 min. After cooling, the AuNPs were separated by centrifugation at 13,000 rotations per minute (rpm) for 15 min. The prepared AuNPs were then re-dispersed in DI water and stored at 4 °C before use.

### 2.3. Preparation of master solution

The master solution, which is the solution that was spiked by $Hg^{2+}$, was prepared with the AuNPs and PDA. All the $Hg^{2+}$ sensing experiments were performed under the optimal PDA and AuNP concentrations. For the preparation of master solution, 0.4 mL of the prepared AuNPs was mixed with 1 mL PDA (10 mM) and 2 mL water. This solution was then heated in a water bath at 80 °C for 10 min. After the solution was cooled to room temperature, the master solution was formed and ready for the addition of $Hg^{2+}$.

### 2.4. Colorimetric sensing of $Hg^{2+}$

Colorimetric sensing of $Hg^{2+}$ was executed by adding different concentrations of $Hg^{2+}$ to the sample of master solution. Then all of the solutions were heated in a water bath around 80 °C for 10 min, resulting in the solutions changing from red to purple to blue depending on the amount of mercury present. The resulting sample solutions were used to perform the UV–Vis measurements. For the selectivity testing of the sensing system, different metal ion solutions ($Al_2(NO_3)_3$, $CaCl_2$, $Co(NO_3)_2$, $CuCl_2$, $FeCl_2$, $FeCl_3$, KCl, LiCl, $MgCl_2$, NaCl, $Ni(NO_3)_2$, $SrCl_2$, $ZnCl_2$, $BaCl_2$, $Pb(COOH)_2$, $AgNO_3$, and $HgCl_2$) were prepared in concentrations of 5 μM. For the practical application test, river water was collected from the City Creek Natural Area in Salt Lake City.

## 3. Results and discussion

### 3.1. Sensing strategy

In the synthesis of AuNPs, we used chitosan as the reducing agent and also as the stabilizer to disperse AuNPs (~23 nm). Chitosan has a chain structure with a free amine group ($-NH_2$). Chitosan can be dissolved in slightly acidic aqueous solution because the free amine group forms $-NH^{3+}$ when pH < 6.5 [31]. After the redox reaction to form zero-valent Au from $Au^{3+}$, chitosan chains can adsorb on the Au surface due to the strong affinity of gold towards the amine group ($-NH_2$) and the chitosan acts as capping agent of the gold nanoparticles [31]. We used PDA as the chelating agent of $Hg^{2+}$, which contains a pyridine ring with two carboxyl groups. After adding PDA in the AuNPs solution and heating to 80 °C to accelerate the adsorption process, the PDA conjugate to the chitosan on the AuNPs surface. Reports [32] have proven that pyridine's nitrogen atom has a strong affinity to $Hg^{2+}$, which causes the formation of coordination complexes. Because of this, upon the addition of $Hg^{2+}$, the solution was heated to provide sufficient energy such that AuNPs/Chitosan/PDA would ligate to $Hg^{2+}$ and form $Hg^{2+}$ complexes. At the same time, Chitosan/PDA on AuNPs form complexes with $Hg^{2+}$, causing the AuNPs to aggregate into clusters, and resulting in a color change of the solution from red to blue. The scheme of sensing mechanism is shown in Fig. 1.

As shown in Fig. 2(A), we conducted UV–Vis spectroscopy with three samples under controlled condition, which are AuNPs/Chitosan (short as AuNPs, indicated by the black curve in the figure), AuNPs after adding 10 mM PDA (AuNPs/PDA, red curve), and AuNPs with PDA in the presence of 10 μM $Hg^{2+}$ (AuNPs/PDA/Hg, blue curve). The inset photograph shows a picture of the samples: AuNPs, AuNPs/PDA, and AuNPs/PDA/Hg from left to right. For the AuNPs sample without PDA and $Hg^{2+}$, a characteristic SPR absorption band at around 525 nm was observed in the spectra, and the sample exhibited a red color. After adding PDA, dispersed AuNPs still exist and no obvious aggregation appears since there is no peak shift in the AuNPs/PDA spectra (red line) compared to the AuNPs spectra (black line), and the color of the sample remained red. After the addition of 10 μM $Hg^{2+}$, the 525 nm SPR band decreased in intensity while a band appeared around 620 nm, which showed almost the same intensity as the 525 nm band. This indicates the aggregation of AuNPs and the corresponding sample exhibited a blue color which can easily be observed by the naked eye (see inset of Fig. 2). This proves that the addition of $Hg^{2+}$ results in the aggregation of AuNPs. Fig. 2(B) shows the TEM images of the AuNPs, and the AuNPs/PDA/Hg which provide further proof of the proposed $Hg^{2+}$ sensing mechanism. The left image shows the dispersed AuNPs, and the right one displays the aggregated AuNPs after adding $Hg^{2+}$ (10 μM).

### 3.2. Effect of the concentration of PDA and AuNPs

To study the effect of concentration of PDA on the performance of the system, we varied the concentration of PDA in the master solution and conducted UV–Vis spectroscopy in the presence of 0.5 μM $Hg^{2+}$ (red column) and 5.4 μM $Hg^{2+}$ (blue column) as is shown in Fig. 3(A). The volume ratio of prepared PDA solution (10 mM) versus AuNPs was varied as 0.25:1, 0.5:1, 1:1, 2.5:1, 5:1, 7.5:1, respectively, while fixing the concentration of AuNPs in the total solution. To attain these ratios, the following concentrations of PDA were used: 0.29 mM,