Instant Sensitive Measurement of Hg Concentration Using Lab-on-a-Phone Colorimetry

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Mercury is one of the most toxic heavy metals in the environment that can seriously damage the human health. Therefore, the identification of mercury in water sources such as rivers, lakes, and bays is very crucial. Many traditional methods are used for the detection of mercury (II) ions (Hg$^{2+}$), but they suffer from dependence on expensive and complicated instruments and need time consuming operating process. Herein, a fast, low cost, and accurate lab-on-a-phone device has been introduced for on-site monitoring of Hg$^{2+}$ in ppb level. It detects Hg$^{2+}$ based on localized surface plasmon resonance property of gold nanoparticles. The apparatus consists of lightweight opto-mechanical attachment, wireless connected to a smart phone. This method presents a sensitive detection of Hg$^{2+}$ in water with a detection limit of 3 nM (≈0.8 ppb). Detection limit of the proposed sensor is well below the maximum allowed containment level of Hg$^{2+}$ for drinking water (6 ppb) by the World Health Organization.

1. Introduction

Mercury is one of the most toxic heavy metals in the environment that in addition to natural sources, is also produced by human activities.[1] The presence of small quantities of mercury in foodstuff and drinking water can seriously damage the human health. According to the World Health Organization (WHO)[2] and the U.S. Environmental Protection Agency (EPA), the maximum allowable amount of Hg$^{2+}$ in drinking water is 6 and 2 ppb, respectively.[3] Current techniques of Hg$^{2+}$ detection mainly include atomic absorption/emission spectroscopy (AAS/AES), inductively coupled plasma mass spectrometry (ICP-MS), high performance liquid chromatography (HPLC), etc.[4–7] These techniques have a high sensitivity and selectivity, but are so expensive and complicated. Thus, development of a new substitute for Hg$^{2+}$ detection is still a challenge. This new instrument requires to be sensitive, reliable, low-cost, fast response, and applicable in various environments. Colorimetric assay is a simple and direct method to determine the amount of analyte based on the color change of a solution.[8,9] For this application, gold nanoparticles (GNPs) possess unique chemical and optical properties such as chemical stability, biocompatibility, functionality, and localized surface Plasmon resonance (LSPR).[10] They have been used widely in colorimetric sensors for detection of variety of materials in a solution including ions, molecules, and biomolecules.[11–13] The optical properties of gold nanoparticles depend on their size, shape, surface structure, agglomeration state, and dielectric medium. Even a small change in the aforementioned parameters leads to the red shift of the LSPR peak which also changes the visual color to the naked eye.[14]

The color change characteristic of gold nanoparticle solution is a well-known behavior for heavy metal ion detection even with very low concentrations. Li et al. detected chromium ions (Cr$^{3+}$ and Cr$^{6+}$) in water samples using sodium hyaluronate functionalized gold nanoparticles simultaneously. By addition of chromium ions to the solution, Cr$^{3+}$ and Cr$^{6+}$ bound onto the gold nanoparticles through the hyaluronate ions and aggregation of these nanoparticles causes a red shift in the plasmon frequency.[15] Chai et al. employed glutathione functionalized gold nanoparticles to detect Pb cations. Glutathione protects gold nanoparticles from aggregation in a salt saturated solution and has simultaneously the ability to capture Pb$^{2+}$. In the presence of Pb$^{2+}$, solution color turns from red to blue due to binding-induced aggregation.[14]

Herein, we introduced a colorimetric sensor based on aptamer-gold nanoparticles on a lab-on-a-phone system that is capable of detecting very tiny amounts of mercury in water samples down to 0.8 ppb range. In this study, the Hg$^{2+}$ detection occurs by measuring the aggregation of gold nanoparticles in presence of Hg$^{2+}$. These ions can bind to aptamers within thymine (T) nucleotide. Aptamer is a DNA or RNA oligonucleotide that is capable of recognizing a target with high affinity and specificity. On the other hand, aptamers can absorb to GNPs and suppress their aggregation in presence of salt solution. Anti-Hg$^{2+}$ aptamer in the presence of Hg$^{2+}$ forms T-Hg$^{2+}$-T stable combinations, which are unable to absorb to GNPs resulting agglomeration of the remaining GNPs in presence of salt.[17] The aggregated gold nanoparticles change the color of solution from red to blue or purple. Eventually, the rate of color change
corresponds to the Hg$^{2+}$ concentration and can be measured using our lab-on-a-phone system.

Recently smart phone platforms have been widely used for environmental monitoring, quality management in food chain and healthcare.[18] Herein, we apply a reliable colorimetric method in order to obtain information about concentration of mercury ions in each sample. Absorbance value can be obtained from RGB (red, green, blue) values. Any image taken by a smart phone camera consists of a number of pixels.[19] Each pixel in an images results in an RGB value from its R, G, and B sensors.

Then, the average of all RGB values taken from all pixels can be determined. Difference measured between R, G, and B of the sample and the reference solutions can be employed to measure concentration of mercury ions in the sample solution. This colorimetry analysis utilizing smart phone, produces accurate, and proper quantitative data.

2. Experimental Details
2.1. Fabrication Process

All chemicals were of analytical reagent grade and used without further purification. Gold nanoparticles were synthesized by sodium citrate reduction. In this approach, 100 mL of 0.5 $\times$ 10$^{-3}$ M HgCl$_2$ solution was boiled at 97°C on a hotplate/stirrer for 5 min.[20] Next, 10 mL of 38.8 $\times$ 10$^{-3}$ M trisodium citrate was added to the solution under stirring. After 10 min mixing, the color of solution turned to red wine and gold nanoparticles were completely synthesized.

Concentration of gold nanoparticles in a synthesized solution was estimated to be 9.7 $\times$ 10$^{-8}$ M by UV-Vis spectroscopy and Beer-Lambert law based on the extinction coefficient 1.01 $\times$ 10$^{-8}$ M$^{-1}$ cm$^{-1}$ for 10 nm nanoparticles at 520 nm. For measuring the Hg$^{2+}$ concentration, we need to compare the colorimetry results of two solutions, a reference solution and a sample solution. Thus, a solution with optimized combination of different materials was prepared. Then, for measuring the Hg$^{2+}$ concentration, one cuvette was filled with the sample solution (solution was prepared with sample water) and another was filled with the reference solution (solution was prepared with DI water without any Hg ions). Preparation of the solution can be done using the following optimized protocol: 75 $\mu$L of 10 $\times$ 10$^{-6}$ M aptamer solution was mixed with 75 $\mu$L of water sample (or DI water). After leaving the solution for 5 min at room temperature, 225 $\mu$L of 9.7 $\times$ 10$^{-9}$ M synthesized gold nanoparticles was added. Again, the solution was left at room temperature for 5 min to react and then 75 $\mu$L of 0.25 M NaCl solution was added. The prepared solution was allowed to rest and react for 15 min at room temperature.

In order to calibrate our sensor, we tested it with different water samples to check the variation of color between red to dark blue.[21] The concentration of mercury in samples S1, S2, S3, and S4 was 0, 500 $\times$ 10$^{-9}$ M, 1 $\times$ 10$^{-6}$ M, 10 $\times$ 10$^{-6}$ M, respectively, and their color variations are shown in Figure 1. As mentioned earlier, higher mercury concentration removes more aptamers from nanoparticle’s surface. Later by adding salt to the solution, more GNPs aggregate together and the solution’s color turns more to dark blue. However, for low concentrations of mercury, the color change of the solution cannot be detected with naked eye. In this case, an expensive UV-Vis spectroscopy system can be utilized to detect the shift in the absorption peak of different spectra or instead, our low-cost device can be installed on a smart phone and detect the exact amount of the Hg ions.

2.2. Structure Design

In this apparatus, two LEDs with 520 nm (green) and 640 nm (red) wavelengths used for measuring the difference between the absorption of two reference and sample solutions.

As shown in Figure 2a, the designed lab-on-a-phone device consists of four chambers; board, light path, cuvette, and camera depth of focus chambers. A 3V battery for power supply, LED board and a diffuser in order to keep the lights uniformly in contact with the cuvettes can be named as the main parts of the device. There are two places for cuvettes, one for sample solution consisting the sample water solution and another one including the DI water reference solution. Both cuvettes are illuminated by red and green LEDs and the passed lights through the cuvettes (separated by four rectangular apertures (see Figure 2b)) finally reaches the smart phone camera. Device can be installed on any smart phone using a mechanical fixture.

Figure 2b illustrates a sample image of the lights after passing through the sample and reference solutions on the mobile screen. As shown in the picture, the red and green rectangles on the left are the captured light from the reference solution and the two rectangles on the right are from the sample solution. In order to have a better estimation of Hg$^{2+}$ concentration and avoiding any false measurement resulting from the edge detection, a centered rectangle of 100 $\times$ 200 pixel was smartly filled with the reference and sample lights were named (separated by four rectangular apertures (see Figure 2b)) finally reaches the smart phone camera. Device can be installed on any smart phone using a mechanical fixture.

Figure 1. Different colors of Solutions with different Hg concentrations. S1, S2, S3, and S4 have respectively 0, 500 $\times$ 10$^{-9}$ M, 1 $\times$ 10$^{-6}$ M, and 10 $\times$ 10$^{-6}$ M concentration.
In order to have a better estimation of the color change and a more comparable set of pictures, the camera settings were always set to specific values using the software. To avoid any miscalculation, it is very important that both cuvettes must be exposed with the same light intensity or a calibration factor must be extracted for the system. Therefore, before any measurement, both cuvettes were filled with DI water and their picture was analyzed. The ratio of RS to RR (RS/RR) named red fraction (RF) and the ratio of GS to GR (GS/GR) named green fraction (GF). If RF and GR are 1 it means the same intensity of green and red light passes through every cuvettes otherwise these values (RF and GF) must be used for calibration of the green and red signal in both cuvettes.

An image was taken with a smartphone camera for each experiment and includes the information of green reference, red reference, green sample, and red sample according to Figure 2b. To get the numerical value of these parameters, the average of all pixels in the corresponding rectangle was calculated. Then for each specific case, the amount of (GS-GR) calculated. This subtraction indicates the amount of green signal changes in the sample relative to the reference solution. (RS-RR) was calculated too. The ratio of green color changes to the red color changes that calculated according to the Equation (1) is a linear function of the mercury concentration. The relation between $\Delta G/\Delta R$ and concentration of mercury ions is expressed with accuracy above 99% in the section 3.2.

$$\frac{\Delta G}{\Delta R} = \frac{GS - GR}{RS - RR}$$  

3. Results and Discussions

Gold nanoparticle solution is stabilized by citrate ions against aggregation because of Van der Waals attraction between GNPs.[22] These stabilized GNPs have LSPR absorption peak at 520 nm and the solution has a red color. This color is influenced by the particles size, in a way that by increasing the size of nanoparticles, the color varies from red to dark blue.[23] By adding NaCl to the GNP solution, the stabilizing agents disappear between the gold atoms and this leads to aggregation of the gold atoms and increasing the particles sizes. Therefore, by adding NaCl solution to GNP, its color changes to dark blue. Single stranded oligonucleotides (ssDNA) can be absorbed by GNPs and suppress their aggregation in presence of salt solution.[24] Binding between nitrogen atoms of ssDNA and gold atom is stronger than electrostatic attraction between the positively charged phosphate backbone and the negatively charged GNP.[24] So if we add salt to a solution including ssDNA and GNPs, ssDNA is adsorbed to GNPs atoms and solution’s color remains unchanged.[17] Hg$^{2+}$ ions are able to bind to thymine (T), so they can change T-T to T-Hg$^{2+}$-T and form double stranded oligonucleotides (dsDNA) that are stable at room temperature.[25] We have used single oligonucleotides (S’-TTTTTTTTT-3’) as the aptamer of Hg ions.[26] This aptamer can easily absorb to Au atoms but in the presence of Hg$^{2+}$ ions in the solution, two ssDNA form a dsDNA structure which cannot be absorbed strongly by GNPs.[24] So if we have a solution containing GNPs, aptamer, and Hg solution, Hg ions and aptamers bind together and if we add a salt solution, GNPs aggregate and the solution’s color changes to dark blue. Using this phenomenon, we can identify mercury ions in a sample water by measuring the amount of its color change compared to the reference solution.

3.1. Characteristics of GNPs

The gold nanoparticle solution was prepared by reduction of sodium citrate, as mentioned earlier. We used transmission electron microscopy (TEM, Philips CM30) to investigate the shape of synthesized gold nanoparticles. Thus, the GNP solution was dispersed on a Formvar/carbon coated grid and dried at room temperature. GNPs were mainly synthesized in spherical shapes with diameter of 10 nm. TEM images of the synthesized and the aggregated functionalized GNPs in the presence of Hg$^{2+}$ are shown in Figure 3.

![Figure 2. a) Design of the proposed device and (b) Left rectangles received from reference cuvette and the right rectangles received from sample cuvette. Inside of each signal zone, a rectangle of 100’200 pixel was considered. The reference cuvette contained GNPs + aptamer + DI water + salts solution and the sample cuvette contained GNPs + aptamer + water sample + salts solution.](image-url)
The gold nanoparticle’s absorption spectrum has been measured using UV-Vis spectroscopy from 400 to 700 nm range that is shown in Figure 4a. According to this figure, the absorption peak of synthesized gold nanoparticles is located at 520 nm. Also, the sharpness and limitation of the absorption peak in this range indicated that the gold nanoparticles have the same and uniform size distribution in the solution. The LSPR absorption peak of nanoparticles depends on their size.[27] Therefore, the change and non-uniformity in size of gold nanoparticles shifts and widens the absorption peak.

The absorption spectra of 10–50 nm GNPs has been simulated using MiePlot software and the results are shown in Figure 4a to compare with the synthesized gold nanoparticle spectrum. The software’s algorithm is based on Mie theory.[28,29] Comparison between results of 10 nm GNPs generated with Mie theory and the synthesized GNPs using UV-Vis have indicated that both of them have similar absorption at 520 nm. Therefore, the synthesized GNPs have a good uniformity and a diameter about 10 nm. As mentioned earlier, changing in size of gold nanoparticles causes variation of the solution color that arises from the change in the surface plasmon resonance frequency. The addition of salt to the sample solution causes aggregation of gold nanoparticles (see Figure 4b).

3.2. Results from the Proposed Device

For each concentration of Hg²⁺, as mentioned earlier, a specific ΔG/ΔR was obtained using the smart phone image. Applying the optimized protocol and an android customized application, we have used the developed lab-on-a-phone system and measured the ΔG/ΔR for different solutions containing different Hg²⁺ concentrations. As shown in Figure 5, by increasing the concentration of Hg²⁺, the ΔG/ΔR value also has increased logarithmically. As earlier mentioned, the increment of ΔG/ΔR value at higher Hg²⁺ concentrations is based on the enhanced aggregation of gold nanoparticles. According to changes in the LSPR under the influence of the accumulation of gold nanoparticles, relative value of red signal decreases and relative value of green signal increases. Therefore, the ΔG/ΔR value increases by increasing of Hg²⁺ concentration that can also be seen in Figure 5a. Under optimum experimental condition, ΔG/ΔR is proportional to Hg²⁺ concentration and the theoretically fitted linear function is expressed in Equation (2):

\[
\Delta G/\Delta R = 0.19 \text{(Hg Conc.)} + 1.25
\]

The linear relationship was found between 3 and 800 nM with a correlation coefficient \((R^2)\) of 0.99. The limit of detection (LOD) is found to be \(3 \times 10^{-9} \text{M} \approx 0.8 \text{ppb}\) for Hg determination with
the signal to noise ratio of 3. According to the World Health Organization (WHO), the maximum allowed amount of mercury in drinking water is $10 \times 10^{-9} \text{ M}$ (6 ppb) that is well above the detection limit of this biosensor. The responses of our designed lab-on-a-phone device were monitored for the repetitive measurements of $100 \times 10^{-9} \text{ M} \text{Hg}^{2+}$ for ten times in each day over 1 week to evaluate the operational stability of the fabricated biosensor. The response of the device exhibited less than 3% variation during a week.

### 3.3. Interference Effects

A series of test solutions containing DI water plus several potent interfering compounds ($\text{Cu}^{2+}$, $\text{Ni}^{2+}$, $\text{Cd}^{2+}$, $\text{Fe}^{3+}$, $\text{Zn}^{2+}$, $\text{K}^{+}$, $\text{Mg}^{2+}$, $\text{Cr}^{3+}$, $\text{Cr}^{6+}$, $\text{Co}^{2+}$, $\text{Ba}^{2+}$, and $\text{Pb}^{2+}$) with the concentration of 500 nM were prepared to evaluate the selectivity of the lab-on-a-phone device. Then, the responses of the device were obtained for the prepared test solutions with the aforementioned process and compared with that obtained for a standard $\text{Hg}^{2+}$ solution. As shown in Figure 6, the analysis of results demonstrate that the $\text{Hg}^{2+}$ sensor response is negligible for other added metal ions. Observation illustrates cross-reactivity for $500 \times 10^{-9} \text{ M} \text{Hg}^{2+}$ was less than 5% in the presence of additional ions and it provides an influential implement for real sample tests.

### 3.4. Real Sample Analysis

The performance of the lab-on-a-phone device for real sample analysis was examined by determination of $\text{Hg}^{2+}$ in tap water and Zam Zam water samples. Specific amounts of $\text{Hg}^{2+}$ were added to them and the responses of the fabricated device were obtained before and after addition of $\text{Hg}^{2+}$. A comparison of the results showed that the average recovery for the added samples is better than 96.5%. The analytical results are shown in Table 1.

### 4. Conclusions

In this study, we introduced and developed a lab-on-a-phone device that can detect and measure various concentrations of mercury in water. This device is easy to operate, on-site, low cost, user-friendly interface, fast response, and the ability of detecting

| Table 1. Lab-on-a-phone results for Tap water and Zam Zam water samples. |
|----------------|-----------------|-----------------|-----------|
| Sample        | Added [$\times 10^{-9}$ M] | Found [$\times 10^{-9}$ M] | Recovery [%] |
| Tap water     | 20               | 18 ± 0.5       | 92.5       |
|               | 100              | 98 ± 0.2       | 98.2       |
|               | 200              | 199 ± 0.1      | 99.5       |
| Zam Zam water | 20               | 19.5 ± 0.2     | 98.5       |
|               | 100              | 91 ± 0.7       | 91.7       |
|               | 200              | 195 ± 0.6      | 98.8       |

Each test repeated for three times and average of three repeat ± standard deviation reported.
mercury at ppb level. For this application, we have provided a solution that its color changes proportional to the concentration of mercury present in the sample solution. For low concentrations of mercury, the amount of color change due to LSPR peak variation is very small but detectable using the camera of a smartphone. Briefly, the color change of the sample solution compared to the reference solution for concentrations above 500 nM can be easily seen by naked eyes. Nevertheless, with the help of mobile friendly software, we are able to detect and measure the concentrations of Hg$^{2+}$ with LOD $3 \times 10^{-9} \text{M} (\approx 0.8 \text{ppb})$.

**Conflict of Interest**
The authors declare no conflict of interest.

**Keywords**
colorimetry, gold nanoparticles, lab-on-a-phone, localized surface, mercury ions, plasmon resonance

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