Arsenic Detection

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Use of Gold Nanoparticles in a Simple Colorimetric and Ultrasensitive Dynamic Light Scattering Assay: Selective Detection of Arsenic in Groundwater**

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The contamination of drinking water with arsenic poses a threat to global health.[1a–e] As many as 140 million people worldwide may have been exposed to drinking water with arsenic contamination levels higher than the World Health Organization's (WHO) guideline of 10 ppb.[1a] The major arsenic species found in environmental samples are inorganic arsenite (As^{III}) and arsenate (As^{V}) salts, organic forms of arsenic, for example, dithioarsenate (DTA), dimethylarsinic acid (DMA), and monomethylarsinic acid (MMA).[1b–f] Current technology based on laboratory-based analytical procedures[2a,b] is time-consuming and relies on a series of enrichment steps. As a result, the development of ultrasensitive assays for the real-time detection of arsenic has attracted considerable research efforts in recent years.[2c–f,3a,b] Noble-metal nanostructures attract much interest because of their unique properties, including large optical field enhancements that result in the strong scattering and absorption of light.[3a–n] In the last 15 years, the field of biological and chemical sensors that use nanomaterials has witnessed an explosion because of the unique optical properties of nanomaterials.[3a–n] Very recently, a surface-enhanced Raman scattering (SERS) based assay[3a] and surface plasmon resonance (SPR) sensors[3b] have been reported for arsenic detection down to 1 ppb, which is an order of magnitude lower than WHO guidelines.[1a] However, these assays are not selective against alkali-, alkaline-earth- and heavy-transition-metal ions. Such selectivity is essential for applications to real environmental samples. Herein, we report a glutathione (GSH), dithiothreitol (DTT), and cysteine (Cys) modified gold-nanoparticle-based dynamic light scattering (DLS) assay for the label-free selective detection of arsenic, with an excellent detection limit (10 ppt) and selectivity over other analytes.

Dynamic light scattering, also known as photon correlation spectroscopy (PCS), is a well-established noninvasive technique for measuring the size of particles in the range from 0.5 nm to 6 μm.[4a–d] DLS is an absolute measurement and is a powerful tool for determining small changes in the size of particles. Recently, it has been shown that DLS can be used to monitor the nanoshell (nanoparticle with a dielectric core covered by a metallic shell) concentration in blood samples, cancer biomarker detection, DNA sensing, and to probe the interaction between enzymes and quantum dots.[4a–d] Our detection method is based on the aggregation of GSH/DTT/Cys-modified gold nanoparticles. Binding between As^{III} and the surface-modified gold nanoparticles results in their aggregation (Figure 1 a–c). The chelating sulfur-containing ligands DTT, GSH, and Cys bind to the gold nanoparticle surface through Au–S bonds;[3b,m–n] it is well known that As^{III} has a very high affinity for these ligands.[2a–f,3b,5a–c] Each As^{III} ion can bind with three DTT-conjugated gold nanoparticles through an As–S linkage (Figure 1 a). In the case of the GSH- or Cys-conjugated gold nanoparticles, however, there is no free SH group available for binding with As^{III} ions. It has been reported that As^{III} can bind with humic acid[2e] and *N*-(dithiocarboxy)-*N*-methyl-D-glucamine[3b] though an As–O linkage.

We proposed that each As^{III} ion can bind with three GSH- or Cys-conjugated gold nanoparticles through an As–O linkage (Figure 1 b,c). The stability constants[5a–c] for As^{III} ions and chelating ligands are $\log K = 32.0$ (GSH), 37.8 (DTT), and 29.84 (Cys). On the other hand, the stability constants between other heavy-metal ions and DTT are $\log K = 11.06$ (Zn), 13.89 (Pb), 14.6 (Cd), 10.7 (Ni), 15.3 (Cu), 17.6 (Hg).[5a,c] The stability constant of the As^{III}–DTT complex is about 30 orders of magnitude higher than that of other interfering metal ions. The colorimetric response of the GSH/DTT/Cys-modified gold nanoparticles upon addition of As^{III} is shown in Figure 2 a, and the corresponding absorption spectrum is shown in Figure 2 b. The strong long-wavelength band in the visible region ($\lambda = 520$ nm) arises from the oscillation of the conduction band electrons. The band that appears around 670 nm after the addition of As^{III} demonstrates the aggregation of gold nanoparticles. When we modified the surface with only DTT and GSH, the assay showed negligible responses toward Sb^{III}, Mn^{II}, Ni^{II}, K^{II}, Cr^{III}, and Sr^{II} (all at 10 ppm) but we have noted a small shift in the plasmon band energy to longer wavelength and a color change from red to blue in the presence of Hg^{II}, Pb^{II}, Fe^{II}, Fe^{III}, Zn^{II}, as well as Cd^{II} (all at 10 ppm). When we modified the surface with all three ligands DTT, GSH, and Cys, we saw

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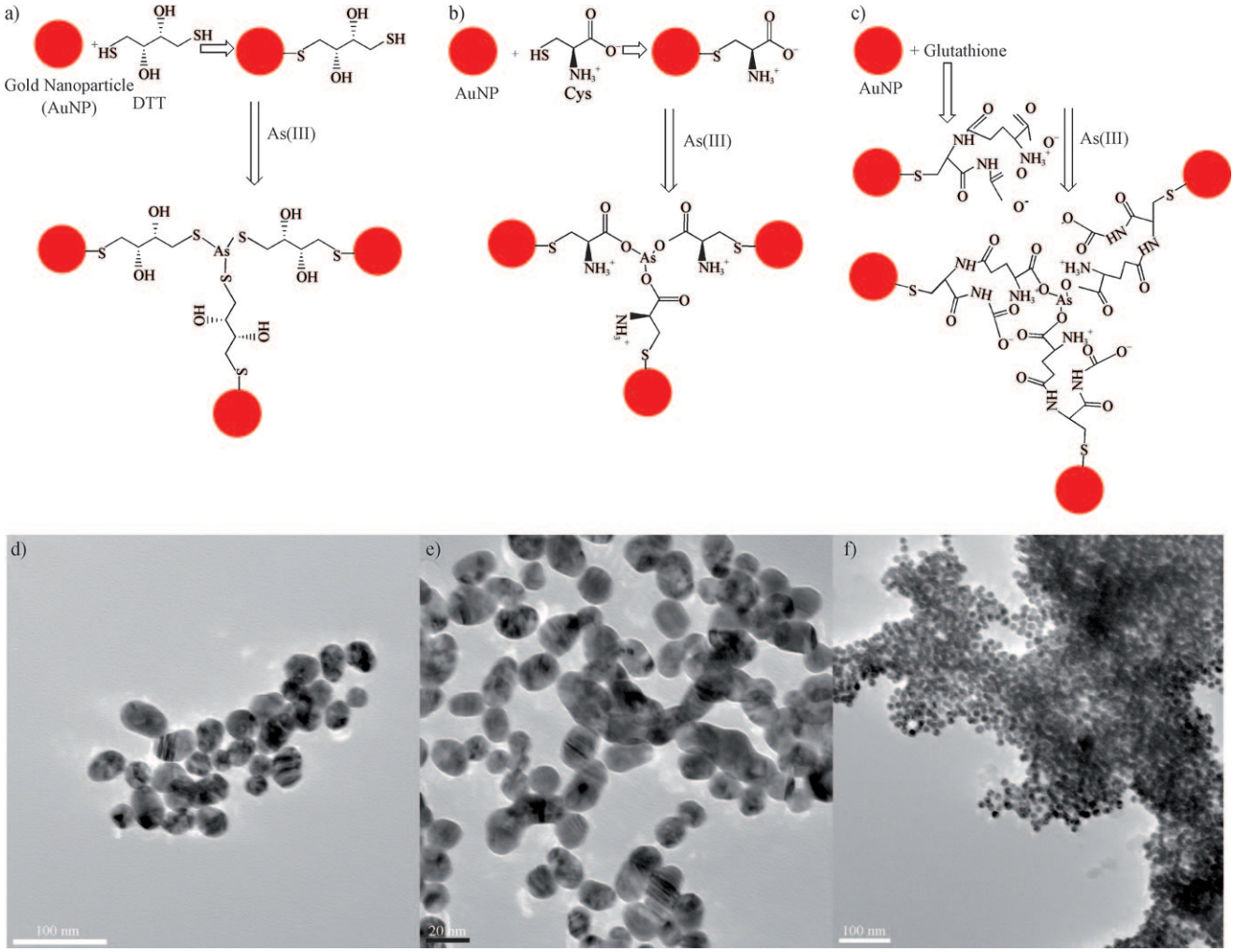

Figure 1. a) Representation of gold-nanoparticle-based arsenic detection. a) DTT-modified gold nanoparticles, b) Cys-modified gold nanoparticles, and c) GSH-modified gold nanoparticles. d) TEM image showing GSH/DTT/Cys-modified gold nanoparticles before addition of As^{III}. e) TEM image demonstrating aggregation of GSH/DTT/Cys-modified gold nanoparticles after addition of 80 ppb As^{III}. f) TEM image after the addition of 250 ppt As^{III}.

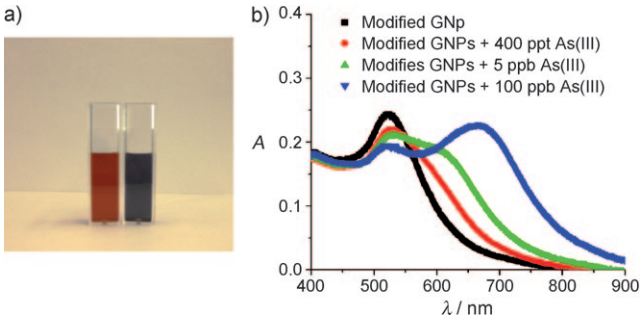

Figure 2. a) Photograph showing colorimetric change of GSH/DTT/Cys-modified gold nanoparticles upon addition of 800 ppb As^{III}. b) Absorption profiles of modified gold nanoparticles before and after addition of As^{III} ions.

negligible responses from Pb^{II}, Fe^{II}, Fe^{III}, Zn^{II}, and Cd^{II}, but a good response from Hg^{II}, even at a concentration of 10 ppm.

The selectivity of the assay toward As^{III} ions was improved even further by the addition of another chelating ligand, 2,6-pyridinedicarboxylic acid (PDCA). The stability constants of heavy-metal ions[6] with PDCA are $\log K(\mathrm{Pb}) = 8.2$ (Pb), 20.2 (Hg), 10.0 (Cd), and 8.5 (Mn). Therefore PDCA will be able to form much more stable complexes with Hg^{II} than with other metal ions. Since PDCA will not be able to link with gold nanoparticles through the SH linkage in the same fashion as DTT, GSH, and Cys, PDCA mostly forms complexes with Hg^{II} ions in the bulk solutions, thus suppressing interference of Hg^{II} ions with the nanoparticle surface. Figure 3 clearly shows excellent selectivity over alkali-, alkaline-earth and heavy-transition-metal ions after the addition of PDCA to DTT/GSH/Cys-modified gold nanoparticles. DTT can also reduce As^{V} to As^{III}, [3b,5c,7] thus, this result clearly demonstrates that the assay can detect As^{III} as well as As^{V} (Figure 3a). To evaluate the sensitivity of this colorimetric technique, different concentrations of As^{III} from one stock solution were evaluated. The colorimetric assay is highly sensitive to the concentration of As^{III} ions (Figure 3b) and its sensitivity is as low as 1 ppb, which is an order of magnitude less than that in the EPA guidelines. We also separately recorded sensitivities of 5 ppb, 20 ppb, and 25 ppb for DTT-

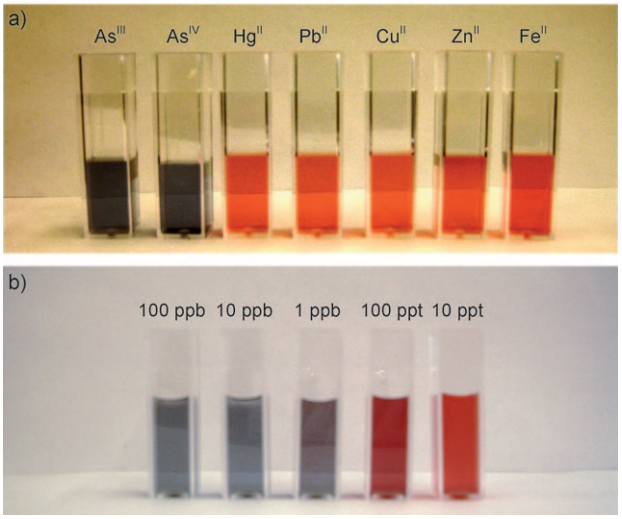

Figure 3. Photograph showing colorimetric changes of GSH/DTT/Cys-modified gold nanoparticles in the presence of PDCA upon addition of a) various metal ions (5 ppb) and b) different concentrations of As^{III}.

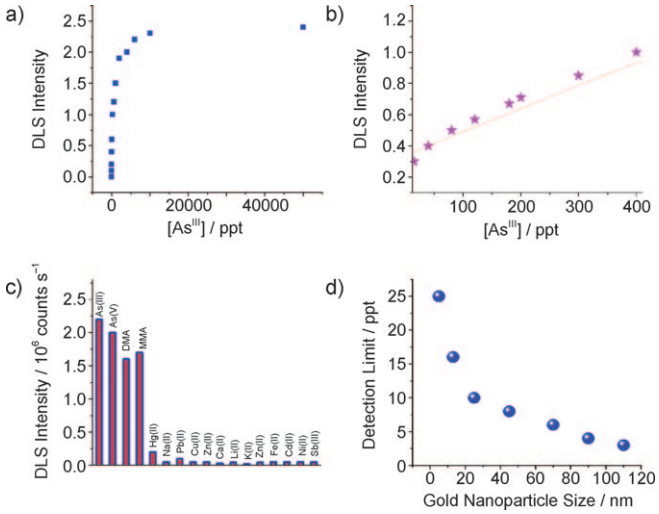

Figure 4. a) Variation of DLS intensity with As^{III} concentration a) from 1 ppt to 50 ppb and b) 0 ppt to 450 ppt. The solid line represents a linear fit of the data ($R = 0.96$). c) DLS response for different arsenic species and selectivity of the DLS assay over heavy metal ions (100 ppb). d) DLS detection limit as a function of gold nanoparticle size.

conjugated, GSH-conjugated, and Cys-conjugated gold nanoparticles, respectively.

We employed DLS to improve the sensitivity of the assay towards As^{III}. DLS is a powerful method for the determination of small changes in particle sizes, and can therefore be used to distinguish dimers from monomers. When As^{III} was added to GSH/DTT/Cys-modified gold nanoparticles, dimers, trimers, and larger aggregates are formed (Figure 1 d–f), depending on the As^{III} concentration. Figure 4 clearly shows that DLS is highly sensitive to the concentration of As^{III}. The experimental results (Figure 4 b) clearly demonstrate that the sensitivity of the DLS assay is as low as 10 ppt for As^{III} detection. To the best of our knowledge, the detection limit achieved with this DLS probe represents the lowest among all the reported methods.[2a–f,3a,b] This result shows that the DLS assay is about two orders of magnitude more sensitive than common colorimetric techniques, and its sensitivity is three orders of magnitude higher than the WHO guidelines.[1a] Several impurities can arise from heavy metal ions in genuine environmental samples. Figure 4 c shows the DLS response of the GSH/DTT/Cys-modified gold nanoparticles in the presence of various environmentally relevant heavy-metal ions. The experimental results clearly demonstrate that this DLS assay can be used for the detection of arsenic in environmental samples. To show whether this assay can detect organoarsenic compounds, we also tested this DLS assay response toward dimethylarsinic acid (DMA) and monomethylarsinic acid (MMA), which are the common organoarsenic species in rice.[8] As shown in Figure 4 c, the experimental results clearly demonstrated that the GSH/DTT/Cys-modified gold nanoparticle based DLS assay can detect compounds such as DMA and MMA.

To understand whether the DLS assay sensitivities vary with the size of the gold nanoparticles, we performed experiments using 5–110 nm gold nanoparticles. The experimental results (Figure 4 d) show that the assay sensitivity is highly dependent on the particle diameter. As the particle diameter increases, the detection limit improves and the data indicate that the assay can detect As^{III} ion levels as low as 3 ppt when the particle size is 110 nm. This variation of sensitivity efficiency with particle size can arise from the reduced surface area of smaller nanoparticles, which will limit accommodation of the chelating ligands by the gold nanoparticles. To demonstrate the potential practical application of the assay to measure the As content in ground water, we collected water samples from Bangladeshi wells that are contaminated with As, and compared the data with samples collected from a tap at Jackson State University and bottled water from Walmart, Jackson, Mississippi, USA.

As shown in Figure 5, the colorimetric assay clearly shows that the amount of As in water from Bangladeshi wells is much more than 1 ppm and the arsenic content in tap water and bottled water from Jackson are much lower than 1 ppm. To measure the actual arsenic quantity, we used the DLS assay, which showed that the amount of As in Bangladeshi well water is 28 ppb whereas the amount of As in Jackson tap water and bottled water are 380 and 15 ppt, respectively. To compare the data with well-established techniques, we also measured the As content using ICP-MS data (ICP = inductively coupled plasma), which indicated that the arsenic content of Bangladeshi well water is 30 ppb, which is comparable with the DLS assay data. Since the sensitivity of ICP-MS is not sufficient to measure the As content in tap and drinking water from Jackson, we are not able to compare the DLS data with the ICP-MS data.

In conclusion, we have demonstrated a label-free, selective colorimetric assay and highly sensitive DLS assay for 3 ppt arsenic recognition in aqueous solution. The experimental results show that As can be detected at the ppt level quickly and accurately without any tagging, and with excellent discrimination against other heavy metals. This DLS

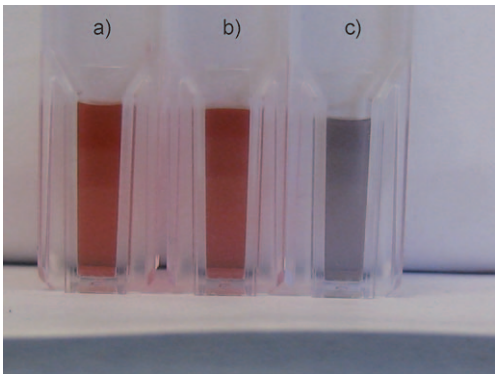

Figure 5. Colorimetric assay response upon addition of a) drinking water from Mississippi, USA; b) Tap water in Mississippi, USA; c) drinking water collected from Bangladeshi wells.

assay is rapid and takes less than 10 min from As binding to detection and analysis. The sensitivity of the assay toward the arsenic level in water is about three orders of magnitude higher than the WHO standard limit. We have also demonstrated that the DLS assay is capable of predicting the amounts of arsenic in Bangladesh well water as well as tap and bottled water from Jackson. The experimental results reported here open up a new possibility of rapid, easy, and reliable diagnosis of arsenic from environmental samples. Given the simplicity, speed, and sensitivity of this approach, the described methodology could easily be extended to a high-throughput format and become a new method of choice in all applications that require an assay for chemical toxin detection.

Experimental Section

Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$), $NaBH_4$, sodium citrate, GSH, DTT, and Cys were purchased from Sigma–Aldrich and used without further purification.

Gold nanoparticles of different sizes and shapes were synthesized by controlling the ratio of $HAuCl_4 \cdot 3H_2O$ and sodium citrate, as recently reported.[3c–f] The nanoparticles were characterized by TEM (JEM-2100F transmission electron microscope) and UV/Vis spectroscopy.

The gold nanoparticle surfaces were modified by addition of GSH (10 mM, 10 μL), DTT (10 mM, 10 μL), and Cys (12 μM, 10 μL) to a solution of gold nanoparticles (1.5 nM, 2 mL) with stirring for 2 h. The mixture was subsequently left for 12 hours without disturbance. For maximum selectivity toward the As^{III} ion, PDCA was added, and the solution was left for one hour.

DLS experiments were performed using a Malvern Zetasizer Nano instrument. To reduce the multiple scattering effect, non-invasive back-scatter (NIBS) technology was used.

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