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ARTICLE TYPE

A paper based microfluidic device for the detection of arsenic using gold nanosensor

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A paper based microfluidic device is fabricated that can rapidly detect very low concentration of As^{3+} ions using gold nanosensor, Au-TA-TG. This simple but efficient system develops a visible bluish-black colour precipitate due to the formation of nanoparticle aggregates through transverse diffusive mixing of Au-TA-TG with As^{3+} ions on paper substrate. The approach is extremely selective for arsenic with a detection limit of 1.0 ppb, which is lower than the WHO's reference standard for drinking water.

Arsenic contamination of ground water is a major concern these days in many developing countries because its exposure can trigger toxic effects in human health.[1, 2] Lots of efforts through optical and electrochemical pathways have been invested for the detection of arsenic ions below 10 ppb, but certain limitations such as cost effectiveness and easy accessibilities of the specific instrument made those approaches non-suitable specifically for onsite real-time analysis of arsenic.[3-6] Lab-based techniques like Atomic Absorption Spectrometry (AAS), Atomic Fluorescence Spectrometry (AFS), and Inductively Coupled Plasma with Mass Spectrometry (ICP-MS) could provide the accurate detection of arsenic, but they are neither cost-effective nor capable of on-site analysis of sample.[4, 5] Electrochemical method for arsenic detection has been proved as an affordable, accurate determination technique with low detection limit, but it still requires a lab setting with bulk electrodes in an electrochemical cell and also suffers from the interferences by the co-deposition of other metals, especially copper if present along with arsenic in water sample.[3, 6] Another reliable approach, i.e. colorimetric method is simple, inexpensive and show good detection limit.[7, 8] However, most of the colorimetric analysis is performed in solution and suffered from safety issues since toxic arsine gas (AsH_3) is produced during the operation. Despite of this drawback, colorimetric technique is widely used throughout the world because of its simplicity and ability to on-site use. [9]

In order to avoid involvement of toxic chemicals in arsenic analysis, researchers have explored and utilized gold nanoparticles (AuNPs) as potential sensor materials in colorimetric assays.[10-13] Due to high extinction coefficients and unique size dependent optical property, AuNPs show visual colour change upon aggregation with arsenic metal ions, demonstrating its outstanding ability for solution based analysis. However, to the best of our knowledge, the use of gold nanoparticles has not received much attention on miniature scale dipstick or chromatographic type arsenic screening devices which would be low cost, safe, disposable, and similar to the in-house glucose or malaria diagnosis kits.

Motivated by these clear demands, we present a simple paper-based microfluidic device in which the proposed gold nanosensor, Au-TA-TG rapidly interacts with As^{3+} ions to develop a visible dark bluish-black precipitate at the interfacial zone. It is worth noting that paper based materials have extensively been investigated in microfluidic research for its potential use in biosensor and electronic applications.[14-16] Due to the capillary action through fibres and pores, paper substrate can develop a self-driven fluid flow with a steady flow-rate of 2-3 µl/min.[17, 18] This low flow-rate allows a very low concentration of arsenic to be retained in microchannel for sufficient period of time for interaction with nanosensor that can produce an intense signal in terms of visible band for analysis. Figure 1 demonstrates the present paper based microfluidic device for arsenic detection using gold nanosensor. Whatman filter paper was used for the

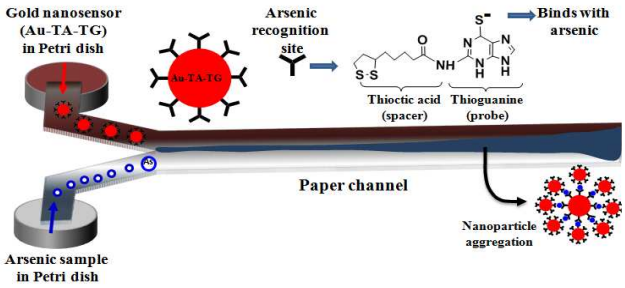

Fig. 1 A schematic representation of paper based microfluidic device for arsenic detection using gold nanosensor, Au-TA-TG.

preparation of 'Y' shaped fluidic channel device by micro-machining method carried out by CO_2 laser engraving system (VLS 2.30, Universal Laser Inc., USA) at 3 Watt. The engraved channel parameters were as follows: length (l) = 15 mm, width (w) = 3 mm and height (h) = 0.1 mm (100 µm). The filter paper channel was soaked in warm HPLC grade water for 30 minutes to remove the impurities, if any. The gold nanosensor was prepared

by step-wise chemical conjugation of gold nanoparticles (AuNPs) with thioctic acid (TA) followed by thioguanine (TG) molecules in presence of EDC/NHS (Supporting Information). Each chemical modification step was followed by hydrodynamic size and surface charge measurements (Table S1). FTIR spectrum of the final nanosensor shown in Fig. S1 confirms the attachment of thioguanine probe with the thioctic acid of AuNPs via amide bond formation (ν_{CO} = 3300 cm^{-1}), keeping –SH functional group free for arsenic recognition through As-S interaction (Fig. 1).[19] The use of thioctic acid as spacer arm can increase the detection ability by decreasing steric hindrance.[20] During detection process, thioguanine probes interact with arsenic ions that lead to the aggregation of nanoparticles. Consequently visual colour change from red to blue is observed because of inter-particles coupled plasmon resonance from aggregated AuNPs (Fig. 2, inset). This distinct colour change forms the basis of the present low-cost, portable, efficient paper based device for very low level (<10 ppb) arsenic detection.

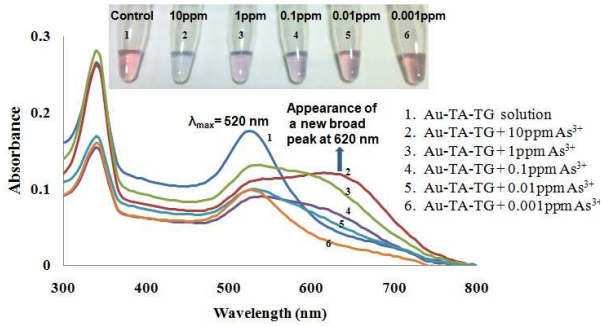

Fig. 2 Differential UV-Vis absorption responses of the Au-TA-TG treated with various concentrations of arsenic. Inset: visual colour changes of the Au-TA-TG.

To explore the affinity of gold nanosensor towards As^{3+} ions, UV-Vis technique was utilized to monitor the colour change in the presence of arsenic ions. Fig. 2 demonstrates the UV-Vis spectra of Au-TA-TG solution in which the nanosensor was stable due to electrostatic repulsion imposed by negative surface charge of particles (-30.1 mV, Peak 1) and after treatment with arsenic at pH~5.5 (Peaks 2-6). The shift in UV-Vis wavelength (λ_{max} = 520 nm to λ_{max} = 620 nm) indicates arsenic binding with Au-TA-TG and formation of aggregates in solution. The gold nanoparticle aggregation was confirmed by FE-SEM experiments (Supporting Information). Fig. S2 explains the increase in size of the nanosensor upon interaction with As^{3+} ions. To determine the sensitivity of the nanosensor, various concentrations (10, 1, 0.1, 0.01, and 0.001 ppm) of arsenic solutions were treated with Au-TA-TG and monitored through UV-Vis technique (Fig. 2). At the concentrations 10, 1, 0.1, and 0.01 ppm, the absorption spectra show a shift in wavelength (λ_{max}) of 100 nm (520 to 620 nm) with a concomitant growth of a new broad peak at 620 nm. In case of 0.001 ppm concentration, this shift was not observed distinctly, which reveals the fact that the nanosensor is not sensitive below 0.01 ppm when UV-Vis technique is employed. The sensitivity of Au-TA-TG was also very poor when visual colour change is considered; the nanosensor did not show significant colour change in test tube below 0.01 ppm (Fig.2, inset). This is quite reasonable, because the number of As^{3+} ions was very less to form precipitate at this concentration and to show the blue colour in solution. This study clearly proves that the colorimetric method has a certain cut-off limit in solution-phase analysis and is not suitable when the concentration of toxic ions is very low. However, the sensitivity at lower level could be increased if the interaction between nanosensor and arsenic ions happens in a confined zone of a microfluidic channel device. In microfluidic device, the probability of transverse diffusive mixing between Au-TA-TG and arsenic may increase many folds even at very low concentration to show the colour change in terms of precipitate formation at the interface of the microchannel.

To investigate the low-level arsenic detection ability using Au-TA-TG as sensor material, we used paper based microfluidic device that works in laminar flow mode. In this study, the Y-shaped paper microchannel was placed on a teflon-wrapped glass slide. Both Au-TA-TG and arsenic solution were kept in two 3 mL petri-dishes, in which the extended arms of the Y-channel were dipped as shown in Fig. 1. As soon as the two arms are touched into the respective solutions, fluids started flowing into the channel by capillary actions as we demonstrated in our previous work.[17] When Au-TA-TG solution meets with As^{3+} ions, a bluish-black precipitate was formed within five minutes resulting in a distinct band at the interface of two fluids. The concentration of arsenic was varied from 10 ppm to 0.001 ppm and a clear band was observed at the interface for each concentration. Though the band became faint at 0.001 ppm, it was easily visualized with naked eye confirming the lowest detection limit. In a control study, HPLC grade water was sent through one of the channel instead of arsenic containing sample, but no band or precipitate formation was observed while interacting with gold nanosensor in microchannel. To evaluate the reproducibility, this study was performed several batches

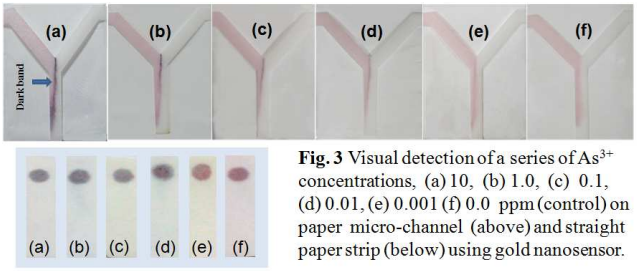

Fig. 3 Visual detection of a series of As^{3+} concentrations, (a) 10, (b) 1.0, (c) 0.1, (d) 0.01, (e) 0.001 (f) 0.0 ppm (control) on paper micro-channel (above) and straight paper strip (below) using gold nanosensor.

(Fig. S3). Fig. 3 compares the images of the microfluidic channel for gold nanosensor treated with a series of As^{3+} concentrations, such as 10 ppm, 1 ppm, 0.1 ppm, 0.01 ppm, and 0.001 ppm, showing the degree of precipitation in the order of 10 ppm>1.0 ppm>0.1 ppm>0.01 ppm>0.001 ppm. Of note, while concentration of 0.001 ppm (1.0 ppb) is hardly detected in solution, it is clearly seen in terms of aggregate precipitation in paper channel. In order to realize arsenic selectivity of gold nanosensor in presence of other common metal, Au-TA-TG was tested against a variety of competitive metal ions, including Ca (II), K(I), Mg (II), Na (I), Fe (III) (normally present in drinking water) in solution as well as on paper channel. In solution-phase, each metal ion (10 ppm) was individually added to the

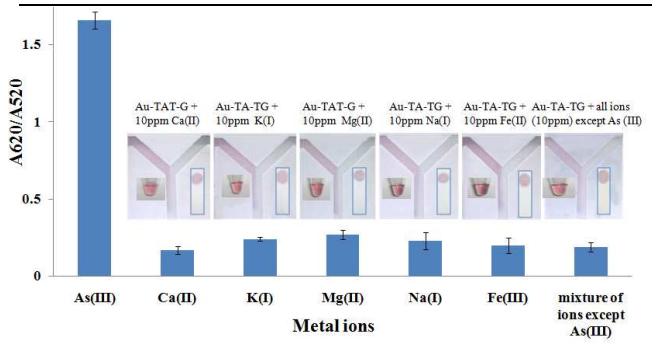

Fig. 4 Selectivity of gold nanosensor for As (III) against a variety of competitive metal ions, Ca (II), K(I), Mg (II), Na (I), Fe (III) and a mixture of them (no arsenic) at 10.0 ppm. Inset: visual colour display of Au-TA-TG in test tubes, Y-shaped paper microchannel and straight paper strip assay after treatment with various metal ions.

nanosensor and the wavelength ratio A620/A520 was calculated. Fig. 4 shows the negligible difference in colour (inset) and A620/520 between different solutions containing 10 ppm of common metal ions. In paper microfluidic device, we passed separately each metal ion and a mixture of them along with Au-TA-TG. As shown in Fig. 4 (inset), not a single metal ion shows any visible colour change or precipitate when mixed with the nanosensor, suggesting the fact that Au-TA-TG is very sensitive and retained high selectivity towards arsenic detection.

After successful detection as lowest as 0.001 ppm (1.0 ppb) of As^{3+} ions in Y-shaped paper channel, we tried to demonstrate this methodology on a straight paper strip made of Whatman filter paper as shown in Fig. 3. The gold nanosensor was immobilized at one end as stationary-phase and the aqueous arsenic samples of various concentrations (10, 1, 0.1, 0.01, and 0.001 ppm) were passed through the strip as a single fluid for interaction. The colorimetric response (red to blue colour change) of the gold nanosensor was clearly observed with each concentration, except 0.001 ppm of arsenic. The result of this study delineates the fact that like two-fluid system in Y-shaped paper microchannel, the lowest detection limit cannot be reached beyond 0.010 ppm with a single lateral flow of fluid in straight paper strip assay. However, this study can certainly be implemented to develop a chromatographic or dipstick type device for 0.010 ppm (10 ppb) level arsenic detection. In order to achieve very low sensitivity limit, the best option is to consider the parallel flow of two fluids, gold nanosensor and arsenic sample in Y-shaped channel, where both can adequately mix at their interface for interaction and produce an intense signal because of nanoparticles aggregation.

We also investigated the performance of the gold nanosensor after long term of storage at 4ºC. We found that Au-TA-TG works fine on paper even after four weeks. The combination of stable Au-TA-TG and paper like substrates therefore develops a highly stable integrated system that easily overcomes the limitations of toxic chemicals handling and requirements of expensive instrumental usages which practically hinder the miniaturization of any sensing systems. The use of paper not only allows self pumping of small volume of the robust nanosensor and arsenic sample with stable flow rates to run the device, it's membraneless architecture also minimizes the fluids crossover to achieve stable precipitation zone within a short period of time. The continuous laminar flow of fluids ensures the constant precipitation of nanosensor-arsenic aggregates at the interface, so even if the concentration is significantly low; a clear cut mark appears for tracer level of arsenic.

In conclusion, we have demonstrated a simple paper-based microfluidic device that can selectively detect arsenic at very low concentration level (1 ppb) using gold nanosensor, Au-TA-TG. The nanosensor forms bluish black colour precipitate after diffusive mixing with As^{3+} ions at the interface. The benefit of this technique is that the paper based substrate has been used for the development of microfluidic device, thus the overall system becomes portable, power-free, cost-effective and safe for arsenic detection. The unique characteristics of gold nanosensor make the detection process rapid and sensitive as it relies on the inter-particle aggregation behaviour of nanoparticles. The present technology proves its potentiality as a completely miniaturized sensing device by removing many challenges we normally encounter with the existing state-of-the-art sensors for arsenic detection.

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Notes

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