On-site Detection of Total Arsenic in Water with DNA Aptamer/CeO$_2$ Nanoconjugates on Arrayed Paper Strips

by

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B.Sc., University of Waterloo/Dalian University of Technology, 2016

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Abstract

An on-site fluorometric method for the qualitative detection of major arsenic species, arsenite, As(III), and arsenate, As(V), in water is presented in this thesis. Both As(III) and As(V) are toxic ions that are found in drinking water or water effluents worldwide. Building upon DNA-capped metal oxide nanoparticles, it has been shown that both As(III) and As(V) can be quantitated with a paper-based fluorometric assay. In particular, fluorescently labelled DNA aptamers were first adsorbed onto CeO$_2$ nanoparticles where the fluorescence was initially quenched. After the addition of either As(III) or As(V), the DNA strands were released from the surface due to strong and specific DNA (aptamer)-As(III/V)(ligand) interactions which resulted in fluorescence recovery. It was demonstrated that such a detection scheme can be realized on an arrayed paper substrate; glass fiber filter paper was first modified to be superhydrophobic and then patterned photochemically to create arrayed reaction sites. The signal readout is based on smartphone-imaging, i.e., analyze the RGB intensities of the assay photo taken under UV irradiation with a smartphone. These paper assays provided great performance for testing water samples containing As(III) and As(V), with detection limits of 29 nM and 38 nM, response ranges from 20 nM to 100 µM and from 10 nM to 100 µM, respectively. The satisfactory detection sensitivity (below the WHO standard, 80 nM), high selectivity, and wide response range augment the potential application of these paper-based fluorometric assays for on-site arsenic detection in water.

Keywords: Arsenic contamination; Metal nanoparticles, DNA aptamers, Fluorescence spectroscopy; Glass fiber filter paper
To my family and friends
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List of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>AFS</td>
<td>Atomic fluorescence spectroscopy</td>
</tr>
<tr>
<td>CMOS</td>
<td>Complementary metal-oxide semiconductor</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy-dispersive X-ray</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FAM</td>
<td>Fluorescein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ICP MS</td>
<td>Inductively coupled plasma mass spectroscopy</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MMER</td>
<td>Metal mining effluent regulation</td>
</tr>
<tr>
<td>MTS</td>
<td>Methyltrichlorosilane</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticle</td>
</tr>
<tr>
<td>ODR</td>
<td>Optical darkness ratio</td>
</tr>
<tr>
<td>OTS</td>
<td>Octadecyltrichlorosilane</td>
</tr>
<tr>
<td>POC</td>
<td>Point of care</td>
</tr>
<tr>
<td>RRS</td>
<td>Raleigh resonance scattering</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>μPAD</td>
<td>Microfluidic paper-based analytical device</td>
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Chapter 1. Introduction

1.1. Previous studies on arsenic detection

Figure 1.1 World map depicting global arsenic occurrences. As indicated in the legend, arsenic-affected aquifers are highlighted in orange; areas with arsenic related to mining are indicated by red dots; and geothermal waters are indicated by green dots.


The abundance of arsenic is more than most of us think as an element; it ranks the 20th in natural abundance (0.00005% of the Earth’s crust), 14th in the seawater and 12th in the human body. The abundance in most rocks is from 0.5 to 2.5 mg/kg1–5. Arsenic is mobilized by natural weathering, biological processes, geochemical reactions, volcanic emissions, and other anthropogenic activities. Soil erosion contributes to 61,200 ton/year and leaching contributes 238,000 ton/year of arsenic, in dissolved and suspended forms in the oceans. Although most environmental arsenic problems are the result of mobilization under natural conditions, human activities such as mining activities,
combustion of fossil fuels and use of arsenic pesticides to livestock feed create significant amounts of arsenic deposition.

Arsenic exists in the oxidation states of -3, 0, +3 and +5 and environmental forms include arsenious acids, arsenic acids, arsenites, arsenates, arsine and so on. Based on the legislative reports and previous studies, typical inorganic forms of arsenic found in water were mainly arsenite, As(III) and arsenate, As(V) species. Arsenate is stable in oxygen rich environments while arsenite species predominate in moderately reducing anaerobic environments such as ground water. The pKₐ values of arsenous acid are 9.22, 12.11, and 13.41; for the Arsenic acid are 2.19, 6.94 and 11.5. While both of them are weak acids, the predominate forms at neutral pH are H₃AsO₃ and HAsO₄²⁻ respectively (Fig. 1.2). Arsenate (AsO₄³⁻) is similar to phosphate (PO₄³⁻) in terms of Lewis structure, size, and metal binding affinity of their metal salts, whereas arsenite (AsO₂⁻), on the other hand, share similar properties with phosphite (PO₂⁻).

Figures 1.2 The structure and size of As(III), As (V) and their phosphor counterparts at neutral pH (7.0), respectively.

Arsenic contamination in water presents a health problem and environmental concern in some areas. Arsenic exposure is a public health problem due to its wide distribution in environment and its toxicity. It has been estimated that about 200 million people are at risk of arsenic exposure. High-level contamination of As in groundwater are also found in some areas in China and Canada; the concentration of arsenic is even higher than 500 ppb in some cases. It was shown that inorganic arsenic has a much higher toxicity and it is the number one toxin on the United States Environmental Protection Agency (USEPA) list. Unlike phosphoric species that are essential to biological systems, arsenic species disrupt ATP production and cause cancer, heart disease, and diabetes. The World Health Organization (WHO) has indicated that
the maximum allowed concentration of arsenic in drinking water is 10 ppb (93.53 nM for As(III) and 71.98 nM for As(V)), and the Metal Mining Effluent Regulations (MMER) of Canada has set the limit to 500 ppb (4.7 μM for As(III) and 3.6 μM for As(V)).

During the past few decades, scientists have developed various methods for the detection of arsenic species. The traditional laboratory-based techniques such as atomic spectroscopy, high-performance liquid chromatography (HPLC), mass spectroscopy, electrophoresis and chromatography offer low detection limits and satisfied sensitivity\(^1\)\(^{10}\)\(^{11}\). Although these powerful analytical instruments have been routinely applied to the detection of arsenic in real-world samples, they are not readily available and accessible for rural regions and the developing world. In addition, highly trained technicians and sample labeling are needed for the operation of the central facilities. The shipment of samples to the central laboratories is also challenging due to the time and cost associated with.

<table>
<thead>
<tr>
<th></th>
<th><strong>Atomic Absorption Spectroscopy (AAS)</strong></th>
<th><strong>Inductively Coupled Plasma Mass Spectroscopy (ICP MS)</strong></th>
<th><strong>Atomic Fluorescence Spectroscopy (AFS)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (ppb)</td>
<td>1 - 50</td>
<td>1</td>
<td>5 – 25</td>
</tr>
<tr>
<td>Sample size (μL)</td>
<td>&gt; 1000</td>
<td>2 – 200</td>
<td>20 – 200</td>
</tr>
<tr>
<td>Skill requirement</td>
<td>Trained laboratory technician</td>
<td>Specialized instrument technician</td>
<td>Trained laboratory technician</td>
</tr>
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</table>

Table 1.1 General comparison of the performance attributes of AAS, ICP-MS and AFS for trace arsenic detection in water samples.


As recommended by the World Health Organization (WHO) and the United Nations International Children’s Emergency Fund (UNICEF), portable field test kits are preferred
Most of the field test kits commercially available today were developed based on the Gutzeit reaction\textsuperscript{13,14}, which give results in less than 20 min (to observe the expected color change). The Gutzeit reaction requires zinc powder to reduce arsenic species (either arsenite or arsenate) to arsine gas in the test vial (Figure 1.3.a) (equation 1-1), and arsine will then react with the test strip and decolor it from white to yellow to tan (Figure 1.3.b) (equation 1-2). The method is inexpensive for on-site detection, which costs \$3 for each detection\textsuperscript{1}. The detection limit is \( \approx \) 20 ppb, which is higher than the WHO limit for drinking water. Furthermore, human eyes are not sensitive to yellow\textsuperscript{1}, and arsine gas is extremely toxic to humans; these are practical challenges when using the kit for the arsenic detection.

\[
\text{H}_3\text{AsO}_4 + 4 \text{ Zn} + 8 \text{ HCl} \rightarrow \text{AsH}_3(\text{g}) + 4 \text{ ZnCl}_2 + 4\text{H}_2\text{O} \quad \text{Equation 1-1}
\]

\[
\text{AsH}_3(\text{g}) + \text{HgBr}_2 \rightarrow \text{AsH}_2\text{HgBr} + \text{HBr} \quad \text{Equation 1-2}
\]

![Figure 1.3 Operation of the “Arsenic Low Range Test Kit” commercialized by Hach Company. A) shows the detection of arsenic with the test strip once the sample was added to the reaction vessel. All arsenic species are reduced to arsine gas, which decolors the test strip (shown as the bottom inset) at the vessel opening. B) Reading the test strip by comparing with the color chart.](image)

Figure 1.3A from Ref. [13]: “Arsenic Low Range Test Kit”. Hach Company, Loveland, CO. [https://ca.hach.com/arsenic-low-range-test-kit/product?id=14533981664](https://ca.hach.com/arsenic-low-range-test-kit/product?id=14533981664).


Recently, there has been increasing interest in employing nanomaterials and colorimetric reactions for analytical and quantitative applications for achieving good sensitivities\textsuperscript{15,16}. Based on their unique electronic and optical properties such as high
surface area over volume ratio and carrying out by human eyes, many chemical and biochemical sensors have been developed to detect arsenic species \(^{17-19}\). Several colorimetric methods were developed based on aggregations of gold \(^{20-23}\), silver \(^{24}\) and bimetallic nanoparticles \(^{25}\). Tang et al. employed ssDNA-protected gold nanoparticles to quantify As(III) in water \(^{23}\), for which the DNA strands contain an aptamer sequence for binding As(III). The AuNPs protected with ssDNA strands are stable in the presence of high concentration of NaCl. Upon addition of As(III), the formation of As(III)-DNA aptamer complex removes the DNA strands from the AuNP, which in turn aggregate to induce an increase in Raleigh resonance scattering (RRS) signal. The detection limit was claimed to be 1.9 ppb, which is substantially lower than the WHO limit.

![Figure 1.4 Principle of the aptamer-nanogold – based resonance Rayleigh scattering (RRS) method for the detection of As(III).](image)


Divsar et al. reported an As(III) detection method combining silver nanoparticles and thiolated DNA aptamers to detect As(III) in water \(^{24}\). Thiolated DNA aptamers were conjugated onto silver nanoparticles via strong Ag-S bonding, which promoted the dispersion of AgNP in solution. The addition of As(III) resulted in aggregation AgNPs due to specific binding between As(III) and DNA aptamers (Figure 1.5). The aggregation leads to a significant decrease in the plasmonic absorbance of AgNP at 403 nm, which is linearly dependent on the cunntation of As(III). The detection limit reported in this is impressive as well (6 ppb).

Figure 1.6 Proposed strategy to achieve the wide range of color variations from red to cyan by the use of dual-color probes. The addition of As(III) aggregated QDs and quench red fluorescence but cyan fluorescence from CDs was not affected. When concentration of As(III) increases, color of fluorescence changes from purple to green to cyan.

In the past decade, there were a number of fluorometric sensors developed for the detection of As(III) and As(V). Zhou et al. developed a dual signal sensor using quantum dots and carbon dots on paper substrate. Quantum dots with red fluorescence (glutathione and dithiothreitol modified CdTe QDs) and carbon dots with cyan fluorescence were synthesized and mixed in a ratio of 1:1 to give a purple fluorescence. Upon the addition of As(III), QDs would be aggregated by forming As-S bonds and the red fluorescence would be quenched. Thus, color of fluorescence would change from purple to green to cyan as the concentration of As(III) increases (Figure 1.6). Semi-quantitative results can be obtained based on the color change, quantitative results can be measured with a fluorometer at 630 nm. They also achieved extremely good detection limit (1.7 ppb).

![Diagram of sensing arsenate by DNA-functionalized iron oxide NPs.](image)

**Figure 1.7** Schematics of sensing arsenate by DNA-functionalized iron oxide NPs. DNA fluorescence is quenched upon adsorption on iron oxide NPs, and recovered upon adding arsenate. Fluorescence photographs demonstrating the sensing scheme using a FAM-labelled 24-mer DNA (500 nms. DNA in 25 mM HEPES, pH 7.6). Fe$_3$O$_4$ = 10 mg/mL, final arsenate concentration = 40 mm


Meanwhile, nanoconjugates prepared by combining DNA strands and metal oxide nanoparticles offer the advantage of low cost and high programmability. Liu et al. reported the arsenic detection using Fe$_3$O$_4$ nanoparticle capped with fluorophore-labelled DNA. The addition of arsenate solution resulted in fluorescence recovery due to the release of DNA strands from the Fe$_3$O$_4$ NPs (Figure 1.7). Fluorescence signal was
measured with a fluorometer at 518 nm. This sensor is sensitive to arsenate, not arsenite. The detection limit was only 300 nM (41.8 ppb) but provided a new method of sensing As(V) rather than As(III) as previously reported.

In 2017, Lopez et al. reported a similar method of arsenate detection with FAM-DNA capped nanoparticles including CeO₂, CePO₄, and Fe₃O₄. The idea was to survey the different particles to find the best combination of the DNA-NP system for the fluorescence detection of As(V). With the same detection scheme as shown in Figure 1.8, it was confirmed that CeO₂ nanoparticles were the best choice. This solution-based arsenate detection method has a detection limit of 29 nM (4.1 ppb), which meets the requirement of WHO standard for drinking water.

![Figure 1.8 Principle of As(V) sensing based on competitive adsorption; DNA is adsorbed on the inorganic NP via its phosphate backbone. Since (As(V)) has a very similar property as phosphate, it displaces the adsorbed DNA. If the material is a quencher, the fluorescence can be recovered.](image)


Besides Fe₃O₄ and CeO₂ NPs, other metal oxide such as ZnO have been tested for the arsenic detection. As of their low costs, they might be good options for practical application providing the satisfactory detection performance can be achieved.

All methods described above can only detect one form of arsenic species in water, either As(III) or As(V), and, most often the detection occurs in bulk solution. Furthermore, most of them require advanced instrumentation such as fluorimeters and UV-Vis spectrometer, which are not suitable for on-site detection.
1.2. Mobile phones for analytical sciences

Smartphones are more common in Europe, U.S., less so in developing countries

Percent of adults who report owning a smartphone

![Smartphone Ownership Map](image)

Note: Percentages based on total sample.
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Figure 1.9 Smartphone usage in the world with percentages of adults using smartphones shown beside the country name.


Smartphones are popular nowadays as essential communication tools for our society. Besides built-in functionalities for regular customers, they can be used to assist applications in the field of healthcare and environment. Especially in the developing regions, in which modern analytical instruments are not accessible, the detection using smartphone can be practical and convenient. Through a survey of 40 countries worldwide, it was shown that smartphone ownership in western countries, such as United States
(72%) and European countries (>60%), is still higher than that in the developing countries, although their percent usage has increased from 21% to 37% since 2015 [33] (Figure 1.9). Since the number of smartphone user is growing rapidly around the world, it is of general interest to design smartphone-based analytical devices [33].

With smartphones, personal communications have become instant and direct, as people can send messages via internet at ease. Along with WIFI transmission and cloud computing, specially designed programs/apps, one can store, send, or analyze data just using their personal phone. Additionally, phone cameras can be used to capture pictures with high resolution, which becomes essential for result recording and data collection. It is entirely possible to use smartphone to collect, analyze data from portable analytical devices (or assays) and communicate with the professionals and technicians in central facilities on time.

![Image: General strategy for performing inexpensive bioassays in remote locations and for exchanging the results of the tests with off-site technicians.](image_url)

**Figure 1.10 General strategy for performing inexpensive bioassays in remote locations and for exchanging the results of the tests with off-site technicians.**


On-site detection means that analysis of samples can be performed at any remote locations. For medical diagnosis or environmental pollutant tests in those situations, it is always difficult to find highly trained personnel and appropriate instrumentation required
for data collection and results analysis. Nowadays, build-in features, such as camera and networking in mobile devices, enable them to work as regular computers to “think” and to “analyze” data. Early research primarily focused on assay imaging as was demonstrated by Martinez et al. 34 over a decade ago. The detection performed on this paper-based assay was colorimetric, i.e., the quantitation was done by image analysis. Upon taking photo of the assay, it was then digitized, and the results were transmitted to an expert for interpretation. Particularly Image editing software, such as Adobe Photoshop and ImageJ, were adapted to digitalize the selected regions on the assay and convert them to a set of color values 34. The principle of so-called “telemedicine” proposed by Martinez et al. is shown in Figure 1.10.

Figure 1.11 The preparation and smartphone reading of the colorimetric barcode assay. Solutions are prepared in a micro-centrifuge tube and transferred to a PDMS channel plate attached to a polycarbonate (PC) base. The PDMS chip with two cut-out windows (highlighted in light orange color) is sealed with a second PC plate (the cover) and the paper-printed barcode is positioned above the PDMS channels. The assembled device can then be scanned by a barcode scanner.


Because of the much improved imaging capability of today’s smartphone cameras in comparison with a decade ago, smartphone-based colorimetric quantitation has attracted increased attention ever since 35–43. For example, Guo et al. developed a mobile
phone-based method to detect pesticide residues on fruits by barcode identification and subsequent colorimetric quantitation (Fig. 1.11). Meanwhile, Lee et al. created a probe composed of gold nanoparticles and antibodies to detect vitamin D in blood samples. The detection system consists a smartphone accessory (an app and the light source), a test strip, which is capable of carrying out colorimetric detection of 25-hydroxyvitamin D (25(OH)D) with an AuNP-based immunoassay. Independently, Ozcan et al. expanded the smartphone-based detections to fluorometric assays, i.e., imaging fluorescence assays with a smartphone camera under UV irradiation. The mobile apps were also adapted for analyzing liquid samples in cuvettes.

However, there are still some drawbacks when using smartphones for quantitative detection. RGB values vary under different lighting conditions for the same image, as a matter of fact. To address this issue, Guo et al. proposed that calibration curves should be constructed prior to the determination of unknown samples. Particularly, they used an enzymatic inhibition assay to detect methyl parathion residues, a toxic pesticide. Acetylcholinesterase (AchE) can catalyze the hydrolysis of acetylcholine iodine to produce thiocholine, which subsequently dissociate dithiobis-nitrobenzoate to deprotonated thio-nitro-benzoate, a yellow product. The presence of the organophosphorus pesticides (e.g., methyl parathion) will inhibit the catalytic activity of the AchE enzyme, which essentially decrease the rate of formation of the thiocholine. Thiocholine dissociates dithiobis-nitrobenzoate to the yellow product. The yellow intensity has been confirmed to be inversely dependent on the concentration of the pesticide. The acquired yellow intensity was plotted as function of the pesticide concentration to construct the calibration curve. The quantitation of pesticide was done by reading yellow intensities (Figure 1.11) with the customized app and subsequent calculation based on the calibration curve.

In addition to directly reading color (RGB) intensities, optical darkness ratio (ODR) has been defined and adapted as a standard protocol or main factor for scannometric/colorimetric detections. It was initially proposed by Gupta et al. and the equation is shown below:

$$\text{ODR} = \frac{I_b - I_a}{I_b}$$

where $I_b$ is the luminosity of background; and $I_a$ is the luminosity of the assay site. As ODR is based on the normalized signal/background ratio, it does reduce the effect of background variation. Wong et al. developed a mobile app that can be installed on any
android phone for the colorimetric/scannometric detections\textsuperscript{45}. It was confirmed that the ODRs determined by the mobile app are comparable to the results obtained with an standard office scanner (picture analyzed with Photoshop on a computer). Wong et al. also carefully considered the determination of the grayscale value, which are in two different ways. One way is to calculate the average grayscale by using the following equation:

$$I = \frac{(R + G + B)}{3} \quad \text{Equation 1-4}$$

Where R, G and B are pixel intensities in each of the selected areas. The other way is to calculate the weighted grayscale value which accounts for a human’s color perception:

$$I = 0.30R + 0.59G + 0.11B \quad \text{Equation 1-5}$$

Wong et al. have shown that the weighted grayscale provided better results and was adapted in the detection with their mobile app for image analysis.

Besides using smartphones for capturing images and subsequent analysis, Su et al. developed a standalone colorimetric reading device \textsuperscript{46}; the so-called Bionic electronic eye (Bionic e-Eye) adapts a smartphone or a pad as detection instrument and portable accessory as illumination provider integrating with a wide-angle lens, a piece of lowest-power electro luminescent and a custom-made dark hood (Figure 1.12a). A 96-well microtiter plate (MTP) was positioned on the electro luminescent and the images were captured by the back camera of smartphone (Figure 1.12b). As a show-case application, the commercially available bicinchoninic acid (BCA) protein assay and cell counting kit (CCK8) assay were carried out to demonstrate the better precision and higher sensitivity than a conventional microtiter plate reader (MTPR).
Figure 1.12 A) The scheme of a Bionic e-Eye and B) detecting processes including image processing algorithm. The dimension of Bionic e-Eye is 23.3 cm × 16.7 cm × 9.5 cm.


1.3. Paper-based microanalytical devices: past and present

If a colorimetric reaction was to adapt for on-site detections based on smartphone imaging, the substrate for preparing the assays should be considered at first. Microfluidic paper-based analytical devices (namely µPAD) has been a fast expanding research area in the last decade, which in principle offers point-of-care medical diagnostic and on-site chemical testing tools 47. They are built from low-price and easily obtained materials (cellulose filter paper), which are compatible with many chemical/biochemical assays and can handle sample delivery without any external devices 12,22,26,48–50.
In 2007, Martinez et al. reported their method of patterning photoresist on a paper substrate to create µPADs, which was generally acknowledged as the first generation of such devices. The initial task was to construct hydrophobic barriers and hydrophilic areas for selectively depositing assay-specific reagents. They soaked chromatography paper into SU-8 photoresist then exposed it to UV irradiation (405 nm) through a photomask, followed by further development with appropriate solvent washing. More importantly, with trial glucose and protein detection, they have shown that this type of patterned paper promises a class of low-cost and portable platforms for running multiplex bioassays (Figure 1.13).

Figure 1.13 Diagram depicting the method for patterning paper into millimeter-sized channels: a) Photolithography was used to pattern SU-8 photoresist embedded into paper; b) the patterned paper was modified for bioassays.

In 2008, Abe et al. proposed a different method of µPAD fabrication. A piece of filter paper was cut into designed shape and was immersed in 1.0 wt% poly(styrene) in toluene to make the surface hydrophobic. Toluene was inkjet-printed onto the surface of the fabricated paper to etch hydrophilic patterns (sensing areas) (Figure 1.14). Chemical sensing “inks” can then be prepared and printed onto the designated areas. They have shown that thus prepared devices can be used for pH, total protein and glucose analysis for urine samples.

Figure 1.14 Schematic representation of the fabrication process of the inkjet-printed microfluidic multianalyte chemical sensing paper featuring microfluidic channels connecting a central sample inlet area with three different sensing areas and a reference area. Steps 2 (patterning) and 3 (chemical sensing reagent application) are performed on the same inkjet printing apparatus (the pen symbol indicates the use of the inkjet printer).


The above methods and many others for the fabrication of µPADs relied on physical deposition of photoresists or polymers on the surface of cellulose fibers, which made them mechanically fragile, i.e., bending or folding the paper substrate may damage the patterns. Conceptually different, He et al. reported the fabrication of µPADs by using octadecltrichlorosilane (OTS) to treat cellulose filter paper followed by patterning with selective UV irradiation (Figure 1.14). In brief, filter paper strips were cut into designed sizes and immersed into 0.1% (v/v) OTS solution in n-hexane; the hydrolysis and
polycondensation of the organosilanes promote the hydrophobic properties; such polysiloxane network can be degraded with UV irradiation through a quartz mask create hydrophilic reaction zones on otherwise hydroponic paper substrate to chemically modify the surface to be hydrophobic.

Figure 1.15 A method of fabricating paper-based microfluidic devices by coupling hydrophobic silane to paper fibers followed by deep UV-lithography. After filter paper being simply immersed in an octadecyltrichlorosilane (OTS) solution in n-hexane, the hydrophilic paper becomes highly hydrophobic. The hydrophobized paper is then exposed to UV-lights through a quartz mask that had the pattern of the to-be-prepared channel network. The UV-exposed regions turn highly hydrophilic whereas the masked regions remain highly hydrophobic, generating hydrophilic channels and reaction zones that are well-defined by the hydrophobic regions.


In comparison with the physical deposition methods, silanization is a simpler approach to prepare paper substrate for µPAD. In most cases, the silanization treated paper showed satisfactory hydrophobic properties with water contact angles between 90° and 120°.

Very recently, Zhang et al. reported a binary silanization protocol for the fabrication of superhydrophobic substrates from off-the-shelf laboratory filter paper. The invention was to use a mixture long (OTS) and short (methyltrichlorosilane, MTS) organosilanes for...
The modification, by which a number of filter paper types showed remarkably high-water contact angles (> 150°) upon modification. As shown in Fig. 1.16, the most popularly used Whatman™ grade 3 filter paper would be wicked by ink solution if not treated. In comparison, the water droplet stays at the top of modified sample, and, the SEM image showed a network of various nanostructures (evenly distributed nanoaggregates in particular). The patterning and assay application of such superhydrophobic paper substrates have been also demonstrated.

![Figure 1.16](image)

Figure 1.16 Water contact angles of modified filter paper strips with different pore size and thickness. All samples were treated with a binary solution of OTS and MTS with a volume ratio of 1:1 (0.2 % in total). Grade 1(△); Grade 3(□), Grade 4(○); Grade 6(◇), Grade 113(▲), Grade 520B (■), Grade 597(●) and Grade 602h(◆). The right insets show the pictures of water droplets (10 µL) on untreated (top) and silanized Grade 3 filter paper (bottom).


1.4. Objective of This Study

As mentioned above, most established methods for arsenic detection were for As(III), for which the conversion of As(V) to As(III) is needed. More importantly, although
many different detection methods have been reported, they work primarily in solution and not suitable for on-site detection. Building upon the success of making superhydrophobic paper substrate in this lab, the objective of this thesis is to develop an on-site method for the quantitative detection of total arsenic in water, i.e., to create paper-based microassays that are capable of quantifying both As(III) and As(V). In the following chapters, I will describe the approach of using DNA aptamer/CeO₂ nanoconjugates to sense the presence of As(III) and As(V), first in solution (chapter 3) and subsequently on arrayed paper strips (chapter 4). Besides the understanding of the sensing mechanism (aptamer-As(III/V) binding induced release of fluorescently labelled DNA strands form CeO₂ particles), the quantitation performance (limits of detection, selectivity, and dynamic response ranges) will be also comprehensively examined.
Chapter 2. Experimental Section

2.1. Reagents and materials

Glass fiber filter (Whatman GF/A) was purchased from GE Healthcare (Mississauga, ON). Tris(hydroxymethyl)aminomethane (Tris, >99.9%) was purchased from Thermofisher Scientific (Waltham, MA). CeO$_2$ nanopowder (99.95%, average particle size < 25 nm), sodium arsenate dibasic heptahydrate (≥98%), sodium (meta)arsenite (purity: ≥90%) and octadecyltrichlorosilane (OTS, CH$_3$(CH$_2$)$_{16}$CH$_2$SiCl$_3$, ≥ 90%) were purchased from Sigma-Aldrich (St. Louis, MO). Hexane was ordered from ACP Chemical Inc. (Montreal, QC). Deionized water (>18.3 MΩ cm) was obtained from a Barnstead EASYpure UV/UF compact water system (Dubuque, IA). n-Hexane (ACS reagent grade) was ordered from ACP Chemical Inc. (Montreal, QC). Two synthetic oligonucleotides were purchased from Integrated DNA Technologies, Inc (Coralville, IA) and their sequences were listed in Table 2.1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>A15 DNA (15 mer)</td>
<td>5'-FAM-AAA AAA AAA AAA AAA-3'</td>
</tr>
<tr>
<td>Ars-3 DNA aptamer</td>
<td>5'-FAM-TTA CAG AAC AAC CAA CGT CGC TCC GGG TAC TTC TTC ATCG-3'</td>
</tr>
</tbody>
</table>

Table 2.1 DNA sequences and modification; fluorescein (FAM) was labelled on the 5’ terminus of both oligonucleotides. FAM has an absorption maximum at 495 nm and emission maximum of 518 nm.

2.2. Sample preparation and detection in solution

The 10 mM Tris buffer was prepared by dissolving 60.6 mg of Tris in 50 mL of deionized water and its pH was adjusted to 7.6 with 1 M HCl.

The CeO$_2$ NPs were used as received; the size and morphology were characterized by using both dynamic light scattering (DLS) spectroscopy and transmittance electron microscopy (TEM). As for DLS, CeO$_2$ was dispersed in water to
make a 10 µg/mL solution. As for the TEM measurements, 10 µg/mL ethanol solution of CeO₂ was dropped onto a TEM grid (Center-marked grids, 50 mesh, 3.0mm O.D., copper) and let the solvent to evaporate completely.

In the detection experiments, CeO₂ stock solution was prepared at 1.0 mg/ml in Tris buffer; 88.9 nmoles of ars-3 DNA powder was dissolved in 889 µL Tris buffer to make the 100 µM DNA stock solution based on the instructions from IDT. Similarly, 66.2 nmoles of A15 DNA powder was dissolved in 627 µL Tris buffer to make the 100 µM A15 DNA stock solution. DNA/CeO₂ nanoconjugates were prepared in a 1.5 mL conical centrifuge tube by mixing final concentrations of 100 nM ars-3 DNA/A15 DNA and 130 µg/mL CeO₂ nanoparticles.

Sodium (meta)arsenite was dissolved in deionized water to make the 1.0 M stock solution, from which different dilutions (from 10 nM to 0.1 M) were prepared. Similarly, sodium arsenate dibasic heptahydrate was dissolved in water to make the 1.0 M stock solution; different dilutions (from 10 nM to 0.1 M) were then prepared accordingly.

The concentrations of DNA solutions were confirmed using Nanodrop UV/Vis spectrophotometer (ND-2000, Thermo Fisher Scientific, Waltham, MA). To start, 1.5 µL of Tris buffer was added onto the pedestal with a micropipette to measure the blank. Upon the drop was wiped with Kimwipe tissue, 1.5 µL of the DNA solution was added onto the pedestal and the probe was closed. The absorbance at 260 nm was determined based on which the DNA concentration was calculated.

All fluorescence spectroscopy measurements were performed with a PTI QuantaMaster fluorometer (8000 series, Horiba Scientific, Piscataway, NJ). A quartz fluorometer cell (4 × 1 × 1 cm, Starna Cells Inc., Atascadero, CA) was used for fluorescence detection. The slit width was set to 3 nm. Based on the absorption and emission properties of the fluorescent tag (FAM), which was used for labelling the DNA strands, the excitation wavelength was set to 495 nm and the emission scan range was set to 500 nm to 650 nm. The emission spectra were collected and processed using the software program (FelixGX, v4.0.1) provided by Horiba Scientific.
2.3. Paper modification and assay preparation

The purchased large piece of glass fiber (GF) filter (11 cm in diameter, pore size 1.6 μm) was cut into 4 × 2.5 cm² strips. As shown in Figure 2.1, they were first immersed in a 0.2% (v/v) OTS solution in n-hexane in a 20-mL conical glass vial. Prior to the immersion, the solution was deoxygenated by bubbling Ar for 15 min. After 7 min, the paper strip was taken out and dried with compressed air.

![Figure 2.1 Fabrication of paper-based assays with embedded FAM-DNA/CeO₂ nanoconjugates for the detection of As (III) and As(V). Glass fiber filter is cut into 4 × 2.5 cm. After filter paper being simply immersed in an octadecyltrichlorosilane (OTS) solution in n-hexane for 7 min, the hydrophilic paper becomes hydrophobic. The hydrophobized paper is then exposed to UV irradiation through an aluminum mask. The UV-exposed regions turn hydrophilic whereas the masked regions remain hydrophobic. FAM-DNA/CeO₂ nanoconjugates are loaded onto the hydrophilic regions (assay sites); then arsenic samples are added. The recovered fluorescence is imaged with a smartphone under UV light.](image-url)
An aluminum mask with eight holes of 0.7 cm in diameter was used to pattern the modified glass fiber filter to create hydrophilic reaction regions. The paper strip was treated in a UV/O$_3$ cleaner (PSD-UV, Novascan Technologies Inc., Milwaukee, WI) with the aluminum mask placed on top. Water contact angles were measured using 1.5 μL of water droplets with a goniometer (AST VCA System, Billerica, MA) before and after the treatment. For each sample, 5 different regions of the paper strip were tested to obtain the average water contact angle and associated uncertainties.

To prepare the assay strip, 20 μL of FAM-DNA/CeO$_2$ solution was individually pipetted onto the hydrophilic regions. Afterwards 5 μL of As(III)/As(V) sample at certain concentration were then added individually into each zone and were mixed with a micropipette. After loading the samples, a photo was taken under a UV lamp through a yellow glass placed in front of the camera in the dark (Figure 2.1). The camera flash was turned off while taking the photo, and the images were saved as PNG files for further analysis.

All photos were taken with an android phone, Oneplus 5T, which utilizes Qualcomm Snapdragon 835 processor and a 16 MP camera with a built-in lens that has an F/1.8 aperture. The Image analysis was performed with ImageJ (ver. 1.8.0), a desktop software program. A certain region (Reaction zone of paper strip) on the photo was selected in ImageJ; then a histogram was opened and all values of green and RGB mean were shown with their standard deviation as well. The values of green or RGB mean were recorded for further analysis and quantitation.
Chapter 3. Detection of Total Arsenic with DNA/CeO₂ Nanoconjugates in Solution

The ultimate goal of this study is to create a paper-based sensor for the detection of both As(III) and As(V), however, it is necessary to study the sensing mechanism and test the detection performance in solution first. As a new DNA aptamer/CeO₂ conjugation system, it is needed to optimize the conditions for the preparation of these probes, i.e., to find the ideal concentrations for both DNA aptamers and CeO₂ to work with.

3.1. Detection Mechanism

Figure 3.1 shows schematically the detection of As(III) and As(V) with the “premade” DNA aptamer/CeO₂ nanoconjugates. Upon mixing the fluorescently labelled DNA strands with CeO₂ nanoparticles in solution, the DNA strands adsorb onto the particle surface via presumably coordinative interactions between cerium cation and the phosphate backbones. It is expected that the fluorescence from the FAM tags is quenched due to the energy transfer between the dye molecules and the adjacent metal oxide. The fluorescence intensity was, in fact, suppressed rapidly upon mixing the DNA and CeO₂ (vide infra).

Based on previous studies, arsenic species interact with the DNA-CeO₂ nanoconjugates and release DNA strands into the solution in two ways: specific DNA aptamer-As(III/V)(ligand) interactions (Figure 3.1A) and competitive adsorptions between the arsenic species and DNA at the particle surface (Figure 3.1B). The Ars-3 DNA aptamer (40-mer) chosen for this study was originally selected by Kim et al., which has strong affinities to both As (III) and As(V) with $K_d$ values of 7.05 nM and 4.95 nM, respectively. The added As(III) and As(V) species would bind to the DNA strands and release them from the surface of NPs (Figure 3.1A). After DNA strands are released from the surface of NPs, the fluorescence should be recovered subsequently. On the other hand, we cannot rule out the contribution of the “non-specific” competitive adsorption of As(III) and As(V) and DNA strands towards the CeO₂ NPs (Figure 3.1B), which can also remove DNA strands from the surface (and subsequent fluorescence recovery). In fact, the earlier studies by Liu et al. have relied on such competitive interactions to use DNA...
Fe₃O₄ conjugates for the detection of As (V)\(^{30}\). The question we cannot answer here is to what extent each of the sensing schemes contributes.

**Figure 3.1 Principle of As(III) and As(V) detection with DNA/CeO₂ nanoconjugate.**

CeO₂ nanoparticles adsorb FAM-DNA strands quench the fluorescence. A) Specific DNA aptamer / As (III)/(V) binding to release DNA strands from NPs. After DNA being released from surface of CeO₂, fluorescence recovers, and the signal will be enhanced. B) Competitive adsorption of As(III)/As(V) and DNA towards the surface of NPs. After the addition of arsenic species, arsenic will be adsorbed onto CeO₂ NPs due to stronger adsorption. DNA strands are released from surface of NPs and fluorescence intensities increase.

It has been confirmed that phosphate, arsenite, and arsenate adsorb on CeO₂ NPs with different affinities\(^{30–32,62}\). Liu and co-workers used CeO₂ NPs to adsorb DNA strands first followed by the addition of arsenic species\(^{30–32}\). Their selectivity tests showed that the DNA-capped NPs respond to the presence of As(V) and phosphate but not to As(III)\(^{30,31}\). This is contradicting from the observation of Xu et al that both As(III) and As(V) strongly adsorb on the surface of CeO₂ (and the phosphate as well)\(^{63}\). One of the experimental considerations is the order of adding reagents, i.e., As(III) and CeO₂ were mixed together first, followed by adding DNA strands (Figure 3.2). It might be possible that the replacement of surface bound As(III) ions are difficult, which ends up high fluorescence intensity upon mixing all there components. This could be also true for the detection of As(V).
Figure 3.2 Competitive adsorption of arsenic and DNA towards surface of CeO$_2$ NPs. As(III)/As(V) was adsorbed onto surface of CeO$_2$ first and occupied all binding sites. FAM-DNA (A15) was added after arsenic species. Because of weaker adsorption than arsenic species, DNA will not be adsorbed on NPs and fluorescence intensity will not decrease.

Figure 3.3 Graph of As(III)/As(V) adsorbed initially to CeO$_2$ before adding FAM-DNA (FAM-A15). 130 μg/mL CeO$_2$ and 5 mM As(III)/As(V) were used in this study. Fluorescence studies of the competitive adsorption of As(III)/(V) and DNA on CeO$_2$ nanoparticles, i.e., excess amount of As(III) (A) or As(V) (B) were first added to CeO$_2$ solution (solid line), the FAM DNA (A15) was added (dash line). Concentration of DNA was 100 nM and the concentration of CeO$_2$ was 130 μg/mL. Final concentrations of arsenic species were 5 mM mixed first then 100 nM DNA was added.

To test the above hypothesis, FAM-A15 DNA, the same sequence as used in Liu and co-workers’ studies $^{30-32}$, was examined. Two samples of CeO$_2$ nanoparticles were initially mixed with excess amount of As(III)/As(V) individually; after centrifugation and washing, the NPs were re-dispersed in solution. Upon adding FAM-A15 to the CeO$_2$ “pre-coated” with As(III) / As(V), the fluorescence signal did not show any discernible decrease.
This result indicates that both As(III) and As(V) can adsorb on CeO$_2$ nanoparticle strongly, and the order of adding reagents is critical to the desired sensing response.

The next experiment was to reveal how strong Ars-3 DNA aptamer interact with As(III) or As(V) and if they can pull As(III)/As(V) off the “precoated” CeO$_2$ particles. As shown in Figure 3.4, there are possible outcomes following this procedure. Firstly, DNA aptamers may bind to the surface-bound As(III) and As(V) species form a sandwich structure of DNA-As-NPs (Figure 3.4A). Secondly, DNA strands cannot bind to the surface-bound As(III) or As(V); they will be left in the solution freely. Thirdly, DNA aptamers bind to the surface-bound As(III) and As(V) and release them from the particle surfaces.

Figure 3.4 Competitive adsorption of As(III)/As(V) and DNA aptamers on CeO$_2$. Excess amount of arsenic species is added to CeO$_2$ solution first, then ars-3 DNA aptamers were added to the solution. A) FAM labelled ars-3 DNA aptamers bind to the surface-bound As(III) and As(V) species to form a sandwich structure of DNA-As-NPs. B) DNA aptamers left in the solution freely rather than bind to arsenic or CeO$_2$. C) FAM-DNA aptamers bind to the surface-bound As(III) and As(V) and release them from the particle surfaces.
Figure 3.5 Fluorescence studies of the competitive adsorption of As(III)/(V) and arc-3 DNA aptamers on CeO2 nanoparticles, i.e., Excess amount of As(III) (A) or As(V) (B) were first added to CeO2 solution (solid line), the FAM-tagged ars-3 DNA aptamers were added (dash red line). The green spectrum shows the signal of the DNA only. Concentration of DNA was 100 nM and the concentration of CeO2 was 130 μg/mL. Final concentrations of arsenic species were 5 mM.

As confirmed in Figure 3.5, in either cases, the fluorescence intensities did drop at all upon mixing the DNA aptamers and the CeO2 particles that are pretreated with excess amount of As(III) or As(V).

Figure 3.6 Revised sequence of adding DNA, As(III)/(V) and CeO2 NPs. FAM-DNA (ars-3 aptamer) was first mixed with As(III)/As(V) before adding CeO2 nanoparticles.

The last set of experiment was to mix DNA aptamers with As(III) or As(V) first before adding CeO2 NPs to it (Fig. 3.6). It was discovered that the fluorescence intensities did not decrease, which indicates the formation of DNA aptamer/As(III)/(V) complex in
solution and that these complexes do not bind to CeO$_2$ nanoparticles due to high fluorescence intensities.

![Image of fluorescence studies](image)

Figure 3.7 Fluorescence studies of the reversed sequence of adding arc-3 DNA aptamers, As(III)/(V), and CeO$_2$ nanoparticles. The green line shows the spectrum of FAM-tagged ars-3 DNA aptamers, the red dash line shows the one upon adding As(III) (A) or As(V) (B) and CeO$_2$.

3.2. Assay optimization

3.2.1. Concentrations of DNA and CeO$_2$ in the sensing system

The main goal of this section is to optimize the concentrations of FAM-DNA and CeO$_2$ in the formation of the nanoconjugates for total arsenic quantitation. The first important parameter is the concentration of FAM-DNA; it is preferentially to provide high fluorescence signal and require low CeO$_2$ concentration for quenching. Figure 3.8 shows the emission spectra of FAM dye from 500 nm to 650 nm with corresponding concentrations listed as the inset. It is clear that the fluorescence signals are very strong, i.e., it reaches as high as $8 \times 10^6$ cps (at 110 nM), and the fluorescence intensity is generally linear with the FAM concentration. To start with a high signal but not too close to the saturation, 100 nM was chosen as the concentration to start the preparation of
nanoconjugates. This choice of the DNA concentration shall be revisited based on the subsequent fabrication steps and the final performance of the assay.

Figure 3.8 A) Fluorescence spectra of FAM dye from 1 nM to 110 nM. The excitation wavelength was 495 nm and the emission spectra were collected from 500 nm to 650 nm. B) Fluorescence intensity at 518 nm as function of the FAM concentration. The red solid line shows the best linear fit to the experimental data (black squares).
The other important parameter is the concentration of CeO₂ NPs. The fluorescence intensity of 100 nM FAM-DNA solution was measured when adding different concentrations of CeO₂, and the results are showed in Figure 3.9. The fluorescence intensity dropped dramatically as the concentration of CeO₂ increased from 10 to 120 µg/ml and maintained constant when the concentration reaches 130 µg/ml (i.e., the lowest fluorescence intensity or the most effective quenching). It should be noted that at higher concentrations (> 150 µg/ml), CeO₂ NPs tend to aggregate, which would interfere with the fluorescence measurements. Therefore, 130 µg/mL CeO₂ was chosen for the preparation of the DNA/CeO₂ nanoconjugates for the subsequent measurements.

![Figure 3.9](image.png)

**Figure 3.9 Optimization of and the concentration of CeO₂ nanoparticles.** The dash line is to guide the eyes only. The concentration of DNA was 100 nM and it was dissolved in 10 mM Tris buffer at a pH 7.6. Concentrations of CeO₂ were changed from 10 µg/mL to 160 µg/mL.

### 3.2.2. Characterization of the CeO₂ NPs

The particle size of CeO₂ measured by DLS was 250±80 nm (Figure 3.10A), which is significantly larger than the dimensions provided by the manufacture (< 25 nm). Because there was no stabilizer on the surface of such “nanopowders”, aggregations are expected when they were dispersed in ultrapure water for the DLS measurements. To further examine the exact size distribution, TEM images were obtained for samples
prepared by drop-casting 10 µg/ml solution on the TEM grid (Figure 3.10B). Although the particles are not as uniform as desired, they are generally within a range of 10 nm to 30 nm, and the aggregation seems to be moderate. It should be noted that morphology of these particles may not be the same as when dispersed in solution, which may exist in more uniformed and dispersed fashion. In fact, up to 100 µg/ml, a clear solution can be obtained in the Tris buffer, and it is generally stable (without precipitation) for up to 24 h.

Figure 3.10 A) DLS spectroscopy of the CeO$_2$ NPs (10 µg/ml) used for this study. B) TEM micrograph of CeO$_2$ nanoparticles. Scale bar = 50 nm. 100 µg/mL CeO$_2$ was dispersed in ethanol before dropping casted on the TEM grid for measurements.
Figure 3.11 A) TEM micrographs of CeO$_2$. EDX analysis of B) oxygen, C) fluorine, D) cerium, E) carbon and F) oxygen., fluorine Images of fluorine and cerium overlaps. Scale bar = 50 nm. 100 μg/mL CeO$_2$ was dispersed in ethanol for TEM measurement.

Besides knowing the size distribution of the CeO$_2$ particles, it is also important to confirm the composition and purity. At the same time of acquiring TEM images, energy-dispersive X-ray spectroscopic analysis (EDX) was also performed. Figure 3.11B and 3.11D showed that the “images” of Ce and O overlapped with the TEM micrograph of CeO$_2$ (Figure 3.11A), confirming that Ce and O are the two main elements. Further EDX analysis showed that fluoride was present in the sample as its image also overlapped with the one of CeO$_2$. Fluorides could be introduced into the particles via production, synthesis and characterization$^{64,65}$. As for carbon (Figure 3.11E), it has been found everywhere on the image. Because the TEM grid was made from carbon, it may not from the sample itself. By overlapping the four elemental EDXs, the image obtained (Figure 3.11F) shows a same morphology as that in Figure 3.11A, indicates that carbon was not a component in the CeO$_2$ particles.
3.2.3. Kinetic studies

Figure 3.12 Kinetic data of A) quenching process of FAM-DNA and by CeO$_2$, B) fluorescence recovery upon the addition of As(III) (red dash line) and As(V) (black dash line), respectively. Concentration of DNA was 100 nM and concentration of CeO$_2$ was 130 μg/mL. Final concentrations of arsenic species were 5.0 μM. All solutions were prepared in 10 mM Tris buffer at pH 7.6.

The reaction kinetics is also important for the optimization of the sensing performance of the DNA/CeO$_2$ nanoconjugates. It testifies if the reaction is rapid enough for on-site detection and when is the best time to read the assay signal. As shown in Figure
3.12, upon the addition of 130 μg/mL CeO$_2$ into a 100 nM DNA solution, the fluorescence intensity decreased immediately. Within 10 s, the signal drops to 61.1% of its initial value. Such a fast adsorption process may be due to the large surface areas of well-dispersed NPs in solution. It is also noted that there is rather slow but continuous decrease after 10 s until 600s, which can be attributed to rearrangement of surface-adsorbed DNA strands. The addition of 5 μM As(III) or As(V) to the conjugates resulted in a monotonic increase on fluorescence signal (Figure 3.12B) for the first 50 s. This is not as fast as the adsorption of DNA strands on the particles; however, it is still feasible for observing the equilibrated signal within one min. Both the fluorescence quenching and recovery processes were rapid, making the proposed system suitable for on-site detection.

3.3. Quantitation of As(III)/As(V) in solution

3.3.1. Construction of the calibration curves

With the optimized conditions to prepare the DNA/ CeO$_2$ nanoconjugates, full sets of standard solutions of As(III) and As(V) (from 1.0 nM to 10 μM) were tested, and the results are shown in Figure 3.13. Clear signals (increased fluorescence intensities) were observed for concentrations as low as 1.0 nM in both cases and increase monotonically with increased concentration of the analytes initially. For As(III) detection, the response reaches the maximum at 100 nM, while for As(V) the signals rises gradually which keeps rising for As(V). It should be also noted that the standard deviations of the observed fluorescence intensities are rather large. These standard deviations were obtained from at least three sets of independent measurements, i.e., both the DNA/CeO$_2$ nanoconjugates and standard solutions were re-prepared each time. This means that the reproducibility of the current detection system needs further improvement for both As(III) and As(V) detection. Nevertheless, the determined limits of detection for As(III) and As(V) are 1.5 nM and 10 nM (shown as the dash lines) are better than those reported by Liu et al. (29 nM) $^{31,32}$, which is only capable of detecting As(V). Although the conjugated system was similar and both systems required ssDNA and CeO$_2$ NPs, our system used arsenic aptamer instead which provided sensitive results on As(III) detection as well. Not only the competitive adsorption on NPs contributed to the fluorescence recovery, but the interactions between DNA aptamer and arsenic species raised the sensitivity. In addition, the system employing DNA aptamer and gold nanoparticles was only sensitive to the
presence of As(III)\textsuperscript{21,23}. To be able to detect both species, the conversion from pentavalent to trivalent is no longer needed, which certainly avoids additional experimental complexities.

Figure 3.13 Detection of A) As(III) and B) As(V) with DNA/CeO\textsubscript{2} nanoconjugates in solution. The nanoconjugates were prepared with 100 nM ars-3 DNA aptamer and 130 μg/mL CeO\textsubscript{2} nanoparticles. The dash lines are to guide the eyes only; the limit detection was indicated with the dotted red lines.
3.3.2. Selectivity test

Figure 3.14 Selectivity test of the DNA/CeO₂ nanoconjugates with 5.0 μM of As(III), As(V) and phosphate, and 5.0 mM of other anions. The nanoconjugates were prepared with 100 nM ars-3 DNA and 130 μg/ml CeO₂.

Selectivity is another extremely important factor for the developed sensing system, especially when real-world samples containing many different interfering species being tested. To be practical, much higher concentrations of other anions (5.0 mM) were used to compare with the signal obtained from 5.0 μM As(III) and As(V) (Figure 3.14). As for iodide, bromide, perchlorate, and nitrate, the increase in the fluorescence intensities are very small (< 10%); in comparison the responses for As(III) and As(V) are as high as 70-80% (despite the fact their concentrations are 1000 times lower). Even for the “problematic” phosphate ions, the response in the current system is significantly lower than that reported in the earlier studies 30,31. They have combined CeO₂ nanoparticles with A15 DNA rather than anti-arsenic aptamers, for which the detection mechanism is different from this study as discussed in section 3.1. Particularly, the selected DNA aptamers have
strong affinities with both As(III) and As(V), but not phosphate. Kim et al. also did selectivity tests of the ars-3 aptamer and showed that Ca²⁺, Cd²⁺, Cu²⁺, and other metal cations did not bind the sequence.

For practical environmental applications, the maximum concentration of phosphate ion in groundwater is around 210 nM according to the Environment Canada⁸, which may not have an impact on the detection of arsenic. If the concentration of phosphate is too high for the detection, Ca²⁺ can be used to remove phosphate prior to the quantification. Suggested amount of the calcium salt added into the sample will be based on the solubility products\(K_{sp}\) ³⁰⁻⁻³². As mentioned in Chapter 1, the main species of Arsenic (III) is \(\text{H}_3\text{AsO}_3\) and Arsenic (V) is \(\text{H}_2\text{AsO}_4^-\); their calcium complex has a much higher \(K_{sp}\) (1.07×10⁻⁷ for CaHAsO₃ and 7.24×10⁻²² for Ca₃(AsO₄)₂) in comparison with that of the phosphate counterpart (2.07×10⁻³³ for Ca₃(PO₄)₂).

In summary, CeO₂ NPs with adsorbed ars-3 DNA aptamers on surface can be employed for the detection of total arsenic in solution with satisfactory sensitivity and selectivity. It was suggested that the detection was realized based on both the specific aptamer/As(III)/(V) binding and competitive adsorption of these species on CeO₂ nanoparticle surface. In both cases, the presence of As(III) and As(V) induces the release of fluorescently labeled DNA from the nanoparticle surface, leading to the substantial increase in the fluorescence intensity.

It was discovered that the order of adding the reagents is crucial for the achieving of the desired performance, i.e., if the As(III) and As(V) were present on the nanoparticle surface, the subsequent addition of either A15 DNA or Ars-3 aptamer is not able to replace the "precoated" analytes. Nonetheless, it has been confirmed that the kinetics of both DNA adsorption on and As(III)/As(V) induced release from CeO₂ nanoparticles is rapid, and that the selectivity with respect to other common anions (including phosphate) is great. More importantly, the superior detection limits (1.5 nM for As(III) and 10 nM for As(V)) in comparison with previous methods that utilizes random DNA sequences, lays the foundation of exploring these nanoconjugates for the creation of paper-based assays for on-site detection of total arsenic in water that will be described in Chapter 4.
Chapter 4. Total Arsenic Detection on Arrayed Paper Strips with Aptamer/CeO₂ Nanoconjugates

As described in Chapter 3, DNA aptamer/CeO₂ nanoconjugates are capable of responding to the presence of both As(III) and As(V) in solution to release the fluorescently labelled DNA strands, thus increase the observed fluorescence intensity. Such a system showed superior sensitivity, good selectivity, and broad responses ranges for the quantitative detection of As(III) and As(V) in solution, which motivated the exploration of transferring such a system to paper substrates, i.e., to create paper-based assays that are potentially applicable for on-site total arsenic detection in water. In this chapter, the preparation of superhydrophobic paper substrates, photolithographic patterning of the paper strips, and the establishment of the color reading protocol will be described first, followed by the construction of the calibration curves and testing of spiked water samples.

4.1. Characterization of arrayed paper strips and assay optimization

The modification of regular filter paper to be hydrophobic (preferentially superhydrophobic) and the subsequent patterning is essential for the creation of water-resist paper strips with arrayed reaction sites (areas that are hydrophilic to hold the reagents). Such arrayed paper strips will essentially serve as the substrate for the fluorescent assays based on the loading of DNA aptamer/CeO₂ nanoconjugates. Upon brief treatment of glass fiber filter papers with 5.1 µM OTS solution for 7 min, the surface of the filter paper becomes superhydrophobic, for which the measured water contact angle is as high as 155±1°. More importantly, the treated filter paper remained hydrophobic upon prolonged storage, i.e., the water contact angle on such a surface showed no sign of decrease in a period of over two weeks (Table 4.1).

The creation of reaction sites, i.e., an array of hydrophilic areas on otherwise hydrophobic paper strip was accomplished by UV/O3 through an aluminum mask (Figure 2.1);
<table>
<thead>
<tr>
<th>Time</th>
<th>Contact angles</th>
</tr>
</thead>
<tbody>
<tr>
<td>As prepared</td>
<td>155±1°</td>
</tr>
<tr>
<td>1 h</td>
<td>156±2°</td>
</tr>
<tr>
<td>1 day</td>
<td>159±2°</td>
</tr>
<tr>
<td>1 week</td>
<td>155±1°</td>
</tr>
<tr>
<td>2 weeks</td>
<td>157±3°</td>
</tr>
</tbody>
</table>

**Table 4.1 Contact angles of one paper strip after silanization.**

A photo of the patterned paper strip with 8 reaction sites (7 mm in diameter) is shown in Figure 4.1. Different types of food color solutions spread evenly on those areas that were activated, while the untreated areas remained superhydrophobic. The three droplets with the same amount dye solutions stay on top of the paper substrates, in contrast to 8 dyed spots as designed for depositing the sensing reagents. The decomposition of silane films on solid substrates has been previous studied 66, which is believed to be contributions from both the UV irradiation and treatment. Further studies of the silanization of filter paper and its photolithographic patterning is currently pursued by other graduate students in our laboratory.

![Figure 4.1](image)

**Figure 4.1** Photo of a modified paper strip with hydrophilic detection areas (assay sites) created via UV/O₃ irradiation. Food dye solutions were dropped on the surface to visualize the different wettabilities of the assay sites and otherwise superhydrophobic substrate.
Upon the successful fabrication of the patterned paper strips, the next task was to examine the color reading protocol, i.e., assay imaging and data analysis. To do this, the same amount (25 μL) and same concentration of FAM solution were added to the 8 reaction zones on the same paper strip; the photo taking under UV irradiation is shown in Fig. 4.2A. It is encouraging that all the spots show bright green color with well-defined edges, and the color seems to be uniform within each of the reaction zone. To quantitively analyze the data, the histogram (RGB values reading) of ImageJ was used to obtain the color intensities on a 0-255 scale. In Figure 4.2B, the directly read green (G) value was plotted with respect to the different assay site. In consideration of both the absolute value and associated uncertainty, the obtained G values were a bit surprising as they were scattered between 120 and 160, with an average of 148±13; the relative standard deviation was as large as 8.5%. Such variations could be caused by many factors, including the shape and position of the UV irradiation source, the optics of the phone camera, and the distribution of the solution on the assay. To accommodate this issue, the average RGB value (a separate channel reading from Image J) was examined at the same time and plotted in Figure 4.2C. The obtained RGB means were distributed from 95 to 130 with an average of 115±9 and a relative standard deviation of 7.8%. The data seems to be less scattered and the relative standard deviation from the average is in fact smaller (7.8%) than that of the G value reading. Stemming from the concept of optical darkness ratio (ODR) that normalize the luminosity of the assay band with respect to the value of background, it was attempted to plot the ratio between G and RGB values to see if the better data uniformity can be achieved with the assay shown in Figure 4.2A. To our satisfaction, the plot of G/RGB ratio shows a much narrower distribution, as indicated in Figure 4.2D. The G/RGB was distributed in a narrower range of 1.2 to 1.4 with an average of 1.29 and a relative standard deviation of 3.6%, which was much smaller than the direct reading from G intensities and RGB means. Thus, G/RGB could be used as an image reading protocol of next step.
Figure 4.2 Smartphone imaging of trial fluorescent assay on paper and the color analysis. A) Photo of arrayed paper strip with 25 µL of 100 nM FAM added to each of the assay sites. B) and C) are G intensities and RGB mean values read directly from the ImageJ software for all the sites; D) is the calculated G/RGB values. The photo was taken in dark under a UV lamp. The average of G intensities, RGB means and G/RGB were calculated and shown as red solid lines with standard deviations shown above and below as the dash lines.
As has been discussed in Chapter 3, the FAM-DNA concentration to prepare the DNA/CeO$_2$ nanoconjugates should be optimized to achieve the best balance between the initial signal, quenching efficiency, and the level of fluorescence recovery; similarly to the solution case, on a single paper strip, 8 different concentrations of FAM-DNA (ranging from 1 nM to 110 nM) were deposited to the arrayed reaction zones for testing their “visibility” when imaged with a smartphone. As shown in Figure 4.3A, except for the very low concentration (1 nM) all other sites show bright green color, indicates that the same
concentration range of FAM DNA employed for solution detection works well on the paper assays. While higher concentrations of FAM DNA resulted in brighter colors, it is clear that the observed G/RGB value no obvious increases at a concentration above 100 nM (Figure 4.3B). It was still rising from 50 nM but G/RGB saturated at 100 nM. It is suggested that using a higher concentration of FAM DNA on paper may not be a perfect choice for image analysis due to potential color saturation; higher concentration of FAM DNA also required higher concentrations of CeO$_2$ NPs to quench fluorescence, which might make the solution more cloudy due to the aggregation of CeO$_2$ and affect the observation under UV light. Therefore, 100 nM DNA was chosen for the subsequent preparation of DNA aptamer/CeO$_2$ nanoconjugates for the paper assay.

4.2. Calibration and quantitation

It is not travailed to “translate” the solution detection protocol established in Chapter 3 once the tasks of fabrication of arrayed paper strips and optimization of color reading are accomplished. With much small volume of the reagents (but at the same concentration as used in the solution tests), the paper-based assay can be prepared and tested. Figure 4.4A shows the image of the situation of adding 100 nM FAM DNA into the reaction zones; strong green colors can be observable, and they are uniform; in contrast upon adding the 130 µg/mL CeO$_2$ nanoparticles, the photo shows that fluorescence were quenched on the detection regions and no color could be observed under UV light (Figure 4.4B).

The hydrophilic assay spots became totally dark and the assay was ready for arsenic samples detection (Figure 4.4B). More remarkably, when different concentrations of aqueous solutions of As(III)/As(V) were individually added onto the assays spots afterwards, the green color varies significantly an UV lamp for both As(III) and As(V) (Figure 4.4C and 4.4D)). From low concentration (1 nM) to high concentration (1 mM), the color changed from basically dark, light green, to bright green. It is significant that the green color can be clearly identified when the concentration was only 100 nM for both arsenic species, which means a rapid and straightforward estimation of the analyte concentration can be made upon eye viewing. As for 1.0 mM of As(III) or As(V) (Figure 4.4C and 4.4D), the brightness of green color seems to be the same as the controls (Figure 4.4A), indicating that all DNA strands were released into solution and the fluorescence intensity was fully recovered.
Figure 4.4 Visualization of the detection of total arsenic with DNA/CeO$_2$ nanoconjugates on arrayed paper strip. (A) 100 nM FAM-DNA added to each of the assay site; (B) Upon adding 130 μg/mL CeO$_2$ to each of the assay site. (C) Different concentrations (as listed) of As(III) were added to the assay sites; (D) Different concentrations As(V) added to the assay sites.
Figure 4.5 Normalized G/RGB as function of the concentration of As(III) (A) and As(V) (B) in the standard solutions, respectively. The dash lines are to guide the eyes only. (C) and (D) are linearized calibration curves for As(III) (20 nM to 100 μM) and As(V) (10 nM to 100 μM), respectively. The red solid lines are the best linear fits to the experimental data, from which the detection limits were determined (see the main text for details).

Figure 4.5 A and B show the dependence of normalized G/RGB values as function of the concentrations of As(III) and (V), respectively. The signals raised rapidly at low concentrations (from 0 to 100 μM) and saturated when their concentrations reached 100 μM. For the purpose of sample quantitative, the linearized calibration curves were constructed by plotting the signal (G/RGB) as function of the logarithmic concentration. As
shown in Figure 4.5C and 4.5D, the linear response ranges for As(III) and As (V) are from 20 nM to 100 μM for As(III), and from 10 nM to 100 μM for As(V), respectively.

\[
\begin{align*}
G/RGB &= 0.0771 \times \log[\text{As(III)}] - 0.0178 \quad \text{Equation 4-1} \\
G/RGB &= 0.0798 \times \log[\text{As(V)}] - 0.0073 \quad \text{Equation 4-2}
\end{align*}
\]

The limits of detection (LOD) determined from the above equations were 29±8 nM and 38±11 nM for As(III) and As(V), respectively. These LOD values are both superior than the commercial arsenic field test kit which has a detection limit of approximately 20 ppb (187 nM) for both arsenic species in water \(^1,12\). The WHO drinking water standard for total arsenic is 10 ppb (87 nM for As(III) and 72 nM for As(V)), therefore the developed paper-based assay can certainly satisfy the need of on-site water testing.

Table 4.2 listed the comparison between the newly developed paper assays and other established arsenic detection methods. It should be noted that the performance of these paper-based assays may not be comparable with centralized laboratory testing\(^1,10,67\) and other more sophisticated method reported before. For example, Zhou et al. reported a detection limit of 1.7 ppb (15.9 nM) for As(III) on fluorescent test paper \(^26\) based on aggregation of modified quantum dots by As(III) and quench of red fluorescence which made the color of fluorescence change from purple, combination of red and cyan to green to cyan, the fluorescence of carbon dots. Nevertheless, the DNA aptamer/CeO\(_2\) nanoconjugate-based paper assays have the advantages of simplified preparation and rapid response, which holds the potential to be applied for on-site, real time testing of water samples. The arsenic species do not require of redox between As(III) and As(V) before tests.

<table>
<thead>
<tr>
<th>Ref.#</th>
<th>Author</th>
<th>Analyte</th>
<th>Detection range</th>
<th>Detection limit</th>
<th>Title</th>
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<tr>
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<td>Authors</td>
<td>Species</td>
<td>Concentration Range</td>
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<td>[23]</td>
<td>Tang et al.</td>
<td>As(III)</td>
<td>3.8 – 230.4 ppb (35.6 nM – 2.15 µM)</td>
<td>1.9 ppb (17.8 nM)</td>
<td>A Simple and Sensitive Resonance Rayleigh Scattering Method for Determination of As(III) Using Aptamer-Modified Nanogold as a Probe.</td>
</tr>
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<td>[20]</td>
<td>Wu et al.</td>
<td>As(III)</td>
<td>1 – 1500 ppb (14 µM)</td>
<td>0.6 ppb (5.6 nM)</td>
<td>Ultrasensitive Aptamer Biosensor for Arsenic(III) Detection in Aqueous Solution Based on Surfactant-Induced Aggregation of Gold Nanoparticles.</td>
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<td>[30]</td>
<td>Liu et al.</td>
<td>As(V)</td>
<td>300 nM – 100 µM (1.4 µM)</td>
<td>300 nM (41 ppb)</td>
<td>DNA Adsorption by Magnetic Iron Oxide Nanoparticles and Its Application for Arsenate Detection.</td>
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<tr>
<td>[26]</td>
<td>Zhou et al.</td>
<td>As(III)</td>
<td>5 –100 ppb</td>
<td>1.7 ppb (15.9 nM)</td>
<td>Color-Multiplexing-Based Fluorescent Test Paper: Dosage-Sensitive Visualization of Arsenic(III) with Discernable Scale as Low as 5 Ppb.</td>
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<td>[9]</td>
<td>Lopez et al.</td>
<td>As(V)</td>
<td>29 nM – 100 µM</td>
<td>29 nM</td>
<td>Tuning DNA Adsorption Affinity and Density on Metal Oxide and Phosphate for Improved Arsenate Detection.</td>
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</tbody>
</table>

Table 4.2 Summary of newly developed arsenic detection methods.

4.3. Testing of spiked samples

To further validate the application potential of the paper assays, tap water samples were spiked with As(III) and As(V) at 200 nM and 2 µM, individually. The concentration of
phosphate in tap water is around 210 nM as reported, calcium chloride (200 nM) was added to precipitate the phosphate first. As shown in Table 1, both 200 nM and 2 μM of As(III) samples resulted in over 97.5% recovery rates; meanwhile the results for As(V) detection are equally impressive (> 97.7% recovery rates). The slightly below unity recovery rates may due to the precipitation of arsenic species at the step of phosphate removal (i.e., adding CaCl₂).

<table>
<thead>
<tr>
<th></th>
<th>As(III)</th>
<th>As(V)</th>
</tr>
</thead>
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<tr>
<td>Spiked concentration</td>
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<td>Normalized G/RGB</td>
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<td>0.2180</td>
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<tr>
<td>Recovery rate (%)</td>
<td>97.5</td>
<td>98.0</td>
</tr>
</tbody>
</table>

Table 4.3 Detection results of Paper-based DNA/CeO₂ nanoconjugate assay for tap water spiked with As(III) and As(V).

4.4. Summary

It was shown in this chapter that the solution detection protocol using DNA aptamer/CeO₂ nanoconjugates for total arsenic detection can be translated to paper-based assays. Not only broad linear responses were confirmed, but also spiked samples were tested successfully. The LOD values for As(III) was 29±0.10 nM (3.1 ppb) and 38±0.15 nM (5.3 ppb) for As(V), which meets the requirements of the WHO standard for drinking water (10 ppb) and the or the industrial effluent as regulated by the MMER Canada (500 ppb). It is also rapid and requires no preparation of the water samples.

It should be emphasized that the assay developed herein is a simple and low-cost fluorometric method based on specially prepared paper substrate and smartphone imaging as the readout method. Particularly, the introduction of DNA aptamer/CeO₂ nanoconjugates to arrayed paper substrates opens new avenues of using nanomaterials for advanced on-site chemical analysis that can be a potential alternative of the traditional instrumental methods.
Chapter 5. General Conclusions and Future Applications

5.1. General Conclusions

In summary, a simple and low-cost fluorometric assay method based on functional nanomaterials, patterned paper substrates, and smartphone imaging protocols was demonstrated for the rapid detection of total arsenic in water. In the presence of either As(III) or As(V), the strong binding with the DNA aptamers resulted in a recovery of fluorescence intensity from releasing the DNA strands precoated on the CeO$_2$ nanoparticles, which can be quantitated with either standard fluorescence spectroscopy in solution or smartphone imaging on paper substrates.

The present method for As(III) and As(V) detection meets the WHO standard for drinking water (10 ppb) and the Mining Metal Effluent Regulations (500 ppb). The LOD of detection in solution is as impressive as 1.5 nM (0.20 ppb) for As(III) and 10 nM (2.10 ppb) for As(V), and on the paper substrate is 29 nM (3.77 ppb) for As(III) and 38 nM (5.28 ppb) for As(V). The linear response ranges for As(III) and As(V) are 20 nM to 100 µM and 10 nM to 100 µM respectively, which provide the practical ranges of using the developed method for on-site detection without any pretreatment of the water samples.

By and large, this thesis research opens up new avenues of translate many other solution-based fluorometric detection protocols for other analytes to paper-based assays, as the modification and patterning of paper substrates as well as the smartphone imaging-based signal reading out are universal to any other colorimetric and fluorometric assays.

5.2. Future directions

From the experimental improvement point of view, further optimization of the assaying conditions should be considered, such as choosing different types of filter paper for better reagent and sample handling, changing the UV irradiation source to be more uniform and power-adjustable, using optical filters for better image collection. In fact, a broad range of filter paper types are being surveyed in the laboratory to find the best choice of making the arrayed substrates; it is also under consideration of using cut-and-paste method or 3D printing technology to prepare the substrates. For the preparation of
the DNA aptamer/CeO$_2$ nanoconjugates, specially prepared CeO$_2$ nanoparticles shall be tested in comparison with the CeO$_2$ nanopowder used in this work. In addition, the length of the DNA aptamers may be truncated to obtain the best performance, as the detection relies on the competition between the aptamer / As(III)/(V) binding and its non-specific adsorption on oxide particle surfaces.

For potential applications of the current assay system for on-site and real time detection of As(III) and As(V) in water, samples from different water sources should be tested and compared with standard instrumental methods. In many cases, pretreatment of the samples such as the precipitation of phosphate and the removal of non-soluble particles.
References


(44) Petrayeva, E.; Algar, W. R. A Job for Quantum Dots: Use of a Smartphone and 3D-Printed Accessory for All-in-One Excitation and Imaging of


Appendix A.

Fluorescence

A1. Fluorescence

Fluorescence occurs in both simple and complex atomic and molecular systems in either gaseous, liquid, or solid phases. The simplest fluorescence is displayed by dilute atomic vapors. The absorption of radiation with wavelengths of 589.6 nm and 589.0 nm can excite the 3s electron of vaporized Na atoms to 3p state. The electrons return to the ground state and emit radiation of the same wavelength to all directions. This type of fluorescence, in which the emitted fluorescence has no change from the absorbed radiation, is known as resonance radiation or resonance fluorescence. Many molecular species also show resonance fluorescence. However, emission band shifts toward longer wavelength than the absorption band, which is called the Stokes shift.

The energy level diagram of photoluminescence is shown in Figure A1. The lowest horizontal line represents the ground-state of a molecule, which is normally a singlet state, and is labelled as $S_0$ (Figure A1). The upper heavy lines are the ground vibrational states of three excited electronic states. The two lines on the left are the first ($S_1$) and second ($S_2$) electronic singlet states; the one on the right ($T_1$) is the energy of the first electronic triplet state. Typically, the energy of the first excited triplet state is lower than the energy of the corresponding singlet state.

A great number of vibrational levels are associated with each of the four electronic states, the lighter horizontal lines. As shown in Figure A1, absorption transitions can occur from the ground singlet electronic state ($S_0$) to different vibrational levels of the excited singlet electronic states ($S_1$ and $S_2$).

Molecules excited to electronic states $S_1$ and $S_2$ lose any excess vibrational energy rapidly and relax to the ground vibrational level of the electronic state. This nonradiational process is named vibrational relaxation.
Figure A1 Partial energy-level diagram for a photoluminescent system, often called a Jablonski diagram.


Absorption is a very fast process, which takes place in about 1 to 10 femtoseconds. Fluorescence emission occurs at a significantly slower rate, which is about 1 microsecond to 1 nanosecond.

An excited molecule returns to its ground state within several different mechanistic steps. As shown by the straight, downward pointing arrows in Figure A1, fluorescence involves photon emission. Other deactivation steps that indicated by wavy arrows are radiationless processes. The favored process to the ground state is the one that has the shortest lifetime of the excited state. Thus, if deactivation by fluorescence is faster than the radiationless process, photon emission is observed. On the other hand, if a radiationless route has a faster rate, fluorescence is either absent or less intense.
As shown in Figure A1, a molecule may be excited to any several vibrational level during the absorption process. Collisions between the excited species and solvent molecules lead to a rapid energy transfer with a minuscule increase in temperature of the solvent. Vibrational relaxation has a lifetime of 1 picosecond or less, which is significantly shorter than the average lifetime of an electronically excited state. As a result, fluorescence from solution always involves a transition from the lowest vibrational level of an excited electronic state ($S_1$ and $S_2$). Emission can terminate in any of the vibrational levels of the ground state (Figure A1).

A result of vibrational relaxation is that the fluorescence emission band for a given electronic transition is displaced toward lower frequencies or longer wavelength from the absorption band (the Stokes shift).

The most intense fluorescence is found in compounds containing aromatic functional groups with low energy $\pi \rightarrow \pi^*$ transitions. Fluorescence may also be found in compounds containing highly conjugated double bonds.

The simple heterocyclic, such as pyridine, furan, thiophene and pyrrole, do not exhibit fluorescence. Fused-ring structures ordinarily do fluoresce. With nitrogen heterocyclic, the lowest energy electronic transition involves an $n \rightarrow \pi^*$ system that rapidly converts to the triplet state and prevents fluorescence. However, fusion of benzene rings to a heterocyclic nucleus results in an increase in the molar absorptivity of the absorption band. The lifetime of an excited state is shorter in those structures.

### A2. Dynamic quenching

Quenching usually refers to nonradiative energy transfer from an excited species to other molecules. Dynamic quenching or collisional quenching requires contact between the excited species and the quenching agent (Q). Dynamic quenching occurs as quickly as the collision partners can diffuse together. The rate is temperature and viscosity dependent. The quencher concentration should be high enough so that there is a higher probability of a collision between the excited species and the quenching agent during the lifetime of the excited state.

Because the fluorescence emission $F$ is directly proportional to the quantum efficiency, the Stern-Volmer equation can be rewritten as
\[
\frac{F_0}{F} = 1 + K_q [Q]
\]

where \(F_0\) and \(F\) are the fluorescence signals in the absence and in the presence of quencher, respectively. The Stern-Volmer constant is the slope of a plot of \(F_0/F\) versus \([Q]\), and the intercept of the plot is unity.

As for \(\text{CeO}_2\) used in this study the oxidation state of Ce is +4, i.e., it has no electrons in its 4f orbital (Figure A2). The energy required for electron transition from oxygen's 2p orbital to cerium's 4f orbital is around 3 eV \(^{62}\). The quenching of the FAM fluorescence upon the adsorption of FAM DNA on CeO2 NPs might be the results of the transitions between 2p to 4f orbitals (Figure A2), yet the detailed mechanism deserves further investigation (beyond the scope of this thesis research).

![Figure A2](image)

**Figure A2** Schematic electronic structures of (a) stoichiometric \(\text{CeO}_2\), (b) partially reduced \(\text{CeO}_{2-x}\) and (c) \(\text{Ce}_2\text{O}_3\). Blue blocks represent filled bands, whereas green and red blocks are drawn as empty boxes.

A3. Fluorescence instrumentation

A3.1. Detection mechanism of fluorometers

The instruments for measuring photoluminescence are similar to UV-Vis spectrophotometers. Figure A2 shows a typical configuration in fluorometers and spectrofluorometers. Nearly all fluorescence instruments use double-beam optics to compensate for fluctuations in radiant power. The upper sample beam passes through an excitation wavelength selector (filter or monochromator), which transmits radiation that excites fluorescence but excludes or limits radiation from the fluorescence emission. Fluorescence is emitted towards all directions but is usually observed at right angles to the excitation beam. The right-angle geometry minimizes the emission wavelength from scattering and from the intense source radiation. The emitted radiation then passes through an emission wavelength selector (filter or monochromator) that transmits the fluorescence only. The isolated radiation then strikes a transducer, which converts light signal into an electrical signal for measurement.

The lower reference beam passes through an attenuator for reducing its power to approximately that of the fluorescence. The attenuated reference beam then strikes a second transducer and is converted to an electrical signal. Computer system then processes the signals to determine the ratio of the fluorescence emission intensity to the excitation source intensity and produce the spectrum or single-wavelength data.

Spectrofluorometers allow to produce a fluorescence excitation spectrum or a fluorescence emission spectrum. The fluorescence excitation spectrum was measured when emission was at a constant wavelength while the excitation wavelength was scanned. With suitable corrections for variations in source output intensity and detector response as a function of wavelength, an excitation spectrum is obtained. The emission spectrum was obtained by fixing the excitation wavelength while the emission wavelength was scanned. These two spectra are approximately mirror images of one another because the vibrational energy differences of the ground state and excited electronic states are roughly the same.
Figure A3 Components of a fluorometer or spectrofluorometer. Source radiation is split into two beams. The sample beam passes through the excitation wavelength selector to the sample. The emitted fluorescence is isolated by the emission wavelength selector before striking the transducer. The reference beam is attenuated before striking the transducer. The electronics and computer system compute the ratio of the fluorescence intensity to the reference beam intensity, which cancels the effect of source intensity fluctuations.


**A3.2. Components of fluorometers**

**Sources: Lamps.** The most commonly used source for filter fluorometers is a low-pressure mercury vapor lamp that equipped with a fused silica window. This source produces lines for exciting fluorescence at 254, 302, 313, 546, 578, 691, and 773 nm. Individual lines can be isolated with suitable interference filters. Because fluorescence can be induced in most fluorescing compounds by a variety of wavelengths, at least one of the mercury lines ordinarily proves suitable.

**Lasers.** Laser sources offer advantages in certain instances, such as, 1) when samples are very small, as in microbore chromatography and capillary electrophoresis where the amount of sample is a microfilter or less; 2) in remote sensing, as in fluorometric detection of hydroxyl radicals in the atmosphere, where the collimated nature of laser beams is vital; or 3) when highly monochromatic excitation is needed to minimize the effects of fluorescing interferences.
Filters and monochromators: Interference and absorption filters are used in fluorometers for wavelength selection. Spectrofluorometers are equipped with at least one and often two grating monochromators. Transducers: Luminescence emission signals are low in intensity. Thus, sensitive transducers are required to enhance the signal. Photomultiplier tubes are the most common transducers applied in fluorometer. Charge-transfer devices, such as charge-coupled devices (CCDs), are also used for spectrofluorometry. This type of transducer provides the rapid recording of both excitation and emission spectra and can be applied in chromatography and electrophoresis.

Cells and cell compartments: Both cylindrical and rectangular cells fabricated of glass or silica are used for fluorescence measurements. Cells are designed to reduce the amount of scattered radiation that can reach the detector, so baffles are introduced into the compartment for this purpose. It is important to avoid fingerprints on cells during measurements because skin oils often fluoresce.

Data manipulation: Computer-based luminescence instruments and software have various data-manipulation schemes. Common data-manipulation and display options include blank signal subtraction, production of corrected excitation and emission spectra, peak detection and processing, by various methods can be found in the software, which can make the measurements and data analysis easier. Specialized software is available for kinetics, for HPLC detection, for analysis of mixtures, and for scanning of spectra from different samples.
Appendix B.

DNA aptamers and selection

B1. General principle of DNA aptamer selection

Figure B1 the systematic evolution of ligands by exponential enrichment (SELEX) process to isolate nucleic acid aptamers from a large random sequence pool.

Aptamers are oligonucleotides which bind to specifically target molecules with high affinity\(^70\). The affinity is determined by the dissociation constant of the aptamer-ligand complex \((K_d)\), which is usually from micromolar to picomolar. Aptamers were first reported independently by Gold in the 1990s\(^71\). Since then, DNA aptamers have been widely applied into various researches as highly promising building elements.

DNA aptamers for specific targets are acquired by in vitro selection or SELEX (systematic evolution of ligands by exponential enrichment). As shown in Figure B1, a very large \((10^{13} – 10^{15} \text{ individuals})\) random sequence DNA library was synthesized and built, in which the individual DNA strands have been allowed to fold into their secondary structures. The large amount of DNA strands in library are typically allowed to flow through a column that the target molecules are immobilized onto. Some DNA molecules bind to target while others do not bind are washed off. The bound DNA molecules are eluted from the column and amplified by PCR (polymerase chain reaction). Thus, the single-stranded DNA library is refreshed. This process is repeated to yield highly specific target-binding DNA aptamers, which can be cloned and sequenced. To obtain high target-specificity, counter-selection is used. It performed against a secondary target with similar structure and properties to the target, which can ensure only aptamers with very high affinity and specificity for the desired target are obtained. In counter-selection, those sequences that bind indiscriminately to both target and its analogue can be eliminated from the selection pool. Once an aptamer has been identified by cloning and sequencing, it can be synthesized by automated chemical synthesis. Its binding activity to the target and its analogues can then be confirmed using different methods to determine its affinity and specificity.

Aptamers have many advantages compared to antibodies, which are proteins generated biologically that can bind to antigens, the target molecules. Aptamers do not require an immune response since they are produced chemically in large quantities, by synthesis of nucleic acid. Antibodies cannot be easily obtained for targets with small sizes such as metal cations or for molecules with less immunogenicity or high toxicity. Although aptamers are selected in vitro, they are also can be used to bind to their targets in vivo with good affinity. Besides, aptamers are readily chemically modified, which allows for their immobilization onto substrates easily. For example, in the electrochemical detection, ssDNA labelled with a thiol group enables the formation of self-assembled DNA monolayers onto gold electrode, which is one of the most popular electrode materials. As
a consequence, the electrochemical properties of immobilized DNA assembled on gold surface can be characterized directly.

The secondary and tertiary folding structures of aptamers are necessary to their binding affinity and specificity. DNA aptamers usually undergo a conformational change upon target-ligand binding: they can form internal loops, three-way junctions, or G-quartet structures, based on their sequence and the structures of the target molecules. The capability of folding ensures the high affinity of aptamers for their targets. As for small target molecules, they can be enfolded in the DNA structure; for larger target molecules such as proteins, the folded DNA aptamer need to bind to a particular epitope.

The aptamers have applied into relevant research and projects, which can controllably bind and release a target molecule to generate signal for detection. In principle, an assay combined DNA aptamer can be used to respond to any protein or ligand. They are widely used to recognize specific elements for biosensing applications, including environmental analysis, and medical diagnostics.

B2. Selection and characterization of arsenic aptamers

The Arsenic aptamer was selected by Kim et al. in 2009. The arsenic affinity column was first prepared by immobilizing 4-aminophenylarsine oxide (PAO) on an Affi-gel 10 resin to select arsenic binding DNA strands. During a total of 10 rounds of the SELEX process, a negative selection step that involved incubation of the DNA aptamers eluted from round 7, was carried out between rounds 7 and 8 to eliminate or minimize non-arsenic binding of the ssDNA aptamer to the Affi-gel 10 resin. The arsenic aptamer binding affinity was visualized and examined with Cy5-labelled aptamer and the arsenic-immobilized affinity column. The arsenic-absent activated Affi-gel 10 resin column was also used. The optional SELEX round was determined by real-time PCR to quantify the arsenic-binding ssDNA aptamers eluted from SELEX rounds 0, 8, 9, and 10. The aptamer pool from the round 9 eluted the largest amount of arsenic-binding ssDNA and it was subsequently amplified by PCR using the unmodified primers.

A quantitative assessment of the arsenic-binding affinities of Ars-1 to Ars-8 acquired from the round 9 aptamer pool was done by a modified SPR assay. The arsenic that lacks an amine group was coupled onto the CM5 sensor chip with 1,8-
diaminoctane, which was first attached to the chip surface and link arsenic based on an electrostatic interaction between the positively charged amine groups and negatively charged arsenic species.

<table>
<thead>
<tr>
<th>Clon</th>
<th>Selected sequences</th>
<th>$K_d$ (nM)</th>
<th>As(V)</th>
<th>As(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ars-1</td>
<td>ACCATCGCGGAGTCCAGTCTGCATCAAAATCCGAGTG</td>
<td>17.8±0.2</td>
<td>5</td>
<td>22.3±1.3</td>
</tr>
<tr>
<td>Ars-2</td>
<td>CACGCGTTCAACCCCGGAATTTAGCAATAGCAGATCACG</td>
<td>67.1±1.3</td>
<td>2</td>
<td>35.7±2.1</td>
</tr>
<tr>
<td>Ars-3</td>
<td>TTACAGAACAACCAACGTCTCCGGGTACTTCTCATCG</td>
<td>4.95±0.3</td>
<td>1</td>
<td>7.05±0.9</td>
</tr>
<tr>
<td>Ars-4</td>
<td>TTCCGCTAGGGGAAACATGATCAACATGGACCAAGTAAAC</td>
<td>12.4±0.2</td>
<td>8</td>
<td>29.2±1.6</td>
</tr>
<tr>
<td>Ars-5</td>
<td>CAGTCAGAATCCGCTCTACCCATTGTTATTGTGGCA</td>
<td>96.6±2.1</td>
<td>6</td>
<td>26.6±2.3</td>
</tr>
<tr>
<td>Ars-6</td>
<td>CAATCTAAGCGAAACCCTGCTCGACAGACCAATTACTCGGCTATA</td>
<td>19.6±0.5</td>
<td>3</td>
<td>51.2±3.2</td>
</tr>
<tr>
<td>Ars-7</td>
<td>ATGCAAAACCCTTAAAGAAGTTGGTGTCCAAAAACCATTTG</td>
<td>15.6±0.3</td>
<td>6</td>
<td>13.0±1.2</td>
</tr>
<tr>
<td>Ars-8</td>
<td>TGGGGATTGTACGTACACCACCCATTACACCGCAGTATAG</td>
<td>13.3±0.6</td>
<td>4</td>
<td>36.6±2.4</td>
</tr>
</tbody>
</table>

The eight sequences were tested for their abilities to bind arsenic in terms of the dissociation constants, \( K_d \) (Table B1). Based on the SPR experiments, the ars-3 aptamer has the lowest \( K_d \) values among them. Kim *et al.* has used the ars-3 aptamer to remove arsenic in water; upon dispersing 2 mg/ml of the DNA aptamer, they concluded that 99.8\% of As(V) and 97.2\% of As(III) can be removed after 30 s of incubation from a solution containing 10 mg/L of arsenic\(^{60}\).