Optical Sensors for On-site Detection of Arsenic (III & V) and other Pollutants (Fe(II), Pb(II), Cr(VI), and F^-)

Thesis

Submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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August, 2023


भारतीय प्रौद्योगिकी संस्थान गुवाहाटी
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Dedication

To My Father

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Statement

I hereby declare that the thesis entitled “**Optical Sensors for On-site Detection of Arsenic (III & V) and other Pollutants (Fe(II), Pb(II), Cr(VI), and F$^-$)** contains original research work. Any content of this thesis has not been taken from anywhere else. This thesis has not not been submitted elsewhere for obtaining the degree of Doctor of Philosophy.

August 2023 **Ravula Rajasekhar**

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Certificate

It is certified that the work contained in the thesis entitled "**Optical Sensors for On-site Detection of Arsenic (III & V) and other Pollutants (Fe(II), Pb(II), Cr(VI), and F^-)** by **Ravula Rajasekhar** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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August 2023

IIT Guwahati

(Ravula Rajasekhar)

Abstract

Water is one of the most important substances on Earth and is essential for all forms of life. It covers about 71% of the Earth's surface and about 60% of the human body. Water is essential for human survival and the environment. It is getting polluted because of the contaminants such as heavy metals etc., and because of this human health and the environment is getting worse day by day. This raises the need for the detection of heavy metals in aquatic water for several detections are available that can broadly classify into two broad categories. Namely laboratory methods and portable methods. Laboratory methods are generally more expensive and time-consuming and require specialized equipment and trained personnel; on the other hand, portable methods are more user-friendly and accessible to non-specialists, allowing for on-site testing of water samples; less expensive and faster but may have lower accuracy and precision. However, there is still a need to develop point-of-care test (POCT) devices and digital sensors that can monitor the water continuously and accurately and precisely detect contaminants in water, eatable and drinkable items accurately and precisely.

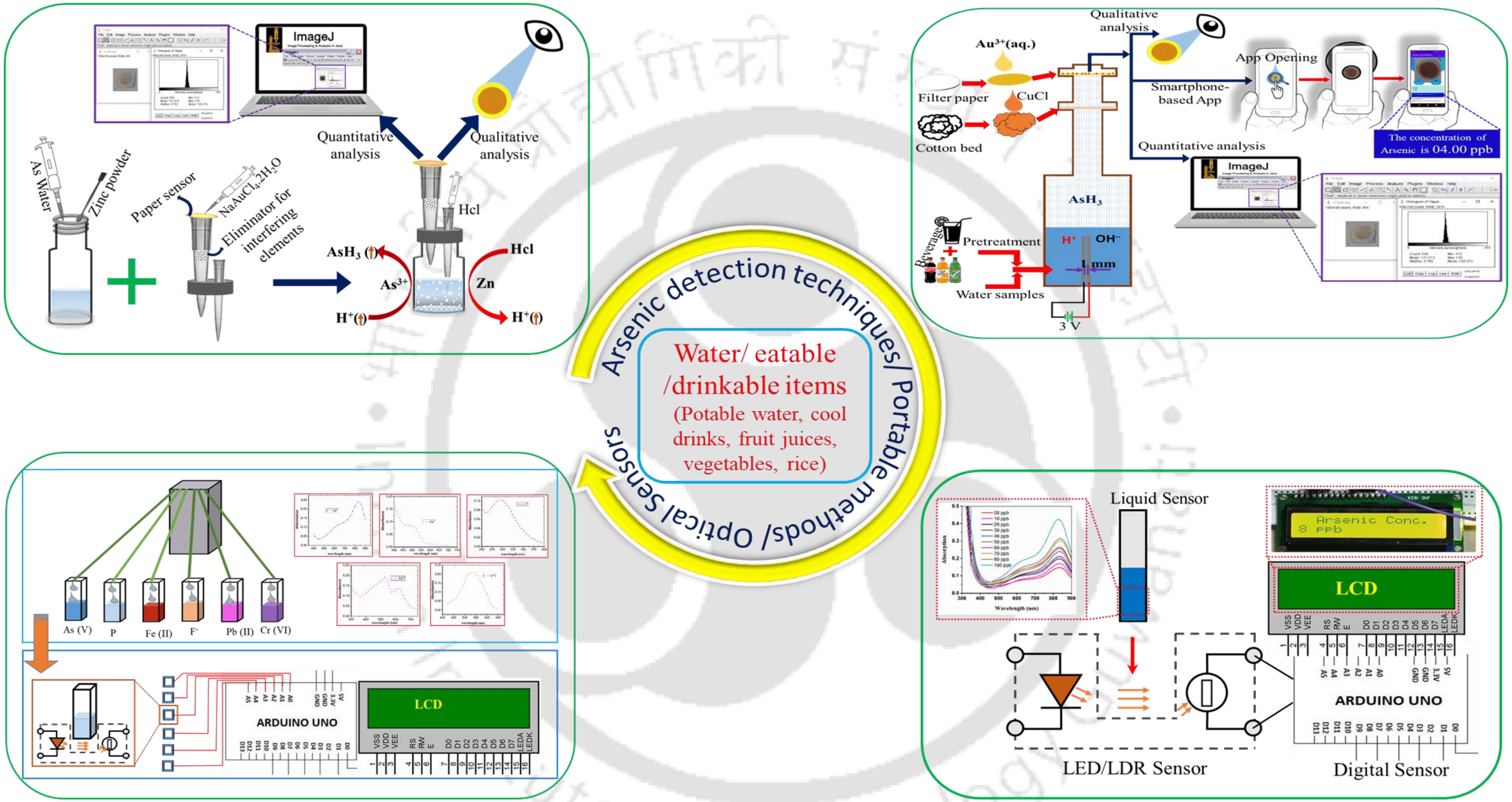
Initially, a rapid, user-friendly POCT device is developed to detect arsenic. The developed method involves the conversion of arsenic to arsine gas, which is then detected using a paper sensor. The sensor was made of impregnated gold chloride ($NaAuCl_4$) on a paper strip, which produces a characteristic color due to the specificity of the reaction. The device demonstrated a low response time, detecting arsenic concentrations of (10-20) µg/L in nearly 4 minutes and concentrations above 20 µg/L up to (20-500) µg/L in just 2 minutes. The device also efficiently detected arsenic in the river and groundwater samples collected from the field.

Further, the work has been extended to develop an indigenous hybrid sensor kit for assessing arsenic in liquid samples, including portable water, soft drinks, and fruit juices. In the kit in situ, hydrogen is generated by electrolysis to convert all arsenic compounds to arsine gas, which reacts with impregnated Au^{3+} on a paper strip. The paper strip changes color from light yellow to a range of grey-red depending on the arsenic concentration. An indigenous smartphone App was developed based on the RGB values of the paper sensor's responses. The App can display the digital value of concentration on the mobile screen. The developed sensor is capable of detecting arsenic concentrations ranging from (04-100) µg/L with a response time of 5 min for concentrations of 04-30 µg/L and 3 min for 30-100 µg/L.

Additionally, this study introduces colorimetric sensors for the on-site quantitative liquid-phase detection of arsenic in water, vegetables, and rice. Through the LDR sensor, this sensor can measure the change in color intensities of different arsenic concentrations. The sensor was transformed into a POCT prototype and calibrated with known samples before testing with real samples. The calibration plot showed that the intensity of the color change was proportional to the resistance change of the LDR. The prototype was further developed into a portable device, which can detect arsenic in the range of 8-100 µg/L and can be used to monitor arsenic in real samples.

Finally, the present study developed the E-Eye, a colorimetric sensor-based POCT device capable of detecting multiple pollutants in water, including arsenic, Iron, Chromium, Fluoride, Lead, and Phosphate. For that, the liquid-based colorimetric sensors was targeted. An LED and a light-dependent resistor were used to measure the color fluctuations. Based on this different calibration curve was developed for the corresponding pollutants. The unknown quantities of pollutants in water samples can be measured using these calibration charts. The sensors were then integrated with an Arduino platform to create a portable and user-friendly POCT device that can monitor water quality. The developed POCT device is stable, user-friendly, and affordable, allowing for on-site detection of contaminants in potable water.

Graphical Abstract



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Nomenclature

Abbreviations

WHO- World Health Organization

IARC- International Agency for Research on Cancer

AAS- Atomic Absorption Spectroscopy

ICP-MS- Inductively Coupled Plasma Mass Spectrometry

AFS- Atomic fluorescence spectrometry

HG-AAS- Hydride generation atomic absorption spectrometry

ETAAS- Electrothermal atomic absorption spectrometry

FI-HG-ICPMS- Flow injection-hydride generation-ICPMS

ASV- anodic stripping voltammetry

CSV- Cathodic stripping volumetry

DPV- differential pulse voltammetry

HPLC- High-performance liquid chromatography

HPLC-HG-ICP-AES

MCL- Maximum Contaminant Level

LOD- Limits of detection

EDX- Energy Dispersive X-ray

POCT- Point-of-Care Testing

UV-Vis spectrophotometer- Ultraviolet-visible spectrophotometer

TEM- Transmission electron microscopes

CHAPTER-I

Introduction and Literature Review


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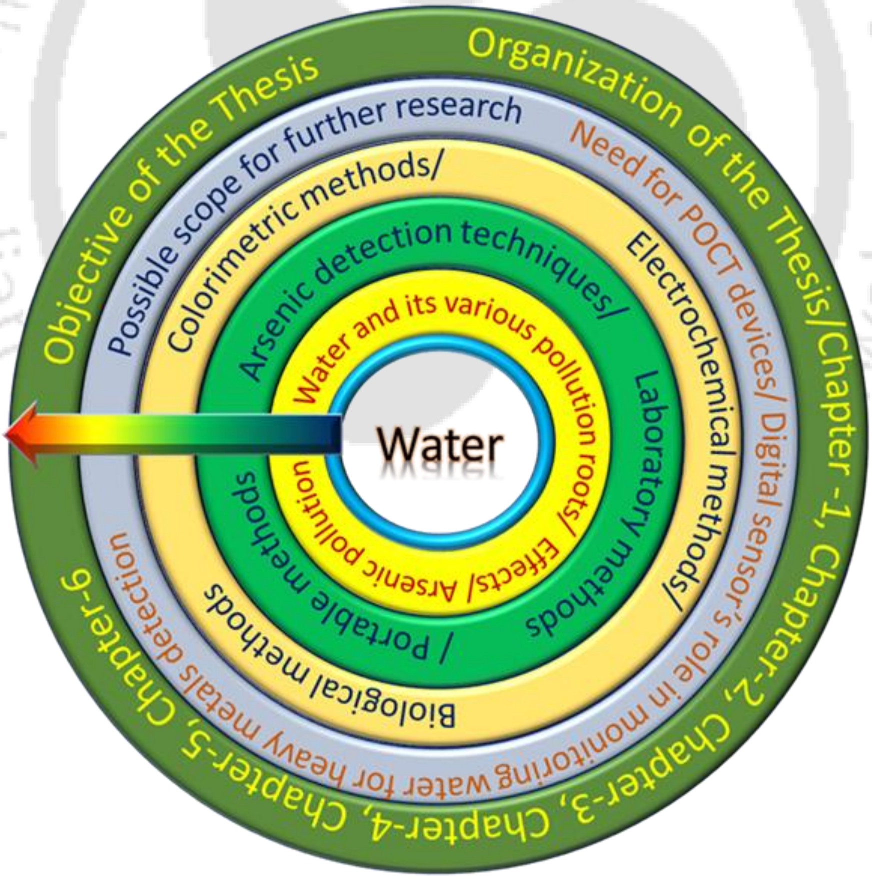
Chapter 1 : Introduction and Literature Review

After presenting a brief overview of water and sources of water contamination due to heavy metals and their effects on humans and the environment *in sections 1.1 and 1.2, respectively, this chapter addresses the summary about arsenic, its effects, and detection methods in section 1.3. Further, this chapter addresses the available prior art in the field of arsenic detection techniques, specifically about the portable methods in section 1.4. Thereby, the potential areas for future research, the thesis's objectives, and the thesis's structure are outlined in sections 1.5, 1.6, and 1.7, respectively.*

Graphical Abstract



1.1 Water as life and its various pollution roots

Water is the most fundamental natural and essential resource for the survival of life on earth, access to safe drinking water is necessary for both humans and the environment [1], [2]. According to a World Health Organization (WHO) report on "Progress on Sanitation and Drinking Water-2015 Update and MDG Assessment," approximately 2.6 billion people had access to improved drinking water sources in 1990, which may increase to 663 million people having access to improved drinking water sources by 2015 [3]. According to the World Water Council, the number of people living in water-stressed areas will rise to roughly 3.9 billion by 2030 [4], [5]. Because of the development of urban, industrial, and natural needs, the current and future demand for water assets is increasing. It is critical to look for arrangements that allow for the effective cleaning of water for possible reuse in various ways [6]–[8].

Water pollution is one of the current issues that society must address in order to improve water quality and reduce the health impacts on humans and ecosystems in the twenty-first century [9]. Water pollution is the contamination of water by foreign matters. The emission of untreated toxic and clean industrial wastes, the disposal of industrial wastewater, and the drainage of agricultural areas are the main causes of freshwater contamination. Such as, organic substances, nutrients, pharmaceuticals, personal care products, poly-, and perfluoroalkyl substances, biocides, heavy metals, dyes, radionuclides, plastics, nanoparticles, and pathogens are all examples of major pollutants [10]–[15]. Nonetheless, over the last few decades, an ever-increasing population, rapid industrialization, increasing urbanization, and careless use of natural resources have all affected water quality [8], [16]. The toxic pollutants released in wastewater can be harmful to aquatic organisms, which also cause the regular waters to be unfit as consumable water sources [17]–[22]. It is astounding that 70–80% of all issues in developing nations-especially those that affect women and children, are related to water contamination [16]. Water bodies such as oceans, lakes, streams, rivers, underground water, and bay are affected by those pollutants. Among all pollutants, heavy metal ions are especially concerning among the most commonly released contaminants because of their potentially devastating effects [23].

1.2 Human and environmental effects of metal contamination

Heavy metals are metallic elements that have a relatively high atomic number (usually greater than 20) and a high density, usually above 5000 kg/m^3 [24], [25]. Almost all heavy metals are toxic to human beings, even at low metal ions concentrations [26]–[32]. Because of their qualities like solubility, oxidation-reduction characteristics, and complex formation, heavy metal decomposition plays a major concern [33]. Heavy metals not only pollute the soil and water, but they also have an impact on food production, quality, and health [34]. In recent years, heavy metals in wastewater have been a major problem in the environment [35]. Those heavy metals are arsenic (As), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), strontium (Sr), tin (Sn), titanium (Ti), vanadium (V) and zinc (Zn), etc., [16], [25]. Due to its malleability, accumulation, nonbiodegradability, and persistence, heavy metal pollution is a significant environmental burden [23], [36]–[40]. These hazardous metals are discharged regularly into the water from a wide range of natural and artificial sources. The paper industry, pesticides, tanneries, metal plating industries, mining operations, etc., release into the environment heavy metal effluent that is nonbiodegradable and poisonous or damaging to human physiology and other biological systems. In the human body, the toxicity of metals reduces brain and nerve function, harms the blood, lungs, kidneys, and other organs, causes weakness, memory loss, an increase in allergies, and raises blood pressure, etc. [41]–[45]. All effluent-generated contaminants were classified as organic or inorganic pollutants, with varying levels of toxicity [8]. Heavy metals fall into the category of inorganic pollutants. At a wide range of places around the world, the average concentrations of As, Cr, Cd, Fe, Co, Mn, and Ni, etc., in surface water bodies are substantially over the maximum permissible limits for drinking water [1], [38], [43], [46]–[48].

1.3 Arsenic pollution of water

Among all heavy metals, arsenic is one of the most toxic. Arsenic is a metalloid, meaning that it has both metallic and non-metallic properties. Considered to be a global health risk factor and is universally present in the earth's environment. Fundamentally, arsenic collects in the earth's crust and bedrock and slowly leaches into the water supply [49]. Arsenic contamination impacts places on every country. Argentina, Bangladesh, China, India, Mexico, Burma, Nepal, Pakistan, Vietnam, and parts of the United States are among the contaminated regions. (*Fig. 1.1*). As a result of its toxicity to people, arsenic (As) pollution has become a major concern in recent years [50] [51]. Arsenic pollutants in groundwater can also impact aquifers' health.

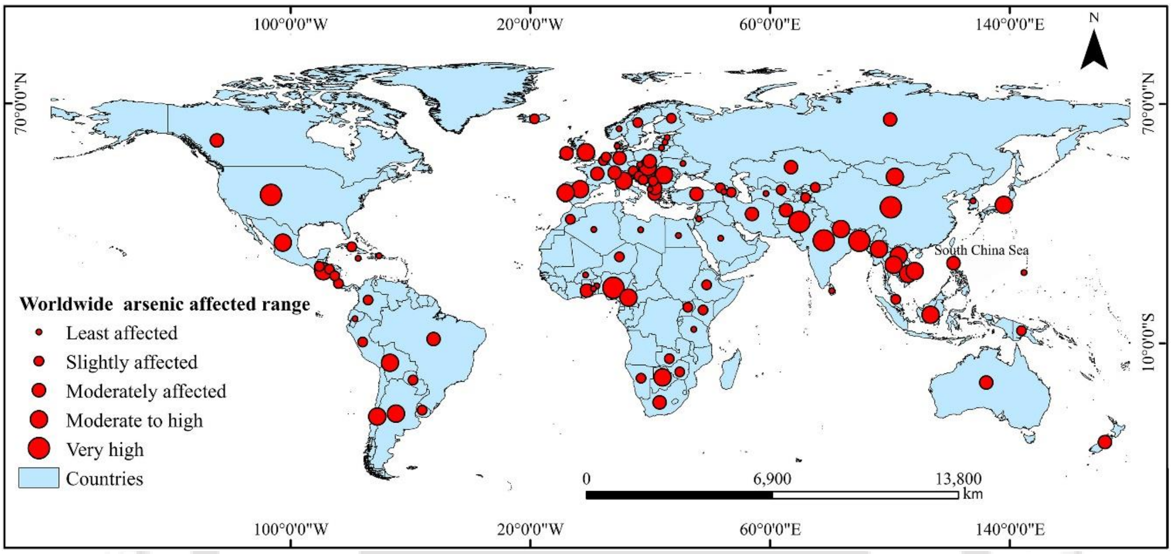


Fig. 1.1: Arsenic afflicted countries around the world intensity shown by the size of the plots.

1.3.1 Geochemistry of arsenic

Arsenic is omnipresent in the environment and ranks about the 12th most common metal in the human body. It is one of the most effective and toxic metals among the other heavy metals. Because of its toxic nature, it affects the environment and humans in various ways [52]. Arsenic is commonly referred to as the "king of poisons" [53]. The permissible concentration of arsenic in drinking water has been set at 10 µg/L since 1993, whereas it was 50 µg/L before that time. Arsenic is classified as a carcinogen, mutagen, and teratogen, with As(III) and As(V) typically combining with oxygen to produce inorganic arsenite. In natural water sources, arsenic is primarily found in these two inorganic oxidation states.

1) Pentavalent arsenic (arsenate or As(V)) is more common in oxidizing groundwater, and surface waters and typically occurs as H_3AsO_4, $H_3AsO_4^{1-}$, $H_3AsO_4^{2-}$ and $H_3AsO_4^{3-}$ depending on pH.

2) Trivalent arsenic (Arsenite or As(III)) mostly exits in low-oxygen (reduction) ground waters and hydrothermal waters. Depending on pH, As(III) may exist in as $H_3AsO_3^0$, $H_3AsO_3^{1-}$, $H_3AsO_3^{2-}$ and $H_3AsO_3^{3-}$.

Inorganic arsenic species are highly toxic in both of their forms [54]. As(III) is 60 times more toxic to humans compared to As(V), and it has a greater degree of mobility in the environment. As(V) is the predominant form of arsenic found in groundwater, which is then reduced to As(III) in anaerobic conditions [55]. Both forms have different chemical properties and stability characteristics. Arsenate is typically more stable than arsenite, especially under oxidizing conditions. This is because arsenate is already in its highest oxidation state (+5), which means that it cannot be oxidized further. As a result, arsenate is less susceptible to chemical reactions that could result in its degradation or conversion to other forms. The stability of arsenate and arsenite can be influenced by a variety of factors, including pH, temperature, and the presence of other chemicals. For example, in acidic conditions, arsenite is more stable than arsenate, while in alkaline conditions, arsenate is more stable than the arsenite. Additionally, the presence of certain chemicals, such as iron or sulfur, can affect the stability of both forms of arsenic.

The South and Southeast Asian Belt, encompassing countries such as India, Bangladesh, Nepal, Vietnam, and China, is regarded as the most severely affected area by arsenic pollution[56]. Developed nations such as the USA and Canada also experience significant levels of arsenic contamination in their groundwater, although the concentrations are typically lower compared to those found in Asian countries [57]. Based on our analysis of global data, it can be inferred that arsenic contamination in groundwater (above the WHO's maximum permissible limit of 10 µg/L) impacts 107 countries.

1.3.2 The scenario in Peninsular India (arsenic pollution in India)

Reports have shown that high levels of arsenic (>10 µg/L) have been detected in shallow aquifers across 10 states in India, although deeper aquifers (>100m) are free from arsenic [CGWB, 2018] [58]. The first case of arsenic contamination in groundwater was reported in the Chandigarh region of North India in 1976 by Datta and Kaul [59], followed by a second case in West Bengal's lower Gangetic plain in 1984 by Garat et al. [59] . This issue has since been reported in various states, including Bihar, Uttar Pradesh, Jharkhand, and West Bengal, as well as Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura, Punjab, Himachal Pradesh, Chhattisgarh, and Andhra Pradesh [60]–[63]. However, our analysis indicates that 20 states (including West Bengal, Jharkhand, Bihar, and Uttar Pradesh), as well as 4 Union territories (Chandigarh, Delhi, Dadra and Nagar Haveli and Daman and Diu , Puducherry, and Jammu and Kashmir), are now affected [63], [64] (*Fig. 1.2*). It is reported that more than 50 million people in India are currently at risk of arsenic contamination in their groundwater.

The high levels of arsenic in groundwater in India have resulted in widespread contamination of drinking water, leading to significant public health concerns. Exposure to arsenic in drinking water has been linked to a variety of health problems, including skin lesions, cancer, cardiovascular disease, and neurological effects [63], [65]–[68]. The most vulnerable populations include pregnant women, children, and people with compromised immune systems or preexisting health conditions.

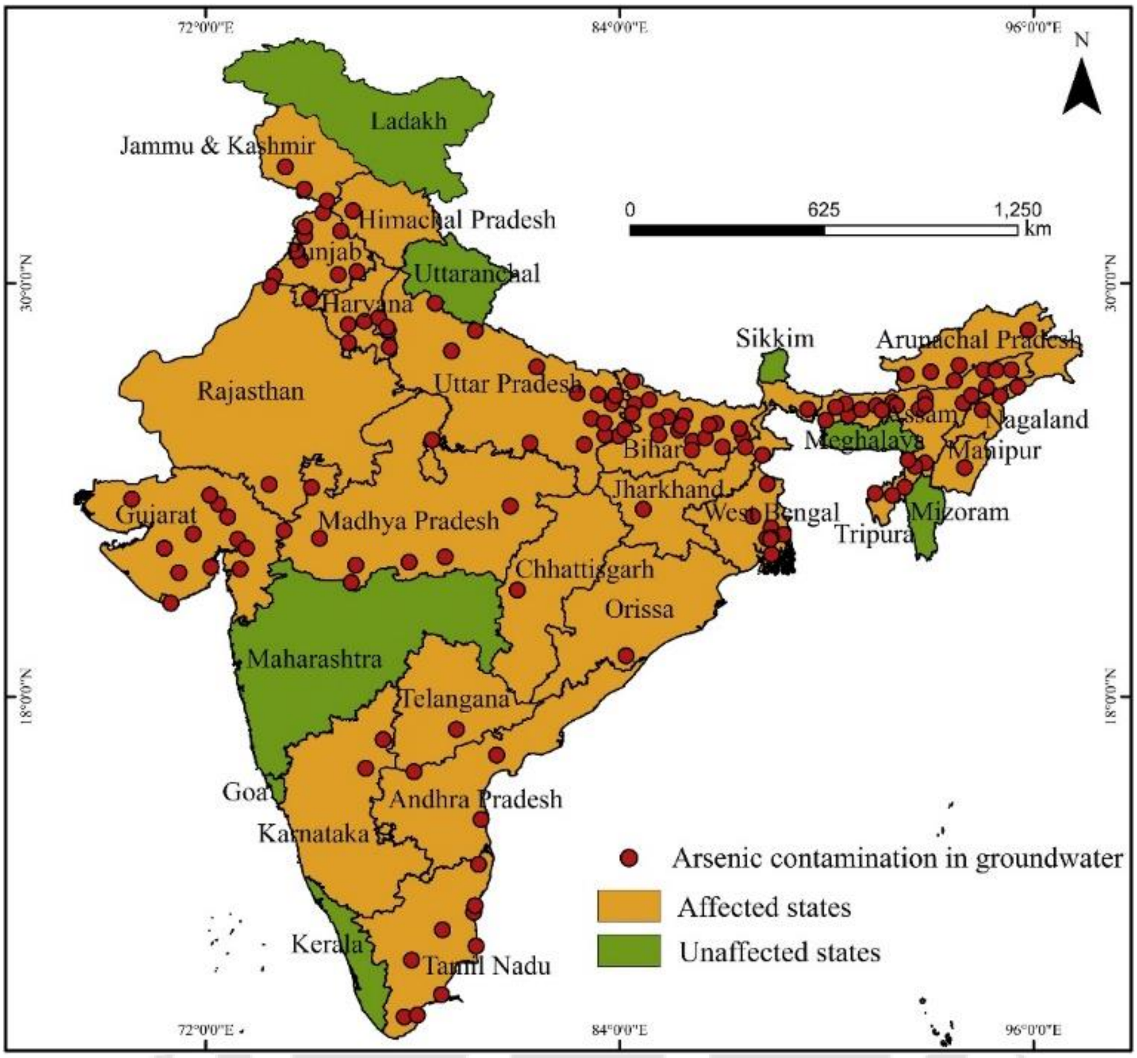


Fig. 1.2: Showing the state wise arsenic contamination areas in India. (Source: Chakraborti et al. 2018 [63], Shaji et al. 2021 [59])

To address the issue of arsenic pollution in India, the government has implemented several initiatives, including the National Water Quality Monitoring Programme and the National Arsenic Mitigation Programme. These programs aim to monitor and regulate arsenic levels in drinking water, promote the use of arsenic-safe technologies, and provide safe drinking water to affected communities. Additionally, there have been efforts to promote sustainable agriculture practices, reduce the use of arsenic-based pesticides and fertilizers, and improve industrial waste management. Despite these efforts, arsenic pollution remains a significant challenge in India, and further action is needed to protect public health and ensure access to safe drinking water for all.

1.3.3 Sources of arsenic contamination

Arsenic contamination can originate from both natural and human activities, as identified by several studies [69]–[72]. Here's a more detailed evaluation of the main sources of arsenic contamination:

1) Natural Sources: Arsenic is a naturally occurring element in the earth's crust. Some rocks and minerals contain higher levels of arsenic than others, and natural weathering and erosion of these materials can release arsenic into groundwater and surface water. Natural sources of arsenic contamination can also be related to geothermal activity, volcanic activity, and coal deposits. In areas with high levels of arsenic in the soil and rock, groundwater can become contaminated with naturally occurring arsenic [73].
2) Anthropogenic Sources: Human activities can also contribute to arsenic contamination in the environment. Industrial activities, such as mining, smelting, and pesticide production, can release arsenic into the air, soil, and water [59]. Coal-fired power plants and coal mining can release arsenic into the air, and this arsenic can settle on land and water sources, contaminating them. Arsenic can also be present in wastewater from these activities, leading to contamination of nearby water sources. Improperly constructed landfills that accept hazardous waste can also be a source of arsenic contamination.

1.3.4 Health risk

Arsenic is a toxic substance that can cause a variety of health problems if consumed in high concentrations over a prolonged period of time. Exposure to arsenic in water and food can lead to both acute and chronic health effects [63], [74]–[77], including:

Skin lesions: Prolonged exposure to arsenic can cause skin lesions, which are typically characterized by thickened, pigmented patches on the palms, soles, and torso.

Cancer: Long-term exposure to high levels of arsenic in water and food has been linked to an increased risk of several types of cancer, including skin, bladder, lung, and liver cancer.

Cardiovascular disease: Exposure to high levels of arsenic has been associated with an increased risk of cardiovascular disease, including hypertension and atherosclerosis.

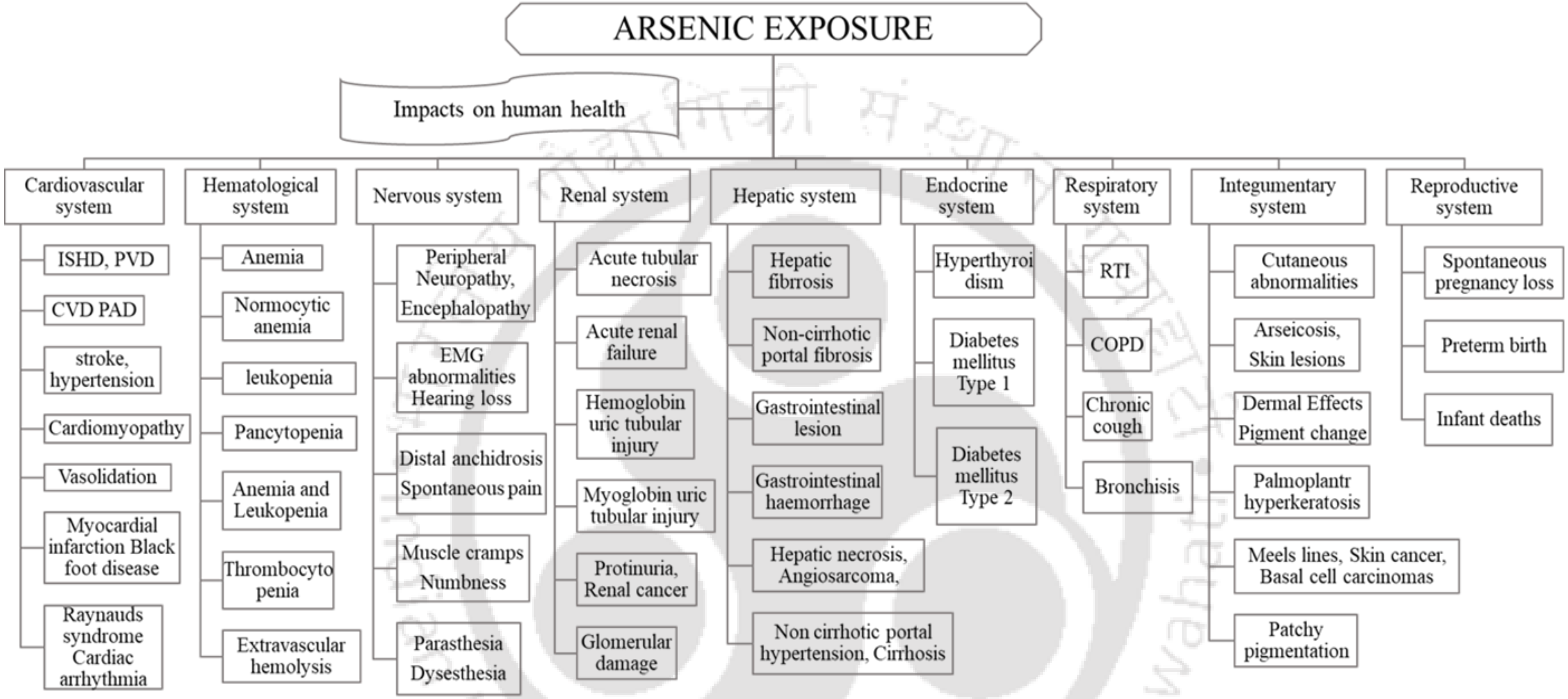


Fig. 1.3: Flow chart shows the overview of the various impacts of arsenic poisoning on human health. (Source: Sahji et al. [59])

Neurological effects: Chronic exposure to arsenic can also cause neurological effects, including peripheral neuropathy, muscle weakness, and cognitive impairment.

Reproductive and developmental effects: Exposure to arsenic in water and food has been linked to adverse reproductive and developmental effects, including decreased fertility and increased risk of spontaneous abortion and stillbirth.

Moreover, arsenic exposure has been associated with newborn mortality, intellectual impairment, and motor dysfunction in children [77]–[82] The health risks associated with arsenic depend on several factors, including the concentration of arsenic in the water or food, the duration of exposure, and individual susceptibility. In general, the most vulnerable populations include pregnant women, children, and people with compromised immune systems or preexisting health conditions. The contamination of groundwater with arsenic is estimated to be impacting 500 million people worldwide. Fig. 1.3 presents a summary chart of the different ways in which arsenic poisoning can affect human health, based on information gathered from different research (Chakraborti et al. 2018 [63]; Saha and Ray, 2019 [77]; Shaji et al. 2021 [59])

1.3.5 Detection techniques for heavy metals in water and their merits and demerits

It is essential to detect and measure the concentration of heavy metal contaminants in water before consumption, utilization, or choosing an effective remediation treatment. The selected technique should be cost-effective, environmentally friendly, selective, and sensitive enough to detect traces accurately [66]. Although meeting all these criteria can be challenging, advancements and combinations of various techniques now enable precise detection of heavy metals and rapid measurement of their concentrations, providing insight into the magnitude of water pollution worldwide. The following sections describe various techniques, including spectroscopic, electrochemical, and optical methods, used for this purpose [1]. However, spectroscopic techniques require expensive and complex equipment, trained personnel working ideally in a laboratory, and relatively challenging sample preparation procedures to stabilize [82]. Moreover, these techniques have higher detection limits compared to spectroscopic and optical methods, making them less preferable when other metal cations coexist in the sample, thereby reducing detection sensitivity. Therefore, electrochemical methods face significant challenges such as selectivity and sensitivity, making them as a best detection method [83] Several optical molecular detectors can guarantee rapid analysis, but poor selectivity limits many of these techniques [66], [84].

1.3.6 Detection techniques for arsenic in water and their merits and demerits

There are several detection techniques available for arsenic in water. Here are some of the most commonly used techniques with their merits and demerits:

(1) Laboratory techniques: Several conventional instrumental techniques are commonly used for arsenic detection. These techniques are Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Atomic fluorescence spectrometry (AFS), Hydride generation atomic absorption spectrometry (HG-AAS), Electrothermal atomic absorption spectrometry (ETAAS), Flow injection-hydride generation-inductively coupled plasma mass spectrometry (FI-HG-ICPMS). Merits: It is a highly sensitive and accurate technique. It can measure both inorganic and organic arsenic species separately. It can be used for both qualitative and quantitative analysis of arsenic.

Demerits: It is an expensive and time-consuming technique. It requires highly trained personnel to operate the equipment. It requires complex sample preparation.

(2) Colorimetric Tests: These tests use chemical reactions to produce a color change proportional to the concentration of arsenic in the sample.

Merits: Those are simple, inexpensive, and easy to use. Those can provide rapid results and be used in the field without specialized equipment.

Demerits: Those are less sensitive than AAS and ICP-MS. They are prone to interference from other substances. Those cannot distinguish between inorganic and organic arsenic species.

(3) Electrochemical Techniques: These techniques measure the electrochemical behavior of arsenic in the sample. The most common electrochemical techniques are anodic stripping voltammetry (ASV) and differential pulse voltammetry (DPV).

Merits: Those are simple, inexpensive, and easy to use. It can provide rapid results and can be used in the field without specialized equipment.

Demerits: They are less sensitive than AAS and ICP-MS. They are prone to interference from other substances. This cannot distinguish between inorganic and organic arsenic species.

(4) Fluorescence Spectroscopy: This technique measures the fluorescence emitted by arsenic compounds when they are excited with a specific wavelength of light.

Merits: It is highly sensitive and accurate. It can measure both inorganic and organic arsenic species separately. It can be used for both qualitative and quantitative analysis of arsenic.

Demerits: It is an expensive and time-consuming technique. It requires highly trained personnel to operate the equipment. It requires complex sample preparation.

(5) Biosensors: Biosensors are devices that use biological materials to detect arsenic. For example, some biosensors use enzymes or antibodies that specifically bind to arsenic, causing a measurable change in the sample's electrical properties.

In summary, the choice of detection technique for arsenic in water depends on several factors, including the required sensitivity, accuracy, and cost. Colorimetric tests and electrochemical methods are inexpensive but less sensitive and cannot distinguish between inorganic and organic arsenic species. AAS, ICP-MS, and fluorescence spectroscopy are highly sensitive and accurate but are expensive, time-consuming, and require specialized equipment and trained personnel.

1.3.7 Merits of colorimetric detection

Colorimetry is one of the most used optical methods to detect the analytes in paper/ liquid-based devices. The intensity of the color is proportional to the analyte concentration, and the results can be interpreted directly without any equipment. The principle of this method lies in the generation of color by chemical/biochemical reaction between the target analyte and reagents. The intensity of the resulting color can be quantified using imaging tools and processing software. The most significant advantage of the colorimetric assay on paper is its simplicity.

Further, the paper substrate is compatible with low-detection systems and provides a white background for colorimetric assays. In addition, advantages like faster analysis, lack of complicated instrumentation, ease of application, low-cost detection system, and low sample volume requirement makes colorimetry-based paper analytical devices highly suitable for point-of-care applications.

Colorimetric assays have gained significant interest because of their inherent advantages, including simple operation, quick response, adaptable sensitivity, and long linear range of the quantitative assay that is based on spectrometry [85].

Colorimetric detection is a widely used technique in various fields, such as chemistry, biology, medicine, environmental science, and food science. Some of the merits of colorimetric detection are:

(1) Simple and low-cost: Colorimetric assays are easy to perform and do not require complex instrumentation or expensive equipment. They are often based on a sample's change in color or absorbance, which can be easily detected with the naked eye or a simple spectrophotometer.

(2) High sensitivity: Colorimetric assays can be highly sensitive and can detect very low concentrations of analytes. This is because the change in color or absorbance is often proportional to the amount of the analyte present in the sample.

(3) High selectivity: Colorimetric assays can be highly selective and can detect specific analytes in complex mixtures. This is because the assays can be designed to target specific chemical or biological species using specific reagents.

(4) Rapid results: Colorimetric assays are often rapid and can provide results within a few minutes or hours. This is important in many applications where fast and reliable results are needed.

(5) Quantitative analysis: Colorimetric assays can be easily quantified and calibrated, allowing for accurate and precise measurements of analyte concentrations.

(6) Versatility: Colorimetric assays can be adapted to a wide range of applications and can be used to detect various types of analytes, such as proteins, nucleic acids, enzymes, and small molecules.

Overall, colorimetric detection is a powerful and versatile technique that can provide rapid, sensitive, selective, and quantitative analysis for a wide range of applications.

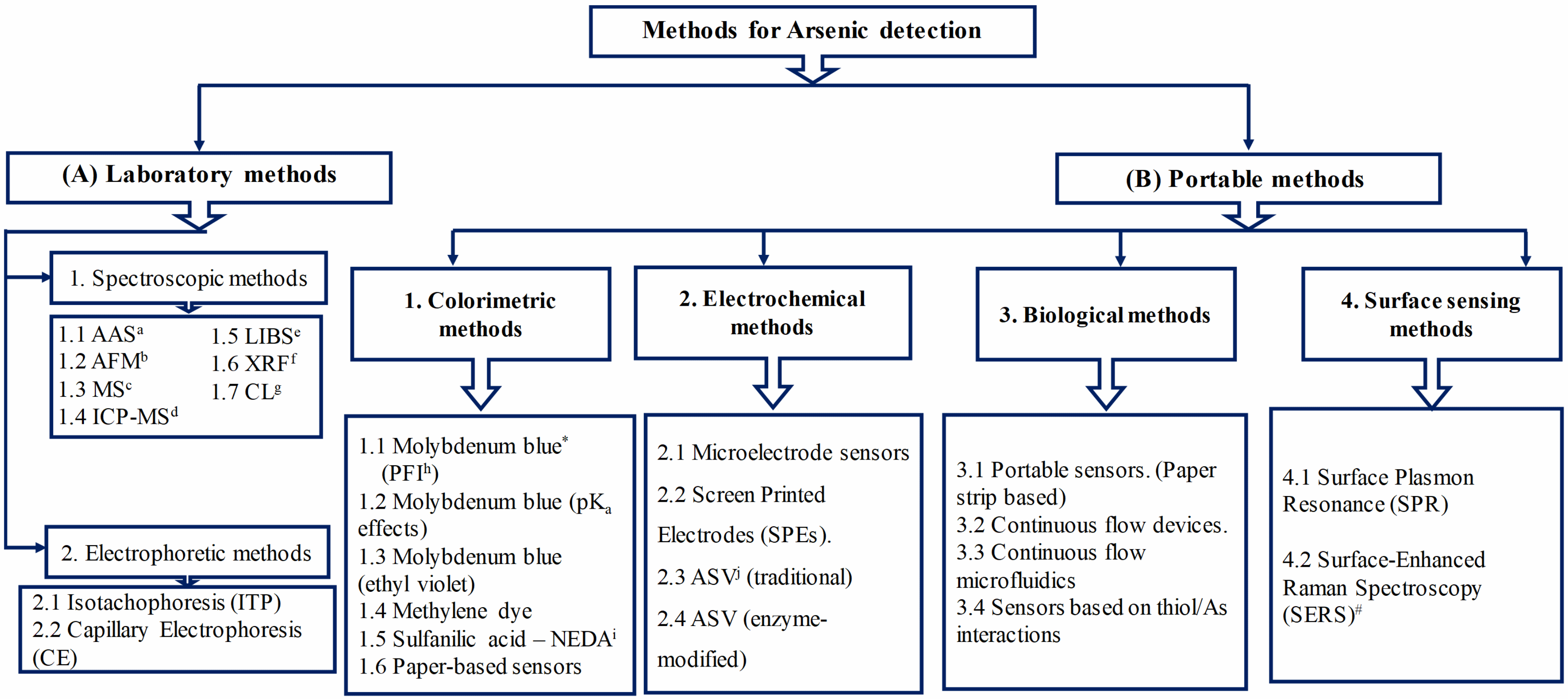
Compared with other methods, the colorimetric method has some obvious advantages, such as low cost, simple instruments (or, in the case of naked eye detection, no instruments), and can be qualitatively or semiquantitatively identified by the naked eye. However, colorimetry is generally less sensitive.

1.4 State-of-the-art

In this section, the available prior art in the field of heavy metals detection, specifically arsenic, is being addressed. The available prior art has been presented for both laboratory and portable methods to detect arsenic in the field of onsite detection. The following sub-sections elaborate for the detection of other pollutants in water.

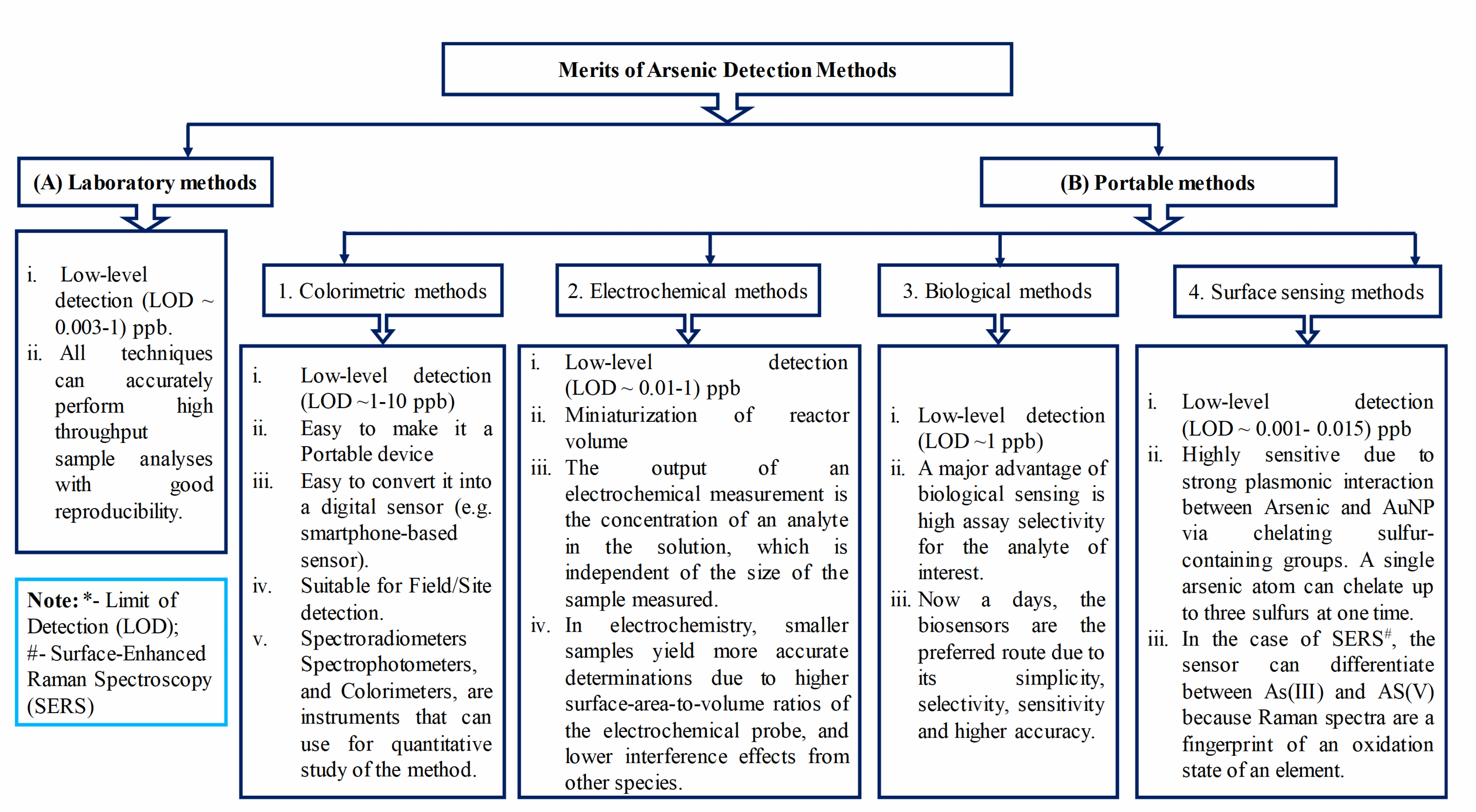
1.4.1 Arsenic detection techniques

Two broad categories of detection techniques are available: laboratory methods and portable methods. While laboratory techniques are more accurate, they require expensive equipment and are not feasible for common use. Hence, this work focuses on low-cost portable techniques with short response times and ease to use. Site-specific testing is also possible with portable techniques. However, these techniques have certain drawbacks that depend on the specific device's working principles. Summarizing the various detection methods and their strengths and limitations is difficult due to their diversity. Therefore, information on the different detection methods and their pros and cons are extracted from the literature and presented in Fig. **1.4**–Fig**. 1.6**



Note: a- Atomic Absorption Spectroscopy; b- Atomic force microscopy; c- mass spectrometry; d- Inductively Coupled Plasma Mass Spectrometry; e- Laser Induced Breakdown Spectroscopy, f- X-Ray Fluorescence, g- Chemiluminescence h- pervaporation flow injection; i- N-(1-naphtyl) ethylene diamine hydrochloride; j- anodic stripping voltammetry; *- since it involves the evaporation, the particular method was not portable; #-Some Raman Spectrometers are portable;

Fig. 1.4: Various arsenic detection techniques.



Merits of Arsenic Detection Methods

(A) Laboratory methods

- i. Low-level detection (LOD ~ 0.003-1) ppb.
- ii. All techniques can accurately perform high throughput sample analyses with good reproducibility.

Note: *- Limit of Detection (LOD); #- Surface-Enhanced Raman Spectroscopy (SERS)

(B) Portable methods

1. Colorimetric methods
   - i. Low-level detection (LOD ~1-10 ppb)
   - ii. Easy to make it a Portable device
   - iii. Easy to convert it into a digital sensor (e.g. smartphone-based sensor).
   - iv. Suitable for Field/Site detection.
   - v. Spectroradiometers Spectrophotometers, and Colorimeters, are instruments that can use for quantitative study of the method.

2. Electrochemical methods
   - i. Low-level detection (LOD ~ 0.01-1) ppb
   - ii. Miniaturization of reactor volume
   - iii. The output of an electrochemical measurement is the concentration of an analyte in the solution, which is independent of the size of the sample measured.
   - iv. In electrochemistry, smaller samples yield more accurate determinations due to higher surface-area-to-volume ratios of the electrochemical probe, and lower interference effects from other species.

3. Biological methods
   - i. Low-level detection (LOD ~1 ppb)
   - ii. A major advantage of biological sensing is high assay selectivity for the analyte of interest.
   - iii. Now a days, the biosensors are the preferred route due to its simplicity, selectivity, sensitivity and higher accuracy.

4. Surface sensing methods
   - i. Low-level detection (LOD ~ 0.001- 0.015) ppb
   - ii. Highly sensitive due to strong plasmonic interaction between Arsenic and AuNP via chelating sulfur-containing groups. A single arsenic atom can chelate up to three sulfurs at one time.
   - iii. In the case of SERS#, the sensor can differentiate between As(III) and AS(V) because Raman spectra are a fingerprint of an oxidation state of an element.

Fig. 1.5: Merits of the available arsenic detection techniques.

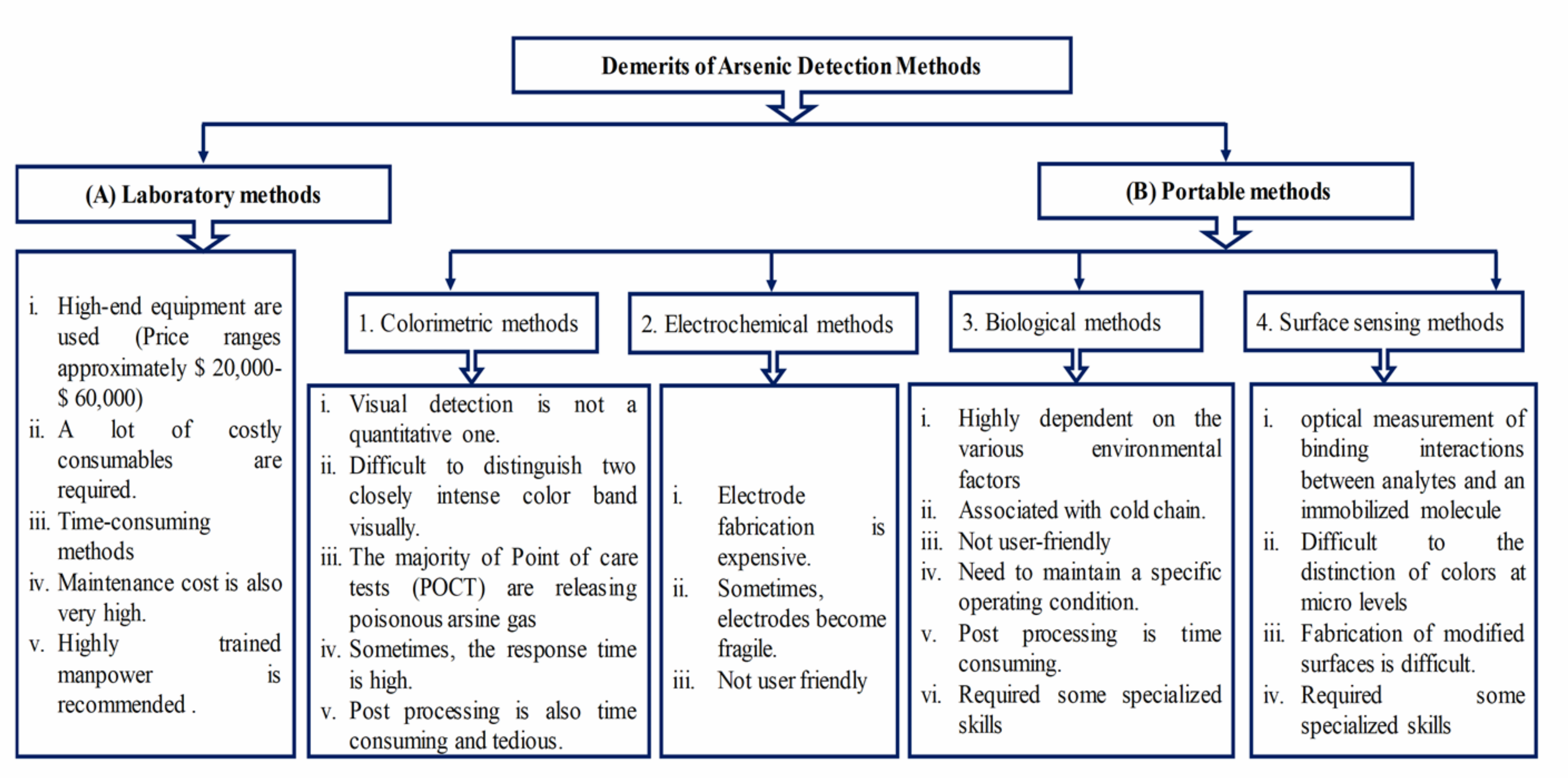


Fig. 1.6: Demerits of the available arsenic detection techniques.

1.4.1.1 Laboratory methods

As described in section 1.4.1, the available literature on laboratory methods is discussed below:

Bose et al. [86] have identified various laboratory methods for arsenic analysis, including atomic spectroscopy, mass spectrometry, neutron-activated analysis, electrophoresis, chromatography, potentiometry, and voltammetry [86]. These techniques typically have a detection limit (LOD) for arsenic of around 1 μg/L or 1 ppb [57], making them suitable for high throughput sample analysis with excellent reproducibility. However, these techniques are expensive, with equipment alone costing more than $30,000, in addition to maintenance, consumables, and the need for highly trained technicians.

The literature predominantly utilizes atomic absorption spectroscopy (AAS), mass spectrometry (MS), and atomic fluorescence spectroscopy (AFS) to calibrate and validate other arsenic detection methods [62], [87]–[89]. Despite being powerful tools for data analysis, they are not practical for routine arsenic monitoring in developing countries due to their high cost and maintenance requirements.

The most commonly used technique for separating different arsenic species is high-performance liquid chromatography (HPLC) and its various forms. The molecular types of arsenic that undergo analysis for evolution are the anions As(III) and As(V). The separation techniques utilized are anion-exchange HPLC with isocratic extraction, as well as ion-pair HPLC.

In 1976, Aggett et al. [90] utilized the well-established hydride evolution technique in combination with atomic absorption spectroscopy to determine the concentration of arsenic in aqueous media. The pH level of the sample was maintained between 4 and 5 using sodium borohydride for the selective determination of arsenite, and the total arsenic concentration was determined by the evolution of hydrochloric acid. The evolved samples were analyzed using the Unicam SP 90A atomic-absorption spectrometer with a photomultiplier tube utilizing a hydrogen-argon flame. This method had a limit of detection of 25 μg/l, and the plotted calibration curves were linear for arsenic concentrations below 300 μg/l.

In 1989, Beauchemin et al. [91] coupled different forms of HPLC with ICP-MS for arsenic speciation to detect the limit of detection (LOD) of As. The study investigated the use of ion-pairing and ion-exchange HPLC to separate As(III) and As(V). It was found that although anion exchange had inferior resolution compared to ion-pairing chromatography, it appeared to be less susceptible to matrix interferences.

Lopez et al. [92] have incorporated the on-line thermos-oxidation to the speciation of As(III) and As(V) and used an anion column with 17mM phosphate at pH 6.0 as the mobile phase hydrochloric acid and sodium borohydride are added on-line to generate the arsine.

In 1994, Demesmay et al. [93] investigated an HPLC system coupled with an ICP-MS detector to determine six different arsenic species, namely As(III), As(V), MMA, DMA, AB, and AC. The interface between the two systems was established using PTFE capillary tubing. An anion-exchange column with a mobile phase of phosphate buffer containing 2% acetonitrile was utilized. An ionic-strength step gradient was necessary to achieve an optimum separation of the arsenic species.

Hydride generation has only been used by a limited number of researchers to detect arsenic in the gas phase through HPLC-HG-ICP-AES, as reported by Raurt et al. [94]. To minimize the volume of solution reaching the plasma torch and improve the separation of volatile hydrides, a silica-based anion-exchange column was used to separate the As(III) and As(V) species of arsenic.

Dedkova et al. [95] performed adsorption of As(V) on a fibrous AV-17 anion exchanger and determined As as a heteropoly acid. The reaction at the adsorbent surface occurred due to the formation of the blue form of molybdoarsenic acid. The concentration of arsenic was determined using X-ray spectrometry and the detection limit was found to be 4 µg/L.

Becker et al. [96] developed a direct method for arsenic determination from a performance comparison of a transversely heated platform atomizer (THPA) with a transversely heated filter atomizer (THFA) Improved sensitivity is achieved in THFA as it reduces the background absorption. The chemical modifier used in both the atomizer was the mixture of 0.1% Pd, 0.03% Mg, and 0.05% Triton-100. Samples were stabilized as a three-component solution. The limit of detection for THPA and THFA was found to be 1.9 and1.8 µg/L, respectively. The limit of quantification was found to be 6.2 and 5.9 µg/L for THPA and THFA, respectively.

In 2011, Bose et al. [86] investigated various laboratory techniques that can be utilized for arsenic analysis, including atomic spectroscopy, mass spectrometry, neutron-activated analysis, electrophoresis, chromatography, potentiometry, and voltammetry. Most established laboratory methods have a limit of detection (LOD) for arsenic in the order of 1 µg/L or 1 ppb. While these techniques can accurately determine the concentration of the sample, their cost and maintenance can be prohibitive, and long-term operation may lead to decreased reliability. Specifically, AAS, MS, and AFS are the most commonly used techniques, and the authors compared some general performance characteristics of these methods. Despite their analytical power, the high cost and maintenance associated with these laboratory methods remain a challenge.

Dominguez-Gonzalez et al. [97] synthesized gold nanoparticles as sensing materials because their optical properties are related to shape and size. A low level of arsenic can be detected by a change in the color of functionalized gold nanoparticles by aggregation. This method was performed with functionalized gold nanoparticles and VIS spectrometry. This method can detect arsenic concentrations in the range of 2-20 µg/L. Gold nanoparticles were functionalized with glutathione-dithiothreitol-cysteine-(2,6-pyridinedicarboxilix acid) (GSHDTT-Cys-PDCA).

Marcinkowska et al. [98] performed an analysis of As, Cr, and Sb in their toxic form. The As(III), As(V), and Cr(VI), were separated using NH_4NO_3 and $(NH_4)_2HPO_4$ in the mobile phase, whereas Sb species were separated using phosphate buffer, carbonate buffer, and potassium hydroxide. A gradient elution program with 3 mM $EDTANa_2$ and 36 mM ammonium nitrate solutions was used to fully separate species. All the samples were analyzed using HPLC-ICP-DRCMS under an optimized condition with pH values 3-7 and 15-35 °C. Detection limits of (0.12-0.6) µg/L for As(III), (0.34-0.5) µg/L for As(V), 0.14, 0.1, and 0.29 µg/L for Sb(III), Sb(V) and Cr(VI) respectively.

1.4.1.1.1 Spectrophotometric method

In 1980, Howard and Arbab-Zavar [99] utilized a spectrophotometric procedure using silver diethyldithiocarbamate (SDDC) to determine arsenite and arsenate. However, this method has interferences from trace metals and methylated arsenic species, which can be prevented using techniques such as chelation, solvent extraction, or ion-exchange.

In 1989, Tamari et al. [100] introduced a new co-precipitation method for the spectrophotometric determination of arsenite and arsenate in groundwater. Both species are co-precipitated with thorium(V) hydroxide at pH 9. After centrifugation and dilution with hydrochloric acid, the absorbance was measured using spectrophotometer by the standard silver diethyldithiocarbamate (SDDS) method.

Palanivelu et al. [101] developed a highly sensitive spectrophotometric method for the precise determination of arsenite and arsenate. The measurement of absorbance was carried out using a Carl Zeiss PMQII spectrophotometer with 10 mm quartz cells. The determination involved extracting arsenic species into benzene followed by measuring total arsenic as arsenic tri-iodine. As(V) was determined by reducing arsenate with potassium iodide. This method was found to be more sensitive than the SDDC method and the fluorescence method based on rhodamine-B. The technique has the advantage of lack of interferences and allows precise determination of trace amounts of arsenic in natural and synthetic samples.

Grudapan et al. (2003) used flow injection spectrophotometry for the determination of As(III) and As(V) in the sample. In the sample containing As(III) and As(V), ammonium molybdate and ascorbic acid solutions were injected into the C18 solid in-valve column. The reduced Mo(V) arsenic complex was absorbed in a column which was monitored by an LED colorimeter to get As(V) concentration. The same process was repeated with the addition of KIO_3 solution to the injected sample, whereby it oxidized the As(III) to As(V), resulting in total arsenic concentration.

Dan Kroll et al. [102] investigated a safe, easy, and accurate process for determining arsenic levels in very low µg/l levels without using expensive equipment. In this study, sulfamic acid and zinc reacted to create a strong reducing agent where the inorganic arsenic compound was converted to arsine gas. In this process, H_2S did not interrupt because it oxidized to sulfate. The evolved arsine gas reacted with mercury bromide-impregnated test paper and formed a mercury /arsenic compound mixer. Its color changed from white to yellow.

Bermejo-Barrera [52] prepared gold nanoparticles for the determination of arsenic. Due to their excellent optical property, it was capable of detecting very low arsenic concentrations up to 2-20 µg/L by spectrophotometer.

1.4.1.1.2 Electrophoresis

Schlegel et al. (1996) developed a modification of high-performance capillary electrophoresis (CE) and capillary zone electrophoresis (CZE) with two detection systems, photometric and conductimetric, for arsenic speciation analysis. The CZE method demonstrated excellent suitability for separating different arsenic species. The photometric detector was able to detect As(III), As(V), DMA, p-aminobenzene arsenate (ABA), and phenylarsonate (PhAs).

Lin et al. (1996) presented an alternative CE method for the determination of As(III), As(V), MMA, and DMA. In this regard, a novel interface of a direct injector nebulizer (DIN) coupled with an ICP-MS detection system was utilized. The DIN interface was optimized independently from the CE system to provide an efficient sample introduction into the ICP-MS under different CE conditions. This approach combined the high separation efficiency of CE with the high elemental sensitivity of ICP-MS.

Magnuson et al. (1997) proposed a technique for the analysis of arsenic species, which involves capillary electrophoresis with hydrodynamically modified electro-diffusion flow. The detection is done using hydride generation inductively coupled plasma mass spectrometry (HG-ICPMS). The method is used to identify As(III), As(V), monomethyl arsenic acid (MMA), and dimethylarsinic acid (DMA).

Vanifatova et al. [103] developed a method for the determination of arsenite using CZE with direct UV detection. In this investigation, arsenite, arsenate, and dimethylarsinic acid were separated in a fused-silica capillary at 25 kV using a 20 mM phosphate buffer with a pH range of 7 to 10.8 as the electrolyte. Detection was carried out at 200 nm, and samples were injected hydrodynamically at the anodic end at 3.45 kPa for 50 seconds. Optimal results were obtained at a pH of 10.6, and the calibration graphs were linear for 0.35-2.5 mg/L As(III), 1-5 mg/L As(V), and 1.3-5 mg/L DMA, with detection limits of 0.15, 1.1, and 1.3 mg/L, respectively.

Tian et al. [104] utilized the movable reduction bed generation system as an interface for CZE and ICP-AES in arsenic speciation analysis. In this method, a sample volume of 400 mL was hydrostatically injected into a fused-silica capillary. Separation of As species was achieved using 50 mM phosphate buffer of pH 6 (buffer A) as the electrolyte under a voltage of 15 kV. The effluent from the CZE column was combined with a flow (10 µg/min) of buffer A before contacting the surface of a tape coated with metal borohydride/salt acid (1:3) housed in a glass chamber.

Dasgupta et al. [105] developed a fast and sensitive method for detecting arsenic at mg/L levels in drinking water using a photometric method based on light-emitting diodes (LEDs). The levels of individual arsenic species, including arsenic(III), arsenic(V), and total arsenic, were determined using reducing agent L-cysteine and oxidizing agent potassium bromate. The detection method involved the formation of arsenomolybdate and reduction to hetero-poly blue. Arsenic(III) in the sample was oxidized to As(V) with potassium bromate. This oxidized stream was mixed with color development solutions of ammonium molybdate, HCl solution, and ascorbic acid solutions in a color development reactor to produce a hetero-poly arsenomolybdate complex. A LED detector then detected the resulting molybdenum blue flow, and the detector output was processed using a PC-based system. This method has a detection limit of 8 µg/L, which is lower than the guideline value for arsenic in potable water recommended by the WHO.

1.4.1.2 Portable methods

Many of these tube-wells were eventually discovered to have arsenic-contaminated water. The World Health Organization, the United Nations Children's Fund, and the World Bank all agreed to address the issue. Since then, millions of dollars have been invested in the manufacture of inexpensive and portable field kits for arsenic detection. Many of the introduced arsenic tests were established to fulfill the Bangladeshi MCL of 50 µg/L for arsenic.

Using these arsenic field kits, nearly one million tube-wells have been evaluated so far. Wells whose arsenic levels were below 50 µg/L were painted green, and the water was deemed "safe" for human consumption. Wells with arsenic levels exceeding this threshold were labeled as "unsafe" and painted red; the majority of wells tested were considered unsafe [62]

All of the test kits used in these original initiatives are based on a method called the Gutzeit reaction, which is 100 years old [106]. The kits work by first turning all of the arsenic in solution into the extremely toxic arsine gas. Then, the gas is mixed with mercuric bromide-coated paper to change the color. During the reaction, the paper strip turns different shades of yellow, depending on how many $–AsH_2$ groups are bound to the mercury.

Field kit evaluations: Due to the huge number of tube-wells yet to be tested for arsenic pollution, it is almost guaranteed that the vast majority of wells will be examined just once.

Consequently, the dependability of that single conclusion is of the greatest importance. In the late 2000s, numerous independent organizations set out to investigate the efficacy of the various test kits on the market. These research findings are somewhat problematic.

Studies reveal that the initial field kits have a high incidence of wrongly identifying wells as either false positive or false negative. Additionally, some of the original field kits are unreliable in detecting arsenic levels below 70 µg/L, and perceptions of "yellowness" vary considerably among people [62]. Certain experts suggest that well samples close to the 50 µg/L threshold should be frequently re-examined to ensure the accuracy of the measurement [87]. Furthermore, nearly half of the arsine gas generated is lost into the environment before detection, and this gas is more hazardous than arsenic in solution. There is a high risk of technicians being exposed to it [65]

Studies of test kits from the last few decades have not shown much improvement. It has been decided that none of them can be used to find arsenic levels of 10 µg/L and that 20 µg/L is the lowest amount that can be found [107] [108]. In some cases, faster reaction times have been linked to better accuracy [61]. In other cases, the accuracy of the methods has been improved by making the reaction times even longer and taking pictures of the test strips with a flatbed scanner [109]. According to a study conducted in Nebraska, USA, the Quick Arsenic and Hach EZ kits were found to be suitable for regular use [88]. The study, which utilized the US Environmental Protection Agency (EPA) standard of 10 µg/L of arsenic in solution, revealed that water samples containing 15 µg/L or more of arsenic were accurately identified as exceeding the Maximum Contaminant Level (MCL). Based on these results, the authors conclude that these field kits provide a viable means of detecting and resolving arsenic contamination. However, it is worth noting that this viewpoint is not widely shared, as most evaluations of field test kits using the Gutzeit method have found them to be unreliable for testing near the 10 µg/L threshold for arsenic toxicity.

Issues with existing field kits: Although there has been considerable research on portable arsenic detection for improved detection of arsenic in water, existing field techniques still lack the robustness and reliability required to accurately classify a water source as "safe" or "unsafe." To effectively monitor drinking water for arsenic contamination, it is important that an ideal arsenic sensor should possess the following five essential characteristics [110].

(a) The arsenic sensor must be sensitive and selective.

Even though the provisional MCL of arsenic in most developing countries is set at 50 μg/L, WHO insists to measure arsenic concentrations as low as 10 μg/L. To obtain an accurate assessment of the contaminated level, it would be beneficial to obtain quantitative measurements across a range of arsenic concentrations. Given that arsenic is typically present in water in trace amounts, other potentially interfering species may be present at much higher concentrations. Thus, it is critical to ensure that the sensor is specific to arsenic. Furthermore, it is desirable to be able to distinguish between various arsenic species, since their bioavailability and toxicity depend on the form in which they are present.

(b) The arsenic assay must proceed quickly and yield reproducible results

To effectively test millions of tube wells in countries like Bangladesh, a high-throughput assay is necessary. It is vital that every sensor generates data that are accurate and reproducible, as there will only be a single test performed on the majority of wells before they are colored green or red.

(c) The arsenic kit must be fully portable and robust enough for field use.

All assays should be performed at the source. This eliminates complicated sample labeling and handling and improves local knowledge of the monitoring process. For effective field use, the sensor and its associated reagents/components must be robust.

(d) The arsenic detection process must reduce health and environmental risks.

Arsenic monitoring prevents arsenicosis and arsenic-related malignancies. Chemical procedures should limit technician exposure and convert arsenic to less harmful forms. Testing millions of tube-wells would generate a considerable volume of chemical waste; the toxicity of these waste-products should be avoided to protect the environment.

(e) The arsenic monitoring plan must be affordable and easy to implement for the local population.

The average cost of a test with today's field kits is less than one dollar. This is an appropriate price point to aim for when marketing goods to consumers in underdeveloped countries. The ideal field sensor should be user-friendly enough to be operated by well owners or local technicians with minimal training. This will make it possible to install the sensor at an affordable cost.

1.4.1.2.1 Colorimetric methods

Paper-based methods have been used in analytical detection from many years. The first recorded history of paper-based colorimetric detection was in the 1st century AD.

The old and well-known spot test technique of filed samples for arsenic detection at the µg/L level is Gutzeit’s Method [111]. It involves the formation of a colored complex due to the formation of arsine by the reduction of arsenic under acidic conditions, either with mercuric bromide [112] or with silver diethyldithiocarbamate. The main advantage of this method is the replacement of expensive instruments, and it can be used as for on-site detection kit of arsenic. Also, the technique is sensitive, rapid, and can be performed easily. But, HCl was used in this method, which is highly corrosive, and also there are handling and transportation problems.

The very familiar Merck kit (Mumbai, India) (with the help of Gutzeit’s method: Merch arsenic test method: colorimeter with test strips, the limit of detection was 0.005-0.5 mg/L As(III) and As(V)) and Hach kit (Loveland, co, USA) [113] are commonly used all over the world for arsenic detection capability of 5-500 mg/L of arsenic and bath of them use solid sulfamic acid in its place of concentrated hydrochloric acid.

Some basic studies that are available in the literature based on Gutzeit's reaction are summarized in Table 1.1**:** Comparison of the performance characteristics of several portable arsenic test kits implementing the Gutzeit reaction It provides a summary of the performance characteristics of different portable arsenic test kits that utilize Gutzeit's reaction for detection adapted from Nevetha et al. [114]. For each method, the table includes information on the theoretical and practical limits of detection, reliability, cost, time required per analysis, required skill level, and type of data generated. If certain information is not available in the literature, the corresponding field is left blank.

Baghel et al. [113] reported the production of arsine gas by reacting magnesium turnings with oxalic acid, and this arsenic reduced auric chloride into metallic gold, which appeared as a

Table 1.1: Comparison of the performance characteristics of several portable arsenic test kits implementing the Gutzeit reaction.

	Portable arsenic test kits based on the Gutzeit's reaction							
	NIPSOM	Merck	GPL	AAIH&PH	AAN	Quick As	Hach EZ	Arsenator
Theoretical LOD (μg/L)	10	10	10	50	10	5	10	0.5-2
Practical LOD(μg/L)	>20	>50	-	>50	>20	-	-	-
Reliability.	Unreliable <70 μg/L Weak concordance with lab results	<70 μg/L. Unreliable	Unreliable <70 μg/L.	Unreliable <70 μg/L	Unreliable <70 μg/L.	Responsible >15 μg/L of arsenic, which is not up to the WHO limit	Responsible >15 μg/L of arsenic, which is not up to the WHO limit.	Reliable in 85% of measurements, Higher sensitivity at <μg/L
Cost (USD)	$0.40	$0.50	$0.40,	$0.40,	$0.40,	$1.00–2.00	<$1.00–2.00	$1.00,
Time (min)	5	30	20		30		20-40	20
Technician capabilities	Sensitivity towards yellow color; working rapidly.	Sensitivity towards yellow color ; working rapidly	Sensitivity towards yellow color ; working rapidly	Sensitivity towards yellow color; working rapidly	Sensitivity towards yellow color; working rapidly	Sensitivity towards yellow color; working rapidly	Sensitivity towards yellow color; working rapidly	Ability to make accurate dilutions
Data type	Array of color degrees	Array of color degrees	Array of color degrees	Array of color degrees	Array of color degrees	Array of color degrees	Array of color degrees	Digital output

pink-violet color. Still, the use of expensive auric chloride showed the major disadvantage of the process. A rapid colorimetric method for arsenic detection was developed. Different reagents with magnesium turnings and a series of acids were tested for an arsenic generation. After that, arsine was allowed to react with gold chloride on Whatman filter paper which showed a color change. It was done that the detection time and detection limit were measured for each acid and found that the oxalic acid was found to be appropriate for all the acids used. It took 10 min to detect 10 µg/L arsenic concentration and only 1 min to detect a concentration higher than 50 µg/L. Several experiments were performed with different acids, and all the acids were also tested for the blank test. Different acids produced distinct colors for the arsenic present in a water sample. The results are summarized in ***Error! Not a valid bookmark self-reference.***.

Joyati et al. [115] successfully demonstrated two user-friendly methods for the detection of arsenic in drinking water. In this study, two kits were prepared (kit –I and kit-II) for arsenic detection with several advantages over existing kits in the market. Both of them could detect the presence of arsenic in water at low concentrations as 10 µg/L of total arsenic. It could detect arsenic in water within 7 min, which is lower than other kits (Hack Kit, Merck kit), timing like 20-30 min. Mercuric bromide and silver nitrate were used in the kit –I and kit-II, respectively.

Nath et al. [116] developed the best sensor for arsenic detection using simple portable paper, which used modified gold nanoparticles for reaction. A Y-shaped paper-based design was made. It is specific for A(III) and can detect arsenic concentrations less than 1 µg/L. The authors proposed using the modified gold nanosensor of Au–TA–TG, which binds to As(III) to produce a black-blue precipitate at the interface where the two species interact. In addition to low cost and portability, the technological advantage of having a paper substrate in this system is the facilitation of a slow, self-driven flow of the sample and Nanosensor flows, allowing for their reaction. This method is one of the best methods for detecting arsenic in water, but making and processing was a bit difficult.

Table 1.2: Arsenic detection limit, time, and color with respect to different acids.

Acid	Detection time (min)	Arsenic concentration (μg/L)	Color
Boric acid	15-20	200	No color
Nitric acid	5.0	200	Dull pink
Hydrochloric acid	5.0	50	Dull pink
Sulfuric acids	10.0	50	Pink-violet
Chloroacetic acid	15.0	200	Dull violet
Sulfamic acid	7.0	200	Violet color
Oxalic acid	1.0	>50	Sharp pink-violet
Oxalic acid	5.0	>10 to 50	Pink –violet
Oxalic acid	10.0	10	Pink -violet

Note: The blank test results for all the tests is no color changes on paper strip. Quantity used in reaction for nitric acid, HCl, and sulfuric acid is 2 ml, and 1 mg used for the remaining acids.

Das et al. [115] developed two test kits for detecting total arsenic in micrograms level in water by using mercuric bromide and silver nitrate as the detector elements. Kearns et al. [109] reported some improvement in the accuracy and precision of the Hach EZ test kit for quantifying inorganic arsenic concentration in drinking water by using digital analysis of the color developed on the detector strip. The digital analysis produced fewer false positive and false negative results. Furthermore, running the test process for 24 hr made the procedure time-consuming. The arsenator digital arsenic test kit commercialized by the watch, plaintext Ltd, United Kingdom, is also a very well-known digitized arsenic detection kit available in the market, but the measurement is costly by this kit.

Okazaki et al. [117] developed a portable device equipped with two attachments of a $CaCO_3$ cartridge and a molybdenum-loaded membrane holder to develop visual colorimetry for the determination of trace arsenic in water. Even though the minimum detection limit was 5 µg/L, the use of a $CaCO_3$ cartridge along with membrane filters made this process costly. Up to- date, no report is available on the use of a dipstick colorimetric for As(V) and the performance evaluation by digital analysis.

Kiso et al. [118] detected the arsenic content in the sample using molybdoarsenic heteropoly acid resulting from the reaction of arsenate and molybdate species. Sodium dichlorocynauric acid was used for oxidation of As(III) to As(V) to get total arsenic content. A small column packed with quaternary ammonium salt-coated PVC particles was prepared. The column was used as a detection tube. The ammonium salt in the column reacted with a heteropoly acid to produce a color band of molybdenum blue inside the tube. The length of the color band correlated linearly with total arsenic content using standard solutions.

Molybdenum blue: The molybdenum blue assay is another arsenic detection method like colorimetric. This test produces the colored hetero poly-acid ion product based on the reaction between arsenate and molybdenum. The molybdenum blue reaction involves passive flows and mixing, and these methods can detect in the range of 1–15 µg/L. The main drawbacks of this method are the bulkiness of instrumentation, the need for high temperatures, and complex sampling handling steps.

The use of molybdenum for arsenic detection may yield negative results due to phosphate interferences in the reaction. However, a different method has been developed to address these interferences, which involves the separation and independent analysis of penta- (pKa ~ 2.2) and trivalent (pKa ~ 9.2) species in solution, as described by Dasgupta et al. [105]. This method allows for the detection of arsenic in the range of 4 to 8 µg/L despite the presence of phosphate interferences.

The use of methylene blue, a cationic organic dye, in direct interaction with arsenic is an advanced colorimetric method for arsenic detection, as described in studies by Ghosh et al. [119] and Kundu et al. [120]. When reduced by arsine gas, methylene blue becomes colorless, allowing for the detection of arsenic in the range of 10-100 µg/L with a detection time of approximately 6 minutes.

Another colorimetric method for arsenic detection is presented by Sharma et al. [121] using sulfanilic acid and N-(1-naphthyl) ethylene diamine hydrochloride (NEDA). In this method, arsenic (III) in solution first reduced the sulfanilic acid, resulting in a product that then reacted with NEDA to produce a magenta-colored product. The entire process was carried out on a disposable thin-layer chromatography (TLC) strip, and the reaction was imaged using a color scanner. MATLAB was then used for the quantification of the detected RGB values. This method had a detection time of 30 minutes and could detect arsenic at concentrations as low as 18 μg/L.

Johnson [122] developed a technique to determine the As(III), As(V), and P(V) concentrations in water samples. Solutions of sodium metabisulphite and sulfuric acid were used for reduction, and sodium thiosulphate solution for successive oxidation of arsenic in the sample. A solution of color development reagent, ammonium molybdate, was added to the prepared sample, reduced sample, and successively oxidized sample. The color-developed samples were analyzed using a spectrophotometer. Concentrations of As(V)+P(V), P(V), and As(III)+As(V) were calculated from absorbance spectra. The method was found to be sensitive to 0.01 µmol/L concentrations.

Lenoble et al. [123] performed the molybdenum blue method for the speciation of arsenite As(III) and arsenate As(V). $KMnO_4$, H_2O_2, NaOCl, and $FeCl_3$ solutions were used to oxidize the arsenite to arsenate in favorable conditions. Color development reagent was prepared with ammonium molybdate solution, potassium antimonyl tartrate solution, and sulphuric acid. Ascorbic acid solution and MnO_2(S) were also prepared. After oxidation and color development of the samples, Arsenio-molybdate complex formed in less than an hour at 20^oC. The absorption peak at 870 nm was observed in the spectrometric analysis, which indicates the presence of As(V). Only $KMnO_4$ and $FeCl_3$ were found promising for oxidation. The detection limit for this method was found to be 20 µg/L.

Dhar et al. [124] improved the calorimetric method developed by Johnson and Pilson to measure arsenic concentration in the range of 0.03-5.3 µmol/L. The optimized method involved increasing the concentrations of reagents used for sample pre-treatment and color development, namely potassium iodate, ascorbic acid, and antimonyl tartrate. The results demonstrated a high level of accuracy, with a reported accuracy of 97±0.5% for the method.

Sounderajan et al. [125] performed the characterization of arsenic(V) and arsenic(III) using the well-known formation of hetero-poly acids when arsenic(V) is reacted with ammonium molybdate in aqueous solutions. Butane was used to extract the heteropoly acid formed from the aqueous solution with the addition of 2 M nitric acid. These extracted samples were characterized using graphite furnace atomic absorption spectroscopy. An amount of 20 mg of palladium were added to the sample extracts as a modifier, and an optimized furnace program was used for the analysis. The recovery and limit of detection of arsenic were found to be >90% and 0.2 µg/L, respectively.

Tsang et al. [126] conducted a quantitative analysis of arsenate and phosphate dissolved in water by modified molybdenum blue method. The method was found to be fast and accurate. This was done by optimizing the composition of the solutions used for color development, namely potassium antimony tartrate, ammonium molybdate, and sulphuric acid. Sodium dithionite was used for the selective reduction of arsenate and analysis of phosphate content. The sequence developed by Johnson and Pilson was used for the speciation of arsenite and arsenate in an aqueous solution. A dual-beam spectrometer was used to analyze the color-developed sample. The limit for calorimetric analysis of arsenic concentrations was about 1 µg/L.

Sharma et al. [127]developed a disposable sensor with present techniques (MATLAB software) for the quantitative detection of arsenic. But the lowest detection limit of this process was 18 µg/L, and unfortunately, it was more significant than MCL of arsenic in groundwater (10 µg/L) as guided by the WHO. Therefore, a significant opportunity is there for the development of a low-cost and environment-friendly simple colorimetric detection of arsenic in groundwater samples.

1.4.1.2.2 Electrochemical methods

Compared to colorimetry, electrochemical methods have an advantage in miniaturization due to their ability to generate electrical signals that can be easily measured even at low analyte concentrations, making them well-suited for small sample sizes. Several electrochemical techniques have been used for arsenic detection, with stripping voltammetric methods being preferred for their ability to pre-concentrate analytes in situ. Anodic stripping voltammetry (ASV) using platinum and gold electrodes and cathodic stripping volumetry (CSV) using a glassy-carbon electrode are examples of such methods. ASV, which has been utilized for over

40 years, can detect arsenic at concentrations as low as ng/L, but requires pre-dilution of samples, regeneration of electrode surfaces, and the use of costly electrodes. The ASV process involves the pre-concentration of arsenic onto the electrode surface via electrodeposition, followed by oxidation of the plated metal using an anodic linear potential sweep, resulting in high sensitivities [128].

Screen Printed Electrodes (SPEs) are another electrochemical method for arsenic detection that can handle challenges in electrochemical [129], [130]. Adapted from microelectronics, screen printing technology allows for the reproducible mass production of inexpensive single-use sensors. The sensitivity of this technique is in the order of µg/L, and the detection time depends on the metabolic rates of enzymes. However, the handling of this technique can have an impact on the environment and human health.

In 1980, Holak [131] developed a CSV procedure for arsenic detection in acidic solutions containing Se(V) using a hanging mercury drop electrode (HMDE). This method offered advantages over solid electrodes and was widely used in routine stripping voltammetry. However, copper interference was a significant issue when using this technique.

Sadana [132] developed a method for determining arsenic in portable water using differential pulse cathodic stripping voltammetry (DPCSV) with a hanging mercury drop electrode (HMDE) as the working electrode and Ag/AgCl as the reference electrode. The optimal analytical conditions included using 0.75 M hydrochloric acid, a Cu^{2+} concentration of 5±1 µg/ml, and a deposition potential of -0.6 V. The method had a lower contamination limit of 1 ng/ml. To determine the total arsenic in water, As(V) was reduced to As(III) through heating for 45 minutes, after which the solution was cooled to room temperature and diluted with a 0.25% (w/v) hydrazinium chloride solution before undergoing CSV analysis.

Henze et al. [133] conducted a study on the speciation of As(V) and As(III) in freshwater samples using CSV. In their procedure, a 10 ml water sample was mixed with 100 µL of hydrogen peroxide and 50 µL of concentrated sulfuric acid and then digested in a UV digester to remove organic material. The sample was subsequently analyzed by CSV using a hanging mercury drop electrode, a double junction Ag/AgCl /3 M KCl reference electrode and a glassy-carbon auxiliary electrode. The method was able to detect As(V) and As(III) at concentrations as low as 0.52 and 0.93 µg/L, respectively.

In 2004, Cui and colleagues created a modified glassy carbon electrode (Au NPs/Fe_3O_4/GCE) using gold nanoparticles and magnetic Fe_3O_4 nanoparticles to detect arsenite ions. The modified glass carbon electrode (GCE) was formed by depositing iron oxide nanoparticles and then electro-depositing gold nanoparticles. Arsenic(III) was detected using square wave anodic stripping voltammetry. The modified GCE displayed remarkable reproducibility and stability, with a detection limit of 0.00097 µg/L under optimal conditions.

Salimi et al. [134] developed a new sensor based on cobalt oxide nanoparticles to detect trace amounts of arsenic in the form of arsenite ion (As(III)) in an aqueous solution. Cobalt nanoparticles were deposited on the surface of the glassy carbon electrode using cyclic voltammetry in the potential range of -1.1 to 1.1 V from the $CoCl_2$ buffer solution (pH 7). The modified glassy carbon electrode showed high catalytic activity towards arsenic oxidation in a wide pH range of 5-11. Cyclic voltammetric analysis (-1.1 to +1.1 V) and hydrodynamic amperometric analysis at 0.75V against Ag/AgCl reference were performed for the response of arsenite ion on the modified glassy carbon electrode. No reduction was found in the performance of the modified electrode stored in ambient conditions for 30 days. The modified glassy carbon electrode offers many advantages, e.g., simple preparation procedure, better reproducibility, excellent catalytic activity, and negligible interference. The limit of detection for arsenic was found to be 1.1 µg/L for the developed sensor.

Rajkumar et al. [135] studied the glass carbon electrode (GCE) and indium-tin-oxide glass electrode by fabricating the surface of electrodes with gold-crystal violet nanoparticles using cyclic voltammetry. Samples were analyzed using differential pulse voltammetry. Characterization peak for arsenic(III) reported at 0.39V using Au-CRV film-modified glassy carbon electrode in phosphate buffer solution (pH 7.0). Limit of detection and sensitivity of the sensor for detection of As(III) was reported to be 36.0 µg/L.

Li et al. studied the detection of arsenic(III) with mercapto ethylamine-modified Au electrode fabricated in neutral media, reported as rapid and sensitive. The modified sensor was reported to offer a wide range of detection of 0.2-300 µg/L. The sensor was found to be advantageous over Cu(II) interference. The arsenic(III) characterization peak at -0.17 to -0.13 As(V) was observed for 0.2 to 300 µg/L concentrations in differential pulse anodic stripping voltammetry (DPASV) response at pH 7.0.

Gao et al. [136] demonstrated that electrochemical methods, such as stripping voltammetry analysis, provide fast and accurate analysis of samples and can detect trace levels of elements. To detect arsenic(III) in the sample, a screen-printed carbon electrode modified with Fe_3O_4-RTIL was used for SWASV. Fe_3O_4-RTIL composites were prepared by ultrasonically agitating RTIL and Fe_3O_4 with 100% alcohol. The Fe_3O_4-RTIL composite film on SPCEs was utilized as the electrode for the anodic stripping of arsenic. The use of RTIL improved the electrode's sensitivity due to its excellent conductivity. However, their method could only detect the presence of As(III) and not As(V).

1.4.1.2.3 Biological methods

Water organisms have the ability to accumulate arsenic and can facilitate the conversion of arsenic to arsenate. Biomethylation reactions can also convert arsenite to arsenate, and certain diatoms can reduce arsenate to arsenite and dimethylarsinic acid. Bacteria and fungi can methylate arsenic compounds, resulting in the production of dimethyl and trimethylamine by replacing oxygen atoms with methyl groups. Methylation is believed to be a detoxification mechanism for these microorganisms and plays a crucial role in the transfer of arsenic from sediment to water and the atmosphere.

Several biological sensors have been developed in a strip-based format for field applications [137]–[139]. One such method involves a paper-based colorimetric detector that utilizes X-gal as a substrate for a β-galactosidase reporter. The paper strips contained pre-dried bacterial cells on their surface, and when exposed to an arsenic-containing solution, color development occurred as β-galactosidase digested X-gal, resulting in a loss of blue coloration. The extent of this change is proportional to the concentration of arsenic in the solution. Another β-galactosidase-based sensor has been developed for arsenic detection, utilizing pH detection. In addition, the ArsoLUX is a portable digital system for the bioluminescence detection of arsenic. This method involved introducing an arsenic water sample into a vial containing live, lyophilized bacteria with the lux reporter gene and measuring the luminosity 10 seconds after sample introduction. The ArsoLUX is sensitive and quick, with performance comparable to the Arsenator and Merck field kits. However, it requires a start-up cost of 4000 € and involves fewer steps and less reagent consumption. Field tests conducted in Vietnam have demonstrated its efficacy.

Microfluidic devices have become increasingly popular for portable bacterial detection, surpassing the convenience of strip-based kits in some cases. Various microfluidic geometries have been designed for arsenic bioassays. For instance, Rothert et al. [140] developed a PMMA compact disk microfluidic biosensor that utilizes centrifugal force for sample flow and mixing. Unlike typical microfluidic designs that rely on external pumps for sample introduction, the disk-shaped design generates outward centrifugal force to introduce the sample on-chip. After mixing, the bacterial GFP response is detected by fluorescence microscopy. Using a large product collection reservoir can enhance the system's portability by enabling fluorescence imaging with a regular camera. Another example is a closed, single-use chip proposed by Theytaz et al. [141] . This channel geometry included a filter to immobilize bacteria while the arsenic solution flows through. The captured bacteria were then exposed to the arsenic solution for a fixed duration, and the response was quantified. Although this technique can detect a 50 µg/L arsenic threshold limit.

Siegfried et al. [142] conveyed filed testing of arsenic using lyophilized bacteria. Further, developed a procedure to detect arsenic through the adsorption of DNA by iron oxide nanoparticles. However, these methods were time-consuming.

Song et al. [143] investigated gold nanoparticles in a new biosensor as Au@Ag core-shell NPs to detect the presence of arsenic. The biosensor contained seed-grown Au@Ag core-shell NPs with SERS used for analysis. The biosensor had a good correlation with the arsenic concentration in the range of 0.5 to 10 µg/L. The developed biosensor was able to detect only As(III), not the total arsenic concentration.

Yuanli et al. [144] investigated a biosystem that can trace inorganic arsenic in water with the help of a highly sensitive enzymatic catalysis system. This had capable of detecting arsenic at low-level concentrations, as per the World Health Organization. The use of an enzyme that produced fluorescent NADH in the presence of arsenate enabled the detection of arsenate at concentrations ranging from 0-200 µg/L. This approach had the potential for integration with automated analytical sampling systems to enable accurate determination of arsenic levels in environmental health applications.

Gudlavalleti et al. [137] introduced a novel bio-electronic sensing approach using EGFP to develop a cost-effective, battery-powered system for measuring the fluorescence emitted by a biosensor strain and displaying the corresponding arsenic concentrations in water samples. The

EGFP was excited by the blue light of 480 nm, resulting in a green fluorescence signal proportional to the arsenic concentration. The device consisted of a compact embedded system that measured the fluorescence signal using a light sensor, programmable controllers, and a liquid crystal display showed the arsenic concentration in µg/L. The experimental results demonstrated that this system could detect and display concentrations within the 5 to 100 µg/L range.

Bonyar et al. [145] devised a colorimetric device to detect arsenic in drinking water that utilizes a commonly used reagent kit in arsenic field-testing. The device is capable of detecting total inorganic arsenic ion concentrations (As(III) and As(V)) with a linear response range of 5-20 µg/L, which is lower than the WHO limit. The obtained arsenic concentration values were stable and reliable after 60 minutes at 22°C (ambient temperature) and after only 25 minutes at 50°C water sample temperature, but with an increased relative standard deviation of up to 16% within the linear range.

Priyanka et al. [139] developed a sensor for detecting arsenic(III) using lead sulphide nanoparticles that were biologically synthesized. The lead sulphide nanoparticles have excellent absorbance properties, allowing for detection of As(III) up to a concentration of 50 µg/L, provided that the pH level of the sample is maintained at 9.0 for optimal results.

Table 1.3 presents a comparison of the performance features of different portable methods for arsenic detection, against the criteria for a best arsenic sensor. The methods are evaluated based on various factors, including sensitivity and selectivity, speed and reproducibility, portability and robustness, health and environmental risks, as well as affordability and ease of implementation. The table summarizes how each candidate method performs with respect to these criteria, organizing the characteristics of each method accordingly.

Table 1.3: The table provides a general comparison of different methods that can be used for arsenic detection, as compared to the criteria for an best arsenic sensor that are described in section 1.4.1. The methods are evaluated based on their sensitivity and selectivity, speed and reproducibility, portability and robustness, potential health and environmental risks, and affordability and ease of implementation.

	Qualities of an optimal arsenic sensing device					
	Sensitive and selective	Quick and reproducible	Portable and robust	Low health and environmental risks	Inexpensive and easy	Refreance
Colorimetric methods						
Molybdenum blue (PFI)	LOD between 1 to 15 µg/L Selective about P and Si	Detection time: above 30 min; Good reproducibility	The instrument is not portable and is bulky	AsH_3 gas generation causes poisoning.	Expensive Requires some specialized	[146]–[148]
Molybdenum blue (pKa effects)	LOD between 4 to 8 µg/L; Only accuracy is 20% at ng/L	Detection time: 7 to 10 min	potential to be made portable	Yes; As a continuously in solution	Economical; Easy setup	[105], [112]
Molybdenum blue (ethyl violet)	LOD between 10 and 25 µg/L. interference from phosphorus (P),	Detection time: ~30 min	potential to be made portable	Yes; As a continuously in solution	Economical;	[149], [150]

	silicon (Si), and fluorine (F).					
Methylene dye	LOD between 10 and 100 µg/L	Detection time: ~ 6 min	potential to be made portable	AsH_3 gas generation causes poisoning, but As continuously in solution	Economical;	[119], [151], [152]
Sulfanilic acid NEDA	LOD about 18 µg/L; Selective about phosphorus (P)	Detection time: ~30 min	Portable- Must prohibit	Yes, As continuously in the solution	Economical;	[121]
Paper-based	LOD: 1 µg/L Selective about phosphorus (P)	Very Quick and Consistent	Portable	Yes	Expensive	[153]
Electrochemical methods						
ASV (traditional)	Copper (Cu) interference can affect sensitivity at the ng/L level.	Require sample pre-dilution and electrode surface regeneration	Portable- Must prohibit; electrodes are brittle	Yes; As continuously in solution;	Expensive to fabricate electrodes- Relatively simple	[154]–[157]

				Require very small volumes of sample		
ASV (enzyme-modified)	sensitivity in the range of μg/L and is tolerant of copper (Cu).	Detection time function of enzyme metabolic rate; Detection of bioavailable As	Portable- Must prohibit; electrodes are brittle	Yes; As continuously in solution -Require very small volumes of sample	Expensive to fabricate electrodes; Relatively simple	[158]–[160]
SPE	sensitivity in the range of μg/L; Tolerant of Copper (Cu)	Detection time function of enzyme metabolic rates; Detection of bioavailable As	Portable	Yes; As continuously in solution -Require very small volumes of sample	Economical;	[161], [162]
Biological methods						
Strip-based	Sensitivity in the range of μg/L and is	Detection time function of	Semi portable; only Strips are portable;	Yes; As continuously in solution	Economical;	[163]–[166]

	highly selective for arsenic (As).	enzyme metabolic rates; Detection of bioavailable As	Sensitive to room conditions	-Require very small volumes of sample		
Continuous flow microfluidics	Sensitivity in the range of µg/L and is highly selective for arsenic (As).	The detection method is capable of identifying bioavailable arsenic (As).	Portable- by potentially; depends on other equipment to function; Sensitive to room conditions	Yes; As continuously in solution; Require very small volumes of sample	Disposable; Requires some specialized skills	[140], [163], [167]–[169]
Electrophoretic methods						
ITP -	Sensitivity in the range of mg/L; Selective about Se; Interference from Nitrate, Carbonate, and Phosphate	Detection time: <10 min and Consistent	Potentially portable depends on other equipment to function.	Yes; As continuously in solution; Require very small volumes of sample	Requires some specialized skills	[170], [171]

CE -	Sensitivity in the range of µg/L (AFS detection) As (III)/As (V) resolution	Detection time: <1 min and Consistent	Potentially portable: depends on other equipment to function	Yes; As continuously in solution; Require very small volumes of sample	Expensive; Requires some specialized skills	[172]
Surface sensing methods						
SPR	LOD: 1–15 µg/L; Selective over P	Very Quick and Consistent	Potentially Portable; depends on other equipment to function	Yes; As continuously in solution; Require very small volumes of sample	Disposable Fabrication of modified surfaces is difficult - Involves some specialized abilities	[173]–[175]
SERS	LOD: 1 µg/L Very selective for As (III)/As (V) resolution	Very Quick and Consistent	Potentially Portable; Selective to Raman spectrometers	Yes; As continuously in solution	Expensive; Relatively simple procedure	[176]

				Require very small volumes of sample		
Spectroscopic methods						
LIBS	LOD: 100 μg/L	Arsenic (As) attachment to the substrate needs time	Potentially portable	Yes; do not evolve to generation of poisoning AsH_3 gas.	Easy setup	[177]
XRF	LOD: 0.7 μg/L	Arsenic (As) attachment to the substrate needs time	Selective to XRF detectors are portable	Yes; do not evolve to generation of poisoning AsH_3 gas	Easy setup	[178], [179]
CL	LOD: 2 μg/L Results reliability with ICP MS data	Reaction time: ~1 min; Very Quick and Consistent	Some are Portable	AsH_3 gas generation causes poisoning	Relatively simple procedure	[180]

1.5 Possible scope for further research

As mentioned in the preceding text, colorimetric methods, among various techniques, are the most effective for detecting heavy metals in water. However, specific critical factors must be met when it comes to onsite detection and measuring the concentration. These factors encompass sensitivity, selectivity, portability, user-friendliness, rapid response time, and cost-effectiveness. To meet these requirements, acquiring suitable POCT digital sensors becomes imperative. Ensuring practicality and efficiency in real-world applications, these criteria play a pivotal role in determining the success of the POCT digital sensor system.

1.5.1 Need for POCT devices for heavy metals detection

In recent years, there has been a significant increase in the development of Point-of-Care Testing (POCT) devices for heavy metal detection. Particularly in drinking water resources such as wells, rivers, etc., and industries such as mining, manufacturing, agriculture, etc., heavy metals can pose a significant risk to human health and the environment. So using POCT devices for heavy metal detection is essential in ensuring the safety of our water resources and protecting human health and the environment. POCT devices are portable, easy-to-use devices that provide quick and accurate results onsite, allowing for rapid detection and monitoring of heavy metal contamination. These devices can help reduce the time and cost associated with laboratory testing and the risks associated with transporting samples to a central lab for analysis.

Heavy metals such as arsenic, lead, chromium, mercury, and cadmium are toxic and can accumulate in the human body over time, leading to serious health problems. In addition, these metals can leach into the environment and contaminate soil, water, and air, posing a risk to plants, animals, and ecosystems. POCT devices for heavy metals detection can be used for a range of applications, including soil and water testing, food safety monitoring, and occupational health and safety. They can identify potential contamination sources, track changes in heavy metal concentrations over time, and inform decision-making around remediation and risk management strategies. POCT devices play an important role in water quality monitoring, particularly in detecting contaminants that can pose a risk to public health.

These devices can provide rapid onsite testing of water samples in remote or underserved areas. Developing and deploying POCT devices for heavy metal detection is essential to ensuring a safe and healthy environment for all. Overall, using POCT devices for water detection has several benefits, including improved public health outcomes, enhanced environmental protection, and increased accessibility to water quality testing in remote or underserved areas.

1.5.2 Digital sensors for heavy metals detection in water

Monitoring water quality is vital for several reasons, including:

(1) Protecting public health: Water is essential for human life, and the water we consume must be safe and free from harmful contaminants such as heavy metals, bacteria, viruses, and chemicals. By monitoring water quality, we can detect the presence of contaminants and take action to ensure that the water is safe for human consumption.

(2) Protecting aquatic life: Water quality is also important for the health and survival of aquatic ecosystems. Contaminants such as heavy metals, pesticides, and nutrients can harm fish, plants, and other organisms that live in or depend on water ecosystems.

(3) Ensuring sustainable use: Water is a finite resource, and we must use it sustainably. Monitoring water quality can help us to understand how human activities such as agriculture, industry, and urban development affect water resources and make informed decisions about how to manage and use water sustainably.

(4) Compliance with regulations: Governments around the world have established rules and guidelines for water quality to protect public health and the environment. Monitoring water quality is essential to ensure these regulations are being met and identify areas where further action may be needed.

So, monitoring water quality is essential to protect public health, maintain healthy ecosystems, ensure sustainable use of water resources, and comply with regulations. By monitoring water quality, we can make informed decisions to protect the environment and promote human well-being.

For fulfilling the need to monitor water quality, digital sensors are the best among all the techniques. Digital sensors, on the other hand, are automated devices that can be easily installed and operated by non-experts. They use various technologies such as electrochemical, optical, or thermal techniques to detect the presence of heavy metals in water samples.

When a water sample is collected and passed over the sensor, the sensor detects the heavy metal ions. It generates an electronic signal proportional to the heavy metal's concentration. The data from the sensor is then transmitted to a computer or other data collection device for analysis and interpretation.

Digital sensors can be integrated into water quality monitoring systems, continuously monitoring heavy metal concentrations in water. This is important because heavy metals can harm human health and the environment, and their concentrations can vary over time and location. By using digital sensors to monitor water quality, environmental scientists and public health officials can quickly detect changes in heavy metal concentrations and take action to protect public health and the environment.

1.6 Objective of the Thesis

Based on the state of the art presented in above sections, the PhD thesis targets the fulfillment of the following major objectives:

1) User-Friendly Point of Care Test Device for Detection of Arsenic in Potable Water: Prototype, Design, and Artifact
2) A Highly Sensitive Hybrid Digital Sensor for Room Temperature Arsenic Detection
3) A Photoresistor-Based Arsenic-Specific Digital Sensor for Rapid and Affordable Colorimetric Detection at the Point-Of-Care
4) Colorimetric Optical Analysis for Simultaneously Determining the Presence of Multiple Pollutants in Water

1.7 Organization of the Thesis

The PhD thesis is organized in six chapters. Precisely the following sections are discussed.

Chapter 1 addresses the existing literature and research on the chosen area of study. This typically includes an examination of prior research to identify gaps or limitations that the current study aims to address. Additionally, the chapter may outline the objectives and provide an overview of the organization and structure of the thesis.

Chapter 2 addresses the design and develop a portable, user-friendly and arsenic test kit that can detect arsenic in gas phase. Precisely it refers to (a) The detection of arsenic involves reducing arsenic compounds into arsine gas using a paper strip impregnated with gold chloride, which act as a sensor. A user-friendly prototype has also been developed, which is efficiently measuring the total arsenic concentration (10-500 μg/L) in water samples. (b) Characterization of paper sensor, using EDX, TEM. (c) The qualitative and quantitate study with filed samples. (d) After getting satisfactory results from the prototype, a POCT device has been designed and fabricated to measure the total arsenic present in water.

Chapter 3 describes the design and develop a highly sensitive hybrid digital sensor for room temperature arsenic detection. The approach involves several key steps: (a) the in-situ generation of hydrogen gas via the electrolysis of an aqueous medium, (b) optimization of the applied voltage by reducing the electrode spacing for generation of arsine gas, (c) generating the paper sensor, and (d) development of a smartphone app to quantify the color intensity of the paper sensor. Finally, App assessment is done under field samples.

A prototype of the hybrid green sensor has been developed as a point-of-care testing (POCT) device. The sensor has demonstrated high accuracy and reproducibility in measuring arsenic concentrations in soft drinks, fruit juice, and portable water. The reliability of the sensor has also been verified with field samples.

Chapter 4 elaborates on designing a digital point-of-care testing (POCT) device based on the optical sensor's principle. This can give fast analysis and accurate diagnostics. More specifically, this refers to (a) the liquid sensor was generated by modifying the Molybdenum Blue method for total arsenic in an aqueous and edible samples. (b) Characterization by UV-

Vis spectroscopy for confirming the formation of arsenocmolybdate complex, (c) design and fabrication of the digital sensor, (d) validation with field samples of water, vegetables and rice.

Chapter 5 describes the design and development of colorimetric based digital POCT device to detect multiple water pollutants simultaneously. The individual sensor is multiplexed here. Initially colorimrteic based liquid sensors was generated with optimized reactants for the targeted pollutants. The color intensity of the liquid sensors changes with the amount of targeted pollutants presents in the sample. The specific complex corresponding to the pollutants was conformed through UV-Vis spectroscopy. And further analyzed all the liquid sensors quantitatively under LDR sensor. The calibration plot shows the monotonic variation of LDR resistance with pollutants concentration. The LED/LDR sensor units are integrated with an LCD screen via an Arduino circuit to obtain the digital response of the multiplexed sensors.

Chapter 6 summarizes the conclusions obtained from the research output in this work. Eventually, based on the insights of the obtained research methodologies and data, the chapter also outlines possible directions for future work.

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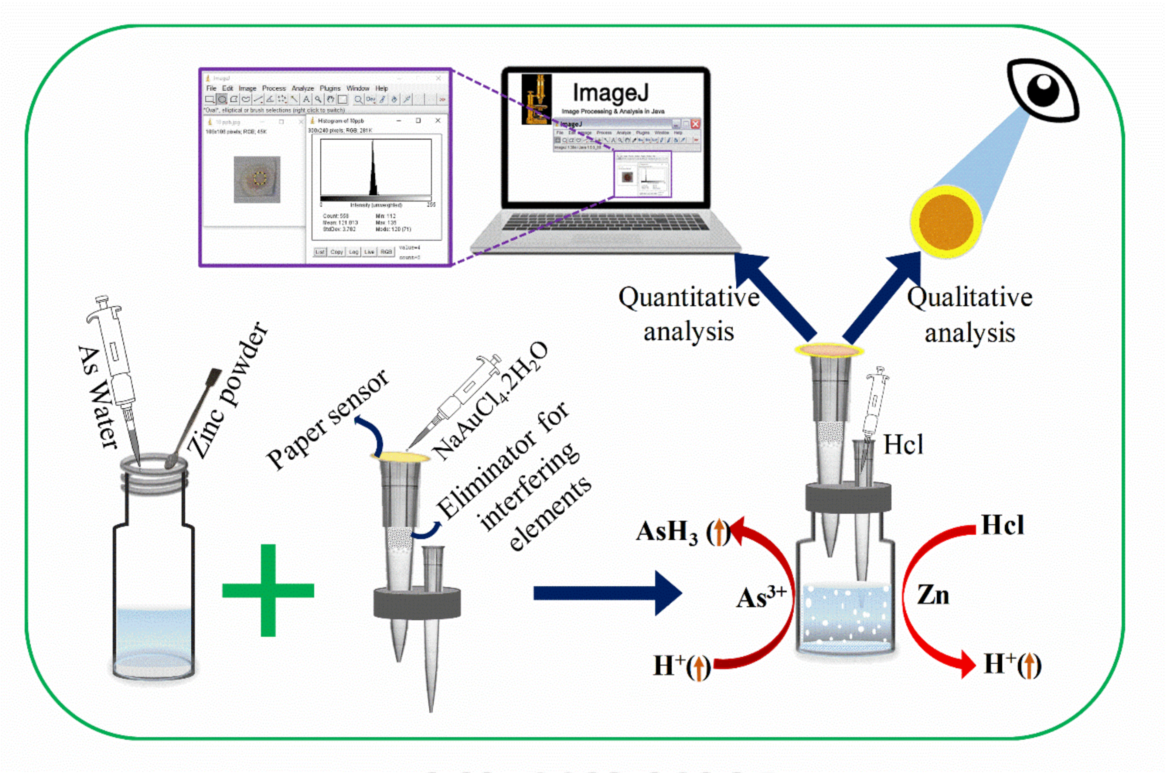
CHAPTER-2

User-Friendly POCT Device for Detection of Arsenic in Potable Water: Prototype, Design, and Artifact

Chapter 2 : User-Friendly POCT Device for Detection of Arsenic in Potable Water: Prototype, Design, and Artifact

Graphical Abstract



Abstract

Arsenic, a common pollutant, harms human health, and its WHO's permissible is 10 μg/L in drinking water. This research aimed to develop a paper-based point of care test (POCT) device for rapid detection of arsenic with a concentration of ~10 μg/L in potable water. In this, arsenic was converted to arsine gas, which was identified by a paper sensor by producing a characteristic color due to specificity of the reaction. The sensor was constructed by impregnating gold chloride ($NaAuCl_4$) on a paper strip. The device had a very good response time, nearly 4 min and 2 min for arsenic concentrations of (10-20) μg/L and above 20 μg/L (20-500) μg/L, respectively. The device also gave decent results for the field samples like river and groundwater. The device could be used for both qualitative and quantitative assays. ImageJ software was used for quantitative estimation, which accorded with AAS results.

Keywords: Paper sensor, Arsenic detection, Gold nanoparticles (AuNPs), POCT device, ImageJ analysis, Field samples

2.1 Introduction

In the world, thousands of people are affected by arsenic toxicity. Arsenic, a well-known carcinogen, is considered one of the world's most hazardous chemicals among heavy metals. The 2014-15 evaluation committee reviewed the presence of high arsenic in groundwater in a few states of India [1], [2] . Elevated concentrations of arsenic in groundwater in West Bengal and Bangladesh have become a key concern in recent years[3]. Arsenic is a metalloid material in a crystal form, brittle in nature and gray or tin-white in color. Arsenic occurs mainly in two forms, arsenite, As^{3+} and arsenate, As^{5+}. Arsenic occurrence in portable water is due to its natural existence in groundwater and on the surface, [4] or as a result of human activities such as disposal of industrial effluents without treatment, [5] use of pesticides, [6] leather, and wood treatments, [7] etc. The threshold value of arsenic contamination in potable water is 10 μg/L, as per WHO’s recommendation [8] [9]. The various health diseases may arise due to the consumption of arsenic polluted water, such as skin, lung, and bladder cancer, [10] pigmentation on the skin, skin hyperkeratosis, etc. For high precision arsenic detection, Atomic absorption spectroscopy (AAS), catholic stripping voltammetry (CSV), inductively coupled plasma mass spectrometry (ICP-MS), and spectrophotometry are used in laboratory [11]. Several years ago, Cortivo et al. [12] developed a spectrophotometric method for detecting arsine gas. In this study, detection of arsenic was done by converting arsenic into arsine gas using silver diethyldithiocarbamate dissolved in pyridine solution. Arsenic in food supplements was also detected by the cathodic stripping voltammetry (CSV) technique, in which a hanging mercury droplet was used as an electrode [13]. Anderson et al. [14] proposed a novel pneumatic nebulization PN/hydride generation (HG) inductively coupled plasma mass spectrometry (ICP-MS) method to measure the concentration of arsenic in liquid samples.

The above-mentioned spectrophotometric methods can detect the lower concentration of arsenic in portable water but are not suitable for on-site detection of arsenic. These techniques are extravagant, time-consuming processes and require well-trained laboratory individuals. Several researchers adapted the functionalized gold nanoparticle techniques to enhance the performance of the sensors. In this direction, bio/nano sensors play an important role. A thorough revision of these types of works was reported by Zong et al. [15]. They highlighted how bio-nano materials boosted the sensitivity and the lower limit of detection of the sensors. Thiol is a widely used compound for functionalizing gold nanoparticles as it gives high chemical compatibility. Thiol functionalized gold nanoparticles enhance the performance of various bio/nano sensors, namely Surface-Enhanced Raman Scattering (SERS), Electrochemical, and colorimetric. In this direction, Nath et al. [16] fabricated a paper-based microfluidic sensor employing Au–TA–TG sensor (gold nanoparticles (AuNPs), thioctic acid (TA), and thioguanine (TG)), which could measure the lower concentration of arsenic. Priyadarshni et al. [17] also constructed a paper sensor with a bio/nano composite (GNR-PEG-DMSA) consisting of gold nanorods (GNR), polyethylene glycol (PEG), and dimercaptosuccinic acid (DMSA), which is capable of detecting a low level of arsenic in a contaminated aqueous sample. These sensors can detect the lower concentration of arsenic in water, but the authors did not report the sensor's applicability to field samples. These sensors are extravagant, time-consuming processes, require experts and laboratory equipment to develop a paper sensor, and have a lower shelf life. In 2011, Sharma et al. [18] developed a use-and-through sensor kit for measuring the arsenic concentration on Thin Layer Chromatography (TLC) color paper empowered with MATLAB coding. The lower detection limit of this kit is 0.018 μg/L. On-site analysis of the produced color on TLC paper is a challenging task, and usage of the software makes the method complicated and time-consuming. Xia et al. [19] established a colorimetric analysis to detect arsenite in water by employing AuNPs and a phyto-chelatin-like peptide. This method is costly and restricted to laboratory research only. The field kits are used frequently to detect the degree of contamination of arsenic in groundwater [20]. Most of the field test kits available in the market have been developed based on Gutzeit methods [21] [22]. In this method, arsine gas (AsH_3) is generated by reducing the arsenic using hydrochloric acid and zinc [23]. The produced arsine can be quantified by capturing arsine gas either in a silver diethyldithiocarbamate solution [24] or on a paper impregnated with mercuric bromide, [25] both of the techniques function well for the arsenic. The main advantage of these field test kits is low detection time at lower arsenic concentrations. But the major disadvantage of these kits is the use of acids in the reaction, which is highly corrosive and leads to handling and transport issues. In addition, another important drawback of these field kits is a false-positive response due to the presence of antimony, phosphorus, and sulfur compounds in the sample. Many investigators have worked to establish new detection methods for arsenic. Magnesium turnings together with oxalic acid were used in one such attempt to generate arsine gas, which reduced auric chloride to metallic gold to map a color band [26]. Although this method's major shortcoming is the usage of auric chloride as a sensor element, which is very expensive. The Merck (Mumbai, India) kit is a widely used test kit for arsenic in groundwater across the globe, which can detect arsenic in the range of 5–500 μg/L. However, in Hach kit (Loveland, CO, USA), solid sulfamic acid is used instead of concentrated hydrochloric acid [26]. It can also detect arsenic in the same concentration level as Merck kit. These kits are costly, and the detection time of both kits is longer (mostly in the range of 20–30 min). Further, Kearns et al. [27] attempted to measure arsenic digitally and followed the digital image analysis technique for this. The foremost disadvantages of these digital kits are the exposure time (~24 hr), cost of the device, and dependency on the use of computers. Recently, Das et al. [28], [29] developed two user-friendly kits to fulfill the demand of low cost. The kits can detect arsenic concentrations of the field samples in the range of 10–250 μg/L, and they claimed 7 min as the detection time. The usage of mercuric bromide, silver nitrate, and strong acids, all hazardous, pose health risks, and pollute the environment, is a vital drawback of this kit. There are still specific challenges regarding suitable user-friendly, ecofriendly, and on-site field test kits in arsenic detection studies.

The present study aims to develop a portable, user-friendly arsenic test kit to fulfill the above-mentioned necessity. For this, the least amount of hydrochloric acid and zinc dust is used to reduce arsenic compounds into arsine gas, and a gold chloride impregnated paper strip acts as a sensor. The detection concentration of arsenic ranges in the order of 10-500 μg/L, and the response time of the sensor ranges from 4-2 min, which is much lower than most of the familiar detection kits available in the market, such as Merck; Hach, USA; Wagtech WTD; Palintest Ltd., United Kingdom; etc. This kit is the first time, in which, we have ever limited the total reagent volume to nearly 2000 μL (2 ml). Hence, the harmful effects of hazardous chemicals on the environment and human health have been significantly minimized. In this work, a user-friendly prototype has been fabricated to efficiently measure water samples' total arsenic concentration (10-500 μg/L). The qualitative results (approximate values) of arsenic concentration obtained from the colored stripe in the proposed technique are quantified by image analysis with the help of ImageJ software. We have also confirmed its applicability and reliability with field samples, namely Brahmaputra river water (BRW), Assam groundwater (AGW), and West Bengal deep tube well water (WBTW). After getting satisfactory results from the prototype, a POCT device was designed and fabricated to measure the total arsenic present in water. The present technique and POCT device to detect arsenic in water samples are very simple, handy, and eco-friendly.

2.2 Materials and methods

2.2.1 Materials

Zinc in powder form, 1 M gold chloride ($NaAuCl_4.2H_2O$) solution, copper chloride (CuCl), and arsenite salt (sodium (meta) arsenite, ($NaAsO_2$)) were procured from Sigma-Aldrich, India. Sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) was purchased from Merck, India. Hydrochloric acid was supplied by Hi-media, India. Micropipettes tips and 5ml glass sample vials were purchased from Tarsons Products Pvt. Ltd. Kolkata, India. Glue guns and glue sticks were purchased from the local market, IIT Guwahati, India.

2.2.2 Preparation of arsenic stock solutions

A stock solution of Arsenite (As^{3+}) 100 mg/L was prepared by adding 0.001 g of sodium meta arsenite into 10 ml of distilled water. Then, diluted arsenic solutions of various concentrations ranging from 1 µg/L to 1000 µg/L were prepared by following the standard dilution method using distilled water and stock solution (100 mg/L). It is important to note that As^{3+} in the stock solution may be oxidized to As^{5+} spontaneously due to its inherent chemical instability in an aqueous solution. So, it is always recommended to use a freshly prepared solution of As^{3+}. Another stock solution of As^{5+} with a concentration of 4803 µg/L was prepared by dissolving 0.002 g of sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) into 100 ml of distilled water. The working standards (10–1000 µg/L) were prepared by adequately diluting the stock solution. As^{5+} stock solutions were prepared freshly in every 2 to 3 months.

2.2.3 Preparation of reagents and paper sensor

In this case, the reducing agent is nascent (in-situ) hydrogen,-produced during the chemical reaction between hydrochloric acid and zinc powder. The acid and powdered zinc were added separately to the arsenic containing water sample.

The paper-based sensor was developed by dipping a filter paper strip (Whatman filter paper No. 1) into a 0.33 M gold chloride solution. The gold chloride solution of 0.33 M was prepared from 1 M of standard gold chloride solution following the dilution method.

2.2.4 Design and development of a sensor prototype and field test kit

To design the prototype of the portable field test kit, two different micropipette tips (long and short) and one 5ml glass bottle were used. Micropipette tips were fixed with the lid of the glass bottle (*Fig. 2.1*a) using a glue gun. The prototype (*Fig. 2.1*c) was assembled by tightening this micropipette attached lid with the main body of the glass bottle (*Fig. 2.1*b). The short tip acted as the inlet of the prototype, and the long one played the role of a reaction chamber for gases generated from interfering elements and arsenic present in the sample. The long column had two well-defined sections - lower and upper. The lower section had a copper chloride soaked cotton bed to absorb interfering gases (*Fig. 2.1*c), and the upper section was closed with gold chloride soaked paper strip, which was working as a sensor (*Fig. 2.1*c).

2.2.5 Experimental procedure

Experiments were performed in a small prototype with a capacity of 5 ml, as shown in *Fig. 2.1*. A mixture of 100 mg of Zinc dust and 2 ml of arsenic solution was taken into the reactor (*Fig. 2.1*b), and then the reactor was closed with the lid (Fig. 1c). 100 µL of 32% Hydrochloric acid of Molarity 10.2 M has been added to the reaction mixture to initiate the conversion of arsenic into arsine (AsH_3) gas (*Fig. 2.1*c). A few other gases, namely PH_3, H_2S, and SbH_3, may flow through the copper chloride-soaked cotton bed kept in the long pipette to arrest the interfering elements P, S, and Sb, respectively (Fig. 1c). PH_3, H_2S, and SbH_3 free arsine gas finally react with a yellowish gold chloride paper strip, which changes the color of the paper sensor to confirm the presence of arsenic as shown in *Fig. 2.1*d and in its inflated view (*Fig. 2.1*e). The color of the sensor varies from red to dark red depending upon the amount of arsenic present in the sample. The color intensity of the paper strip gives the concentration of arsenic present in the sample. Experiments have been repeated thrice to check their reproducibility. Several other experiments have also been performed with the other arsenic solution, -with concentrations ranging from 0-1000 µg/L. Red to dark red color appears due to the formation of gold nanoparticles, AuNPs (GNPs), inside the paper strip, which is confirmed by Transmission Electron Microscopy (TEM) (Make: JEOL, Model: JEM 2100). The characterization has also been repeated at different arsenic concentrations. Energy dispersive X-ray (EDX) analysis (Make: Zeiss, Model: Sigma) has been used to ensure the reaction mechanism.

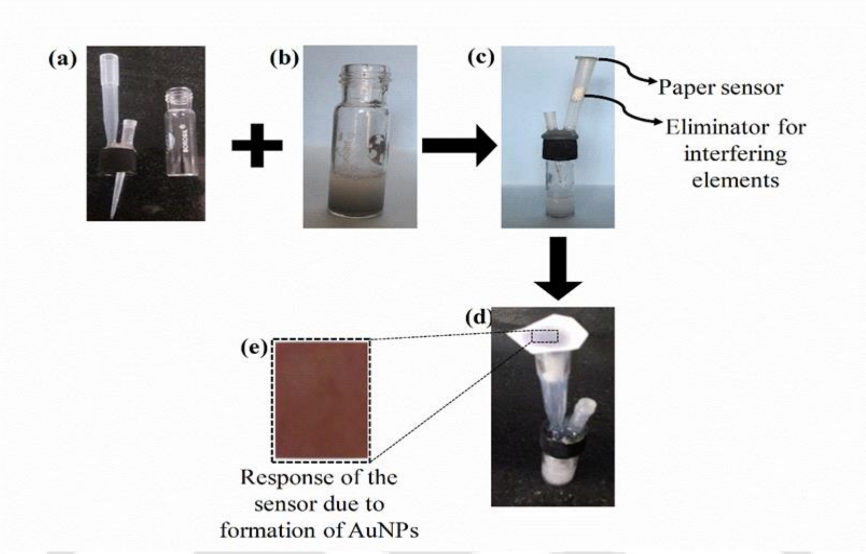


Fig. 2.1: Prototype and scheme for detection of arsenic in water sample, (a) reactor lid connected with two pipette tips, (b) reagent mixture in the reactor, (c) assembled reactor, (d) paper strip after reaction, (e) inflated view of the paper sensor.

2.2.6 Interpretation of arsenic concentration from image analysis

For this purpose, we have adopted a free image processing software, namely ImageJ. The ImageJ can display, edit, analyze, process, and save, the pixel value statistics of an area-based grayscale value. For color images, RGB pixels are transformed into grayscale following the equation given below,

$$V = 0.229R + 0.587G + 0.114B \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where V, R, G, and B represent the grayscale value, Red, Green, and Blue, respectively. The conversion values are displayed in the form of a histogram, and results are obtained based on the best fit statistical of the distribution function. The mean of the best fit distribution is correlated with arsenic concentration present in the paper strips as the color intensity of the paper changes due to variation in arsenic concentration. Hence, the grayscale values can easily be quantified with known arsenic concentration, and a calibrated plot can be established.

2.3 Results and discussions

2.3.1 Performance of the prototype and development of the color chart

The performance of the prototype has been studied in a wide range of arsenic concentrations (10-1000 μg/L). The sensor prototype gives a particular color intensity depending on the amount of arsenic in the samples. Based on the results obtained from the sensors, a standard color chart has been prepared and shown in *Fig. 2.2*. The yellow color slot in the extreme left of the chart indicates the absence of arsenic in the samples (blank test). Then the gradual variations of color intensity from red to dark red represent the different arsenic concentrations from 10-1000 μg/L, as indicated in *Fig. 2.2*. These colors appear due to the gold nanoparticle (AuNPs) formation on the paper strips acting as a sensor. Commonly, arsenic is present in water in the form of arsenite (As^{3+}) and arsenate (As^{5+}) compounds. According to the following reactions (Eq. 2 & 3), these compounds have been reduced into arsine gas (AsH_3) in the presence of nascent hydrogen. AsH_3 gas can produce AuNPs by reducing trivalent gold (Au^{3+}) to zerovalent gold (Au^0), rendering the reaction mentioned in Eq. 4.

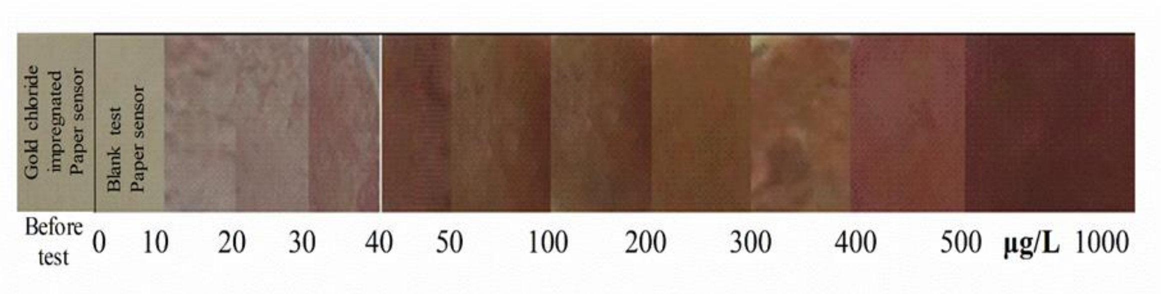


Fig. 2.2: Color chart for various arsenic concentrations.

$As^{3+} + 3Zn + 3H^{+} \rightarrow AsH_3 (\uparrow) + 3Zn^{+}$………………………………. (2)

$As (O_4)^{3-} + 4Zn + 11H^{+} \rightarrow AsH_3 (\uparrow) + 4Zn + 8H_2O$………………... (3)

$AsH_3 + 2Au^{3+} \rightarrow As^{3+} + 2Au (\downarrow)$ ……………………………………. (4)

In favor of the above statements, an Energy Dispersive X-ray (EDX) analysis of the paper sensor before and after use has been depicted in *Fig. 2.3a* and *Fig. 2.3b*, respectively. The inset table of *Fig. 2.3a* and *Fig. 2.3b* show gold and chlorine percentages of (Au 51.3% and Cl

48.7%) and (Au 82.8% and Cl 17.2%), respectively. *Fig. 2.3b* indicates that amount of chloride decreases and the amount of gold increases due to their chemical transformation, which is strong evidence for the reaction characterized by Eq. 3.

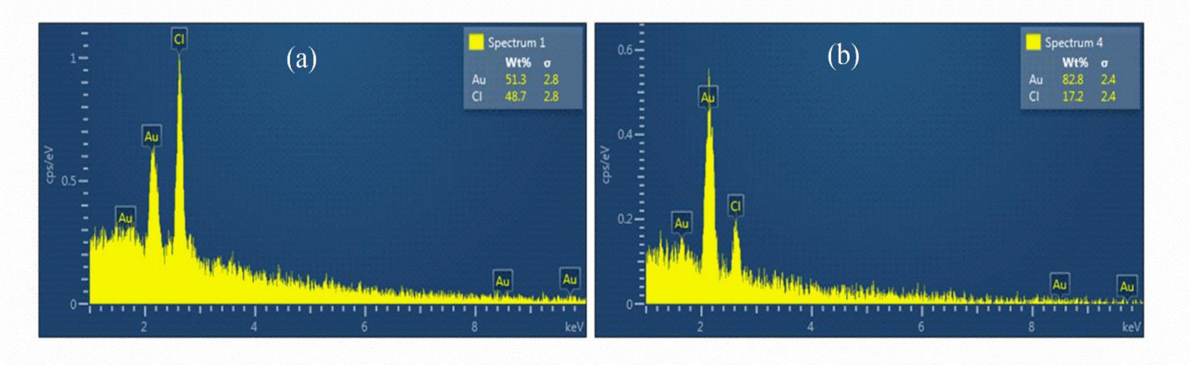


Fig. 2.3: EDX spectrum proves the conversion of Au^{3+} to Au^0 (AuNPs), (a) before and (b) after reaction.

The color contrasts, from red to dark red in *Fig. 2.2*, are due to the size variation of AuNPs. To reveal the dependency of color intensity on particle sizes (i.e., AuNPs), we have presented TEM analysis results in *Fig. 2.4* for four different arsenic concentrations in μg/L units (10, 30, 70, and 100). The figure specifies that particle size changes with concentrations of arsenic. The size of gold nanoparticles increases with concentration. Also, there is a strong tendency for agglomeration in higher concentrations due to the large size and number of AuNPs. In summary, the TEM images (*Fig. 2.4*) reveal that the morphologies of the AuNPs are different at different concentrations of arsenic. These figures show that the morphology of the AuNPs (~34 nm) gradually changes from mono-dispersion to aggregation with increasing concentrations of arsenic. The degree of aggregation also increases if the arsenic concentration goes up. [30], [31]

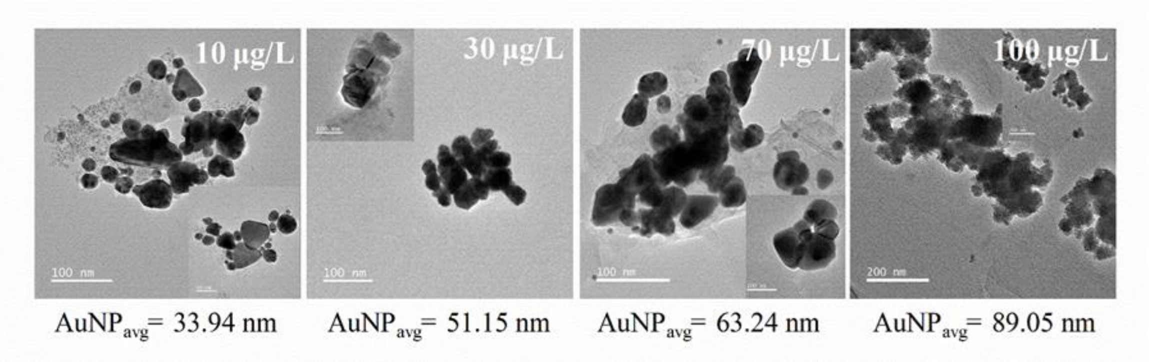


Fig. 2.4: TEM images of AuNPs at various arsenic concentrations such as 10 µg/L, 30 µg/L, 70 µg/L and 100 µg/L . In the figure, AuNPavg means the average size of AuNPs in nm unit.

The proposed color chart in the present work is highly effective in determining the arsenic concentration range in portable and field samples via visual observations. Arsenic concentration above 500 µg/L always gives a dark red color (*Fig. 2.2*), making the visual distinction between any two concentration ranges difficult. So, this method is ineffective for more than 500 µg/L. It is also important to note that the existence of phosphorus, antimony, and sulfur in the sample can mislead the results as they can produce phosphine, stibine, and hydrogen sulfide, respectively. These compounds can reduce trivalent gold (Au^{3+}) to zerovalent gold (Au^0) and may bias the observers. So, it is essential to take enough persuasion to nullify the interfering effects of phosphorus, antimony, and sulfur compounds in the 3rd reaction (Eq. 4). To ensure this, a cotton bed soaked with cuprous chloride (CuCl), is kept below the paper sensor (*Fig. 2.1*c), acting as a shield for the interfering elements. The copper chloride is removing the P, S, and Sb, as per the following given reactions:

$3CuCl + PH_3 \rightarrow Cu_3P + 3HCl$ ……………………………………(5)

$2CuCl + H_2S \rightarrow Cu_2S + 2HCl$……………………………………..(6)

$3CuCl + SbH_3 \rightarrow Cu_3Sb + 3HCl$ ………………………………….....(7)

The above reactions (Eq. 5 - Eq. 7) show that CuCl helps to arrest the interfering elements P, S, and Sb in the solid form, namely Cu_3P, Cu_2S, and Cu_3Sb. The Copper chloride bed has been kept well below the gold paper sensor, which is why all the interfering elements are trapped in this bed, leaving AsH_3 gas alone. That is why the proposed sensor does not give false-positive results and becomes robust.

2.3.2 Quantitative assessment of arsenic concentration using ImageJ software and validation with AAS results

Fig. 2.2 gives a concentration range against a color chart, which is not very much quantized; - it is superficial in nature. To overcome this limitation of visual observation, a quantitative estimation is necessary. An authentic and popular technique, image analysis, has been adopted here for that purpose. The ImageJ software can read, distinguish and quantify the color intensity of the sensor. The software initially transforms the color images (RGB) into a gray scale to spawn a binary image. So, the process converts an analog signal into a digital signal. In the present case, we have extracted all the gray values (i.e., color intensity) corresponding to RGB values of the sensors and tabulated them in *Table 2.1* along with the 4th statistical moment. The concentration vs gray value plot has also been constructed using the tabulated data to understand better. In fact, we have observed that the entire range of the data is not suitable for a single-liner fitting. That is why, we have segregated the data accordingly and plotted it separately in *Fig. 2.5* and *Fig. 2.6*. Those graphs give very good linear fitting with two different sets of concentration ranges (10-100) µg/L and (100-1000) µg/L, respectively, with corresponding regression coefficients (R^2) 0.990 and 0.992. The best-fitting equations (Eq. 8 & 9) have been conveyed below those that can be directly used to predict an unknown sample's concentrations using the contrived prototype.

$$Y_1 = -1.147X_1 + 147.6 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(8)}$$

$$\text{Arsenic concentation} = -1.147 \text{ times Gray value of the paper sensor plus } 147.6$$

$$Y_2 = -39.381\, X_2 + 1872.1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(9)}$$

$$\text{Arsenic concentration} = -39.381 \text{ times Gray values of the paper sensor plus } 1872.1$$

Table 2.1: Gray values (Color Intensity) with respect to arsenic concentration.

Arsenic Concentration µg/L	Gray Value (color Intensity)	Stand Dev.
10	121.013	3.782
20	109.801	2.958
30	105.012	2.041
40	93.66	2.442
50	85.79	2.218
60	73.313	3.469
70	63.692	5.355
80	58.908	1.586
90	50.392	1.694
100	45.882	2.643
200	42.427	4.352
300	38.95	1.267
400	37.418	1.355
500	34.463	0.968
1000	22.615	1.253

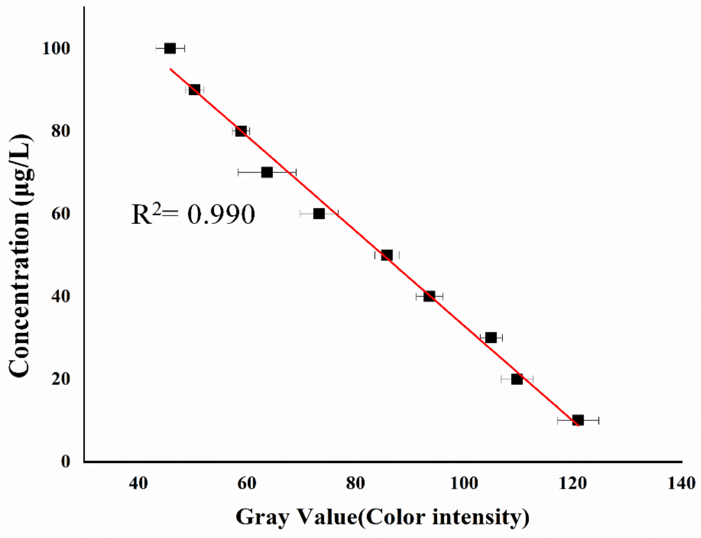


Fig. 2.5: Concentration vs Gray value (10-100 µg/L).

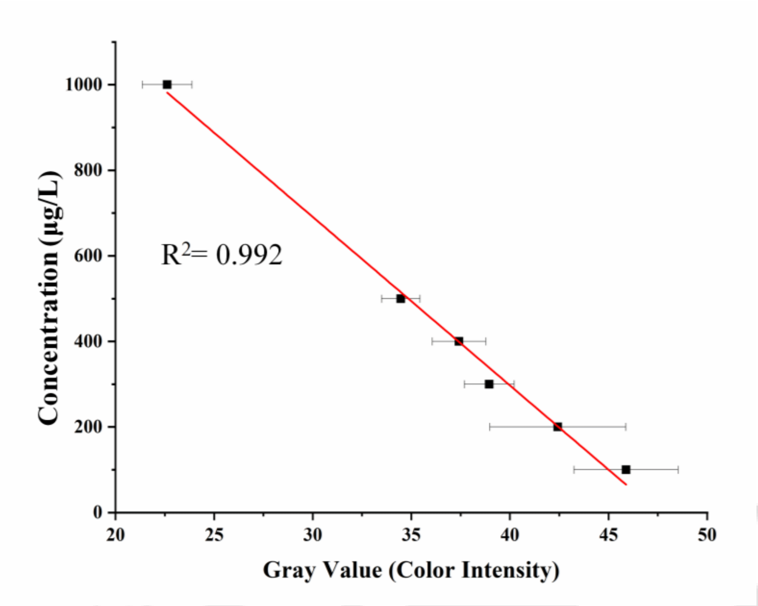


Fig. 2.6: Concentration vs Gray value (100-1000 μg/L).

To acquire better confidence level on the performance of the concocted field kit, present results have been validated with AAS analysis data as it is one of the authentic techniques to measure arsenic concentration accurately. We have followed standard protocol to perform AAS experiments and repeated them thrice to check the reproducibility, and the average values have been reported along with their standard deviation values in *Table 2.2*. The tabulated data have also been presented in a graphical form (*Fig. 2.7***:** Performance of POCT prototype with respect to AAS results. Avg. PD means Average percentage deviation.) and fit well with a straight line having an R^2 value close to 1 (0.999). The data show a little variation in the entire range, with an average percentage deviation of about 1.61%. Our kit visually recognizes the arsenic concentration up to 500 μg/L. However, the samples with a concentration of more than 500 μg/L, can be diluted properly following a standard dilution method, and the actual concentration can be determined with the help of the dilution factor.

Table 2.2: Comparison of the arsenic concentration detected by concocted POCT prototype and AAS.

Present POCT Kit (µg/L)	AAS (µg/L)
10	10.153 ± 0.212
20	20.916 ± 0.427
30	30.487 ± 0.373
40	40.396 ± 0.695
50	50.493 ± 0.639
60	61.345 ± 0.643
70	70.337 ± 0.428
80	80.510 ± 0.453
90	90.797 ± 0.432
100	102.533 ± 1.003

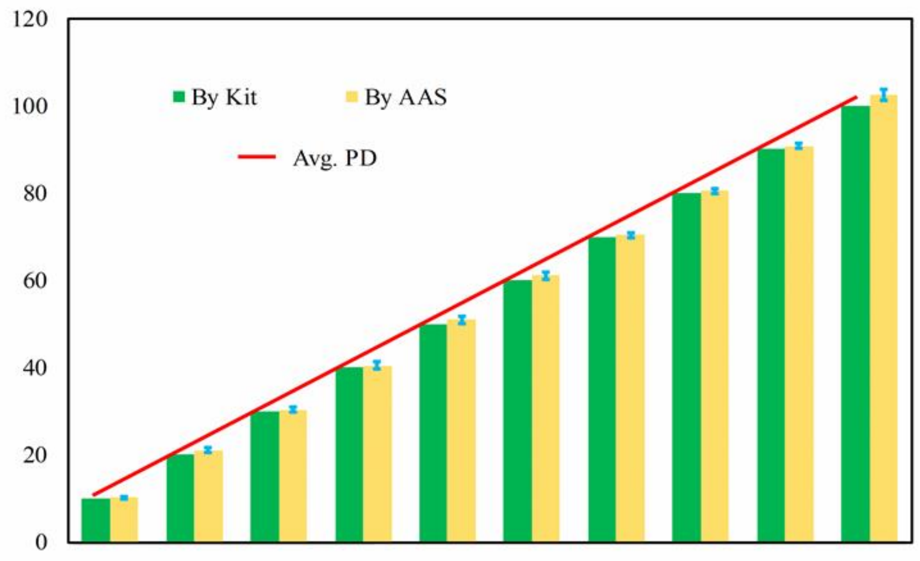


Fig. 2.7: Performance of POCT prototype with respect to AAS results. Avg. PD means Average percentage deviation.

2.3.3 POCT prototype's performance under field sample

Success in the above study encourages us to check the efficiency of this indigenously designed prototype of the POCT kit to detect the arsenic in the field samples. For this purpose, we have collected water samples from three different sources, namely the BRW located at North Amingaon, Guwahati, Assam (26°11'09.7"N 91°43'17.4"E and 26°10'55.2"N 91°41'50.3"E), AGW taken from Ghoramara, North Guwahati, Assam (26°11'23.6"N 91°42'32.3"E), and WBTW situated at North 24 Parganas, Gaighata Region, West Bengal (22° 56′ 4.28″ N, 88° 43′ 50.71″ E). Among these samples, BRW is turbid in nature due to the presence of many impurities such as heavy metals (Al, Cd, Co, Cr, Cu, Pb, Hg, Ni, Mn, Zn, etc.) and Total Dissolved Solids (TDS) [30] [32] and this turbidity is also varying season to season. So, we have collected BRW samples in three different seasons, namely Summer (June - July, Sample - S1), Monsoon (September - October, Sample - S2), and Winter (December - January, Sample - S3), and centrifuged all the BRW sample before further analysis. Then we measured the arsenic concertation of all the raw samples by AAS. As we found, the arsenic concentration of all the raw samples was nil. So, we simulated field samples following the spiking technique. For this, we mixed different amounts of arsenic with BRW and AGW to mimic arsenic-contaminated field samples. Finally, we used these spiked samples to check the performance of our developed POCT kit.

Further, we quantified the visual observations with the help of the ImageJ analysis technique. We applied the AAS technique to meter the actual amount of arsenic present in the samples. The outcome of the whole analysis has been summarized in *Table 2.3*: Arsenic concentration (μg/L) estimated from field sample analysis, and for a better understanding of the results, tabulated values were compared via bar chart in *Fig. 2.8*. The table shows that the developed paper-based POCT kit is highly efficient for the qualitative detection of arsenic in the field samples. The visually observed results of the prototype of the POCT kit show a small absolute deviation (4.59 %) concerning AAS measurement. On the other hand, ImageJ analysis gives a deviation of 9.13%, which is a little higher due to the camera's limitation and the surrounding light intensity available during imaging of the paper sensors. Both the table and plot confirm the high accuracy of the proposed technique.

Table 2.3: Arsenic concentration (μg/L) estimated from field sample analysis.

Samples	Before spiking	After spiking	Detected by POCT prototype	Estimated from ImageJ analysis	Measured by AAS
Distilled water	Nil	0	0	0	0
	Nil	10	10	9.87	10.6
	Nil	50	50	51.57	49.2
	Nil	100	100	98.93	103.5
Brahmaputra river water Sample 1	Nil	0	0	0	0
	Nil	10	10	7.53	09.4
	Nil	50	50	51.87	48.9
	Nil	100	100	97.15	98.6
Brahmaputra river water Sample 2	Nil	0	0	0	0
	Nil	10	10	10.58	09.1
	Nil	50	50	49.28	51.2
	Nil	100	100	98.701	100.7
Brahmaputra river water Sample 3	2	0	NA	0	2
	2	10	10	10.15	11.5
	2	50	50	59.34	51.8
	2	100	100	96.01	101.3
West Bengal deep tube well water	0	Nil	0	0	0
Ground water	Nil	10	10	8.54	10.9

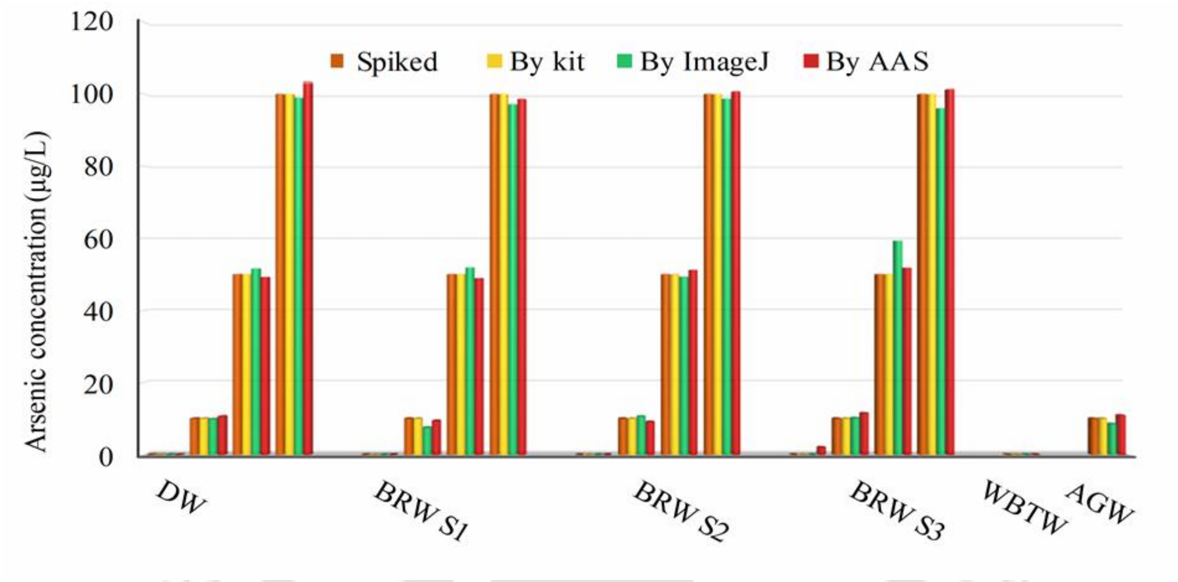
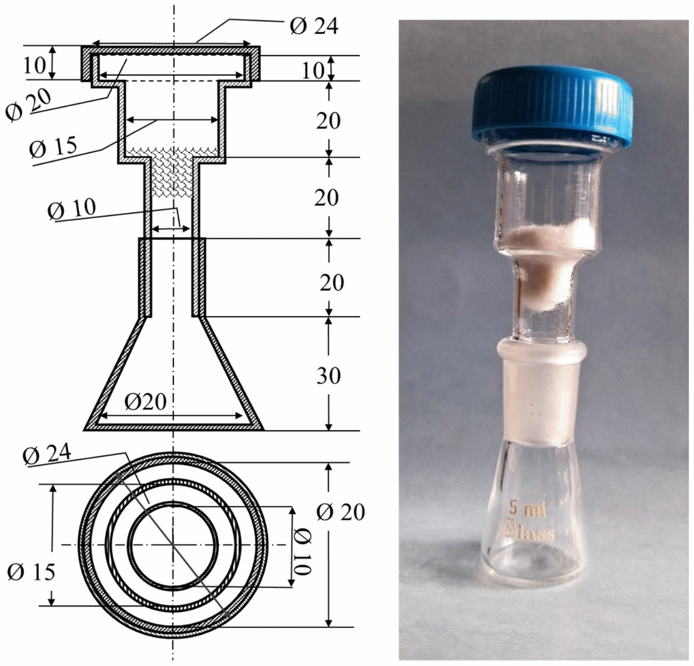


Fig. 2.8: Comparison of performance of the present POCT kit with ImageJ and AAS analysis. The abbreviations represent samples collected from various sources. DW - distilled water, BRW S1 - Brahmaputra River water collected in summer, BRW S2 - Brahmaputra River water collected in monsoon, BRW S3 - Brahmaputra River water collected in winter, AGW - Assam groundwater, WBTW - West Bengal deep tube well water.

2.3.4 Design and development of a POCT device for measurement of arsenic concentration

Based on the above-discussed prototype, a device was designed and fabricated, as shown in *Fig. 2.9*. This device is the desired POCT device and will be used to detect arsenic concentration in the field samples. The detailed dimensions of the kit have been mentioned in Fig. 9a. The design was sent to a glassware fabricator, -Zenith Glassware & Instruments Corporation, Kolkata, India, and fabricated the device (*Fig. 2.9b*).



(a) (b)

Fig. 2.9: Deliverable portable test kit for detecting of arsenic (Gutzeit's method) (a) Engineering drawing of POCT kit (b) photograph of the fabricated POCT kit.

The device can detect minimum and maximum arsenic concentrations of 10 μg/L and 500 μg/L, respectively by visual observation. The device has a very good response time in low and high concentration ranges. It is nearly 4 min and 2 min for the range of (10-20) μg/L and above 20 μg/L (20-500) μg/L, respectively, which is much superior to the kits available in the present market. In the case of commercially available test kits like the Merck kit and Hach kit, response time is 20 min and 30 min, respectively.

On the other hand, the scientifically reported best response time is 7 min, as claimed by Das et al. [26] For a better understanding of the usefulness of the proposed device, we have compared its performance with the various existing kits in Table 4, which endorses the robustness of the developed POCT device. In this device, the whole process, from the first reaction (Eq. 1) to the last reaction (Eq. 3), has been executed in a scientific sequence, as shown in the previous section (Fig. 1). Since the device's size has been squeezed to a miniaturized reaction chamber, it can be used as a portable, eco-friendly and user-friendly POCT device for rapid detection of arsenic in the fields. The size of the reaction chamber will play an important role in the costing, environment, and user-friendliness of the prototype and device. In addition, we have estimated the approximate cost of the device to get an idea about its market potential. Considering the current market price of the raw materials, fabrication charge of the device, and expenses behind the workforce utilized for this development, it becomes about $0.14 per test. The device's overall performance has been compared with commercially available test kits, including Merck kit (*Table 2.4*).

Table 2.4: Comparison with literature data and commercially available kits.

Kits	Sulfide interference	Shelf life	Cost ($ per test)	Detection limit in µg/L	Evolution time in min
MERCK	2 mg/L	2 years	1.84	0-500	20
HACH COMPANY, USA	Not available	Not available	1.42	0-500	30
wagtech WTD, palintest Ltd., United Kingdom	Not available	Not available	2.05	2-100	< 20
MACHEREY-NAGEL GmbH&Co. KG, Duren, Germany	Not available	Not available	1.01	0-500	10
Das et al. 2014 KIT-1(Mercuric bromide)	10 mg/L	One year	0.6	10-250	07
Das et al. 2014 KIT-2 (Silver nitrate)	10 mg/L	Not applicable	0.6	10-250	07
POCT kit (Present study)	**5 mg/L**	**2 years**	**0.14**	**10-500**	**~4**

2.4 Conclusion

This study has successfully developed a user-friendly POCT device to estimate arsenic in potable water. This device is advantageous in terms of low detection time, easy operation, and the use of trace amounts of raw materials compared to the devices available in the market. The device response time is nearly 4 min and 2 min for the range of (10-20) µg/L and (20-500) µg/L of arsenic concentration, respectively. This study was made for qualitative and quantitative estimation of actual samples' unknown concentrations and validated with AAS results. In the qualitative analysis, -visually, it is observed that our device can recognize the arsenic concentration in the range of (10-500) µg/L using the proposed color chart. ImageJ analysis was adopted for quantitative measurement, and the outcome was in good agreement with AAS results with an average deviation of 1.61 %. In the case of field samples, this device is also highly efficient. Both the results obtained from visual observation and ImageJ analysis accord ASS measurements with an average deviation of 4.59 % and 9.13 %, respectively. The size of the device has been squeezed to a miniaturized one; hence it can be used as a portable, eco-friendly, and user-friendly POCT device for rapid detection of arsenic in the fields. Furthermore, the estimated price of a single sample is ~\$0.14, which is less than the cost of currently available testing kits, confirming this concocted device's market potential.

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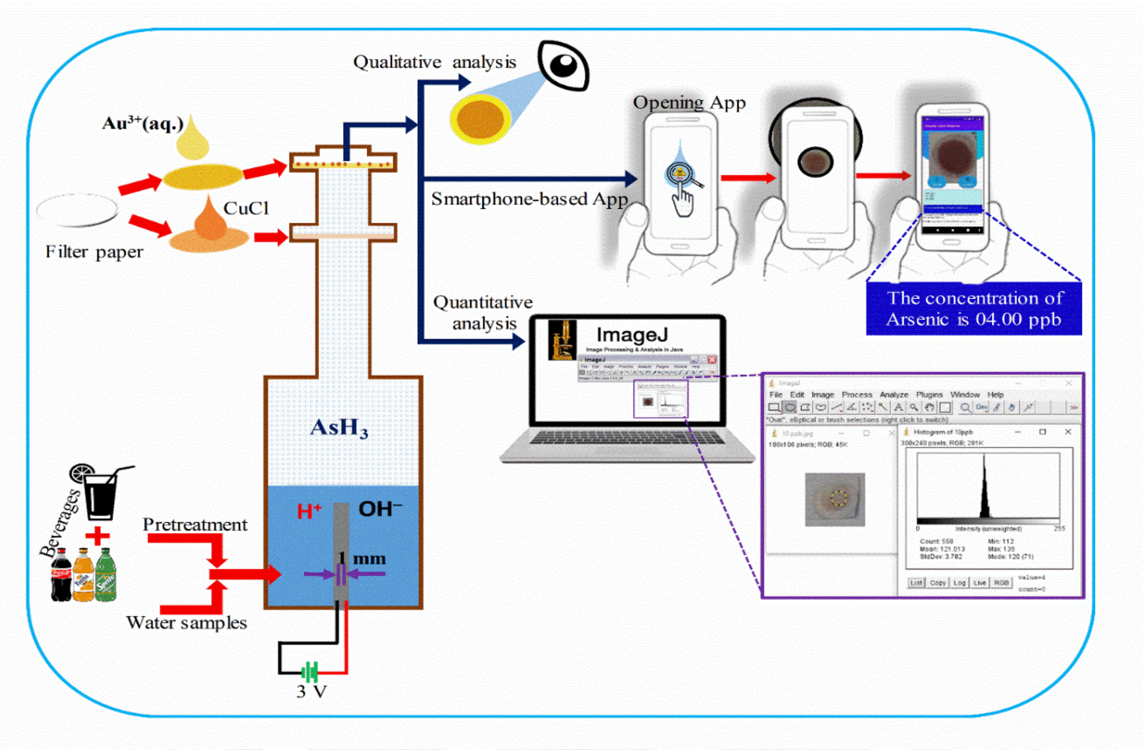
CHAPTER-3

A Highly Sensitive Hybrid Digital Sensor for Room Temperature Arsenic Detection

Chapter 3 : A Highly Sensitive Hybrid Digital Sensor for Room Temperature Arsenic Detection

Graphical abstract



Abstract

Arsenic is a significant water pollutant that causes a severe threat to human health due to its high toxicity. Therefore, the development of sensitive digital point-of-care test (POCT) Kits is highly relevant in monitoring the quality of potable water and beverage. Hence, the present work has proposed an indigenous green sensor Kit to assess the arsenic in liquid samples, namely portable water, soft drinks, and fruit juices. In this test Kit, in-situ hydrogen generated by electrolysis converted all the arsenic compounds into arsine gas and reacted with impregnated Au^{3+} onto a paper strip. Due to the specific nature of the reaction, the paper strip acted as a paper sensor and identified arsenic by changing its color from light yellow to a wide range of gray-red depending upon the arsenic concentration. From the known RGB values of the paper sensor's responses, an indigenous smartphone App was developed for the quantitative assay of arsenic. The sensor was translated into a prototype, and the prototype successfully detected the arsenic in spiked water and beverages collected from various sources. The performance of the developed hybrid sensor on the unknown sample was in good agreement with the Atomic Absorption Spectrophotometer (AAS). It can detect arsenic concentrations ranging from (04-100) µg/L with a very good response time; - 5 min for 04-30 µg/L and 3 min for 30-100 µg/L. The present work manifested many merit incentives, such as lower sensitivity and response time, reusability, user-friendliness, eco-friendliness, and green technology.

Keywords: paper-based optical green sensor, electrochemically empowered, hybrid digital sensor, smartphone-based App, digital sensors prototype

3.1 Introduction

Nowadays, arsenic exposure is one of the significant threats to public health worldwide [1–3]. The most recent estimation of arsenic affected people exposed to levels exceeding the WHO safety limit for drinking water of 10 µg/L [4] is 140 million, - a number that has significantly increased over the decade past [5]. A potential source of arsenic in the ecosystem is attributed to anthropogenic activities and natural resources such as volcanic and granitic rocks, ore deposits, etc., [6]. Exposure to arsenic in drinking water has been associated with the development of skin and internal cancers and also noncarcinogenic effects such as diabetes, peripheral neuropathy, and cardiovascular diseases [7–9]. Apart from drinking water, an excess amount of arsenic exposure has also been accounted for in beverages, like soft drinks and fruit juices [10], [11]. So, there is an urgent need for the development of an arsenic monitoring system as a precautionary step against the adverse effect of arsenic on community health. A highly sensitive smartphone-based digital sensor can give a promising solution to this problem.

Several detection techniques are available those can be broadly classified into two categories, - i) laboratory methods and ii) portable methods. The laboratory techniques are more accurate. However, the analysis cannot be performed without a piece of high-end equipment, which makes the process costlier and out of reach to the common people. That is why laboratory methods are not a concern of the present work. On the other hand, portable techniques are good items of low cost, short response time, ease of use, etc., and site-specific tests are also possible by this technique. Apart from all these merits, certain drawbacks are also associated, which depends on the working principles of the specific device. It is also challenging to generalize all the merits and demerits in a single line or paragraph. So, the various detection methods and their eminences and limitations are extracted from the literature and summarized in section 1.4.1.

Laboratory techniques may sketchily be categorized into two, namely spectroscopic methods and electrophoretic methods. Spectroscopic methods can be further sub-classified into emission spectroscopy like AAS, AFM, etc., [12–15] and mass spectroscopy such as MS and ICPMS [16–18]. All these spectroscopic methods can detect very low arsenic concentrations of about 1 µg/L (1 µg/L), even lower, with very high reproducibility (%RDS<10). Electrophoretic methods [19] are subclassified into two,- isotachophoresis [20] and capillary electrophoresis

[21]. These methods are also very accurate but unable to measure the concentration in µg/L range [21]. On the other hand, the portable methods can be subdivided into four sub-categories, - namely, (i) colorimetric [22–26], (ii) electrochemical [27–29], (iii) biological [30,31], and (iv) surface sensing [32–34] methods. The main concern of the electrochemical methods is the electrode materials, which is sometimes costly, fragile, and difficult to fabricate. But it can be very tiny in size and give highly precise results [28]. For electrochemical sensors, electrodes were modified using a variety of nanomaterials, including carbon nanoparticles, noble metallic nanoparticles, metal oxide nanoparticles, and bimetallic nanoparticles [35]. Some researchers deposited Pt, Ag, and Au noble metal nanoparticles on sensing electrodes to achieve high sensitivity [36–38]. Recently, a chitosan (CT)-modified glassy carbon electrode (GCE) with built-in Silver nanoparticles (AgNPs) based sensor was developed by Prakash et al. [38] for the direct detection of As(III) in the presence of interfering molecules, where the additional arrangement was not required. The review work reported by Zong et al. [39] described that the biosensor made of AgNPs/CT/GCE gave high sensitivity and low detection limit of 1.2 µg/L towards arsenic. Biological sensors have been widely used in this field for a long, and they are highly selective and accurate, with inherent difficulties in handling issues related to biosafety and security [31]. Side by side, surface modified nano-sensors are developed based on the interaction between gold nanoparticles (AuNPs) and thiols groups, which can sense the very low concentration of arsenic in the sample. The main challenge of this technique is to develop a suitable optical sensor that distinguishes the color intensity at very low (pico-nano) and very high levels (sub-milligram onwards) [38].

On the other hand, among all these four methods, colorimetric methods have become popular in the last few decades in the field of test Kit fabrication. These methods follow either Molybdenum blue or Gutzeit's route [40]. AuNPs base Gutzeit's method gives the best performance of the sensor. That is why AuNPs and modified AuNPs methods acquired a vast domain in this field. Specifically, several researchers also adapted the functionalized AuNPs techniques to enhance the performance of the sensors [41] because it is highly stable and low in price w.r.t. AgNPs and PtNPs, respectively. Graphite, glassy carbon (GC), carbon nanotubes (CNT), and Boron-doped diamond (BBD) electrodes have all been modified with AuNPs of various shapes and sizes for use in sensing applications. Subsequently, Nath et al. [25] fabricated a paper-based microfluidic sensor, which could measure the lower concentration of arsenic. The sensor was made based on a composite of gold nanoparticles (AuNPs), thioctic acid (TA), and thioguanine (TG). Priyadarshni et al. [42] also constructed a bio/nano-composite based colorimetric paper sensor. The composite comprised of gold nanorods (GNR), polyethylene glycol (PEG), and dimercaptosuccinic acid (DMSA). The sensor could detect very low levels of arsenic in aquatic samples. It is important to note that some commercial field test Kits have been developed and commercialized based on colorimetric techniques [43],[44]. However, they can only spot the presence and absence and are unable to tell the exact concentration of arsenic in the specimen. That is why the present work has targeted developing a sensor that can quantitatively estimate arsenic concentration in a sample. For this, gold nanoparticle based Gutzeit's method has been modified by integrating an electrochemical technique.

The arsenic concentrations in the portable water may vary concerning time, seasons, and water flow rate in the case of rivers. In beverages and health drinks, it may vary batch-wise depending upon the input water quality of the production plant. arsenic ions are imperative for life in trace quantity; however, they are hazardous to human health at higher concentrations. Thus, real-time monitoring of arsenic in potable water is essential nowadays because Real-Time Monitoring will give vital information and awareness about arsenic contamination with time. This can be achieved through the implementation of the digital sensor system. This system can maintain a stringent real-time checkup for the pollutants in the water resources. Keeping all these points in mind, the present work aims to develop a susceptible hybrid digital sensor following the green technology route for Point of Care Testing (POCT) applications. It is a mobile App-based paper sensor planned to detect arsenic at room temperature. It follows AuNPs-based Gutzeit's method, which is further empowered by integrating electrochemical technique to achieve the detection limit below 10 µg/L. The strategy consists of (i) in-situ generation of hydrogen gas by electrolysis of aqueous medium, (ii) optimization of applied voltage by minimizing the electrode spacing, (iii) Generate the arsine gas by converting all arsenic compounds, (iv) strategic placement of paper sensor to nullify the interference, and (v) development of the smartphone-based App for quantification of color intensity of paper sensor. Finally, a prototype of the hybrid green sensor has been fabricated as a POCT device. The sensor successfully measures the arsenic concentration in soft drinks, fruit juice, and portable water with high accuracy and reproducibility. A good reliability of the sensor has also been observed with the filed samples.

3.2 Materials and methods

3.2.1 Chemicals

The chemicals used in the experiments were of the highest possible purity analytical grade. Arsenite salt (Sodium (meta) arsenite ($NaAsO_2$)) was procured (purchased) in Sigma-Aldrich, India. 1 M Sodium tetrachloroaurate(III) dehydrate ($NaAuCl_4.2H_2O$) solution, Zinc in powder form, and copper chloride (CuCl) were purchased from Merck, India. Hydrochloric acid was procured from Hi-media, India. Syringe filter holders were procured from Axiva Laboratory Products, India. Micropipette tips and 5ml glass sample vials were purchased from Tarsons Products Pvt. Ltd., Kolkata, India. Graphite electrodes (30 mm length and 1mm diameter), Gunn Power Supply Meter, 1.5 V batteries, and beverages were procured from the local market of IIT Guwahati, India. All solutions were prepared with Mille Pore water.

All the glassware used was of Borosil. Prior to all analyses, the reagent bottles, beakers, and volumetric flasks were cleaned by soaking overnight in 2N hydrochloric acid and rinsed with a water oven. All solutions were prepared using Millipore water, and all the arsenic solutions were prepared by diluting the stock solution. Arsenic stock solutions and various concentrations of the arsenic sample ranging from 1 µg/L to 1000 µg/L were prepared by dilution technique as described in Ravula et al., 2022 [45] .

3.2.2 Preparation of paper sensor

A strip of filter paper (Whatman filter paper No. 1) was deep into 0.33 M Sodium tetrachloroaurate(III) dehydrate solution to make the paper sensor. Gold chloride solution was prepared by diluting a standard gold chloride solution of 1 M.

3.2.3 Pretreatment of beverages

3.2.3.1 Pretreatment process for Juice

All commonly used fruit juices were taken from the local market of IIT Guwahati, such as apples, pineapples, oranges, and grapes. For detecting arsenic content in each juice, 5 ml of fruit juice sample was transferred into a 20 ml centrifuge tube. It was diluted with 5 ml of DI water. Then the centrifuge tube was capped tightly and shaken for 30 s to get a clear solution.

Sometimes the samples became turbi, and then further centrifugation was recommended at high speed, say 4,000 rpm for 2 min. A 0.45 μm crodisc polyether sulfone (PES) syringe filter (Agilent Captiva filtration products, India.) was used to filter, and filtrates were kept in pre-labeled 15 ml centrifuge tubes for further use. The filtered sample was instantly subjected to analysis.

3.2.3.2 Pretreatment process for soft drinks

Four samples of soft drinks, namely Thums Up, Sprite, Coca-Cola, and 7up, were purchased from local food stores at IIT Guwahati Commercial Complex. Prior to the experiments, the materials were pretreated following Wallace's technique (2001) [46]. Firstly, it is essential to get gas-free soft drinks by sonicating them for 30 minutes. Then, 20 ml of the sample was mixed with 10 ml of 69% concentrated nitric acid. The mixture was evaporated on a hot plate in a fume cupboard until the brown vapors disappeared, leaving only white vapors. If brown fumes persist, then bring the sample to room temperature, and add 5 ml of 69% concentrated nitric acid and 5 ml of 30% H_2O_2. Refluxed the sample until it was reduced to 2-5 ml at 90^0C. Then, make up the total sample volume to 100 ml with distilled water, and the mixture was filtered with Whatman filter paper. This filtrate was ready for analysis. Each sample was tested in the present fabricated hybrid digital sensor and atomic absorption spectrophotometer (AAS) to measure the total arsenic content.

3.2.4 Design of the miniaturized in-situ hydrogen generator

The conversion of arsenic compound into arsine gas is the key factor of the proposed sensor. This reduction process governs the lower detection limit of this sensor. So, a potent reducing agent is required for the system. It is well established that atomic hydrogen has more reducing power than hydrogen molecules. To achieve the atomic hydrogen, a miniaturized in-situ hydrogen generator was designed based on the concept of electrolysis of water. The designed nascent hydrogen generator consisted of a small reaction chamber of a capacity of 5 ml, two shrimpy graphite rods with a dimension of 25 mm × 5 mm as electrodes, and two 1.5 V batteries as a power source (*Fig. 3.1*a). The electrodes were inserted from the bottom into the reactor (*Fig. 3.1*b) by maintaining a small gap of 1 mm between them. The arrangement assisted in maintaining a very high voltage intensity of 3 kV/m. Further, those graphite rods are connected to the power supply, where two batteries are connected in series to get a 3 V supply (*Fig. 3.1*b).

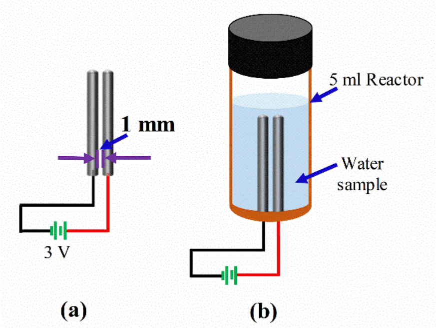


Fig. 3.1: Schematic of the miniaturized (5 ml in volume) in-situ hydrogen generator; (a) space between two electrodes (i.e., 1 mm), (b) arrangement of the electrodes in the miniaturized in-situ hydrogen generator.

3.2.5 Design and development of a hybrid sensor prototype

One miniaturized reactor with a lid, two syringe filters holder, and one micropipette tip were used to design the sensor prototype. The prototype had an upper section and a lower section, as shown in the *Fig. 3.2*b. Using a glue gun, the micropipette tip was initially fixed to the reactor's lid, then two syringe filter holders were joined head-to-head from the reactor's lid, which built up the complete arrangement of the upper section of the prototype. The lower section was prepared by connecting the aforementioned in-situ hydrogen generator and power supply (*Fig. 3.1*b). Now, the overall prototype was assembled by combining the upper and lower section, as shown in *Fig. 3.2*. This will act as a POCT kit. The lower section of the prototype acted as a reduction chamber to convert the arsenic compounds into arsine gas. The upper section of the prototype performed dual roles, - which removed the interfering elements and established an interference-free Gutzeit's reaction, as shown in the sketch of the figure. Copper chloride-soaked cotton is inserted in the lower syringe filter holder to remove interfering gases. Likewise, the upper syringe filter holder is closed with a sensor made of the gold chloride-soaked paper strip. The bottom section of the prototype is used for arsine gas generation through the water-splitting technique.

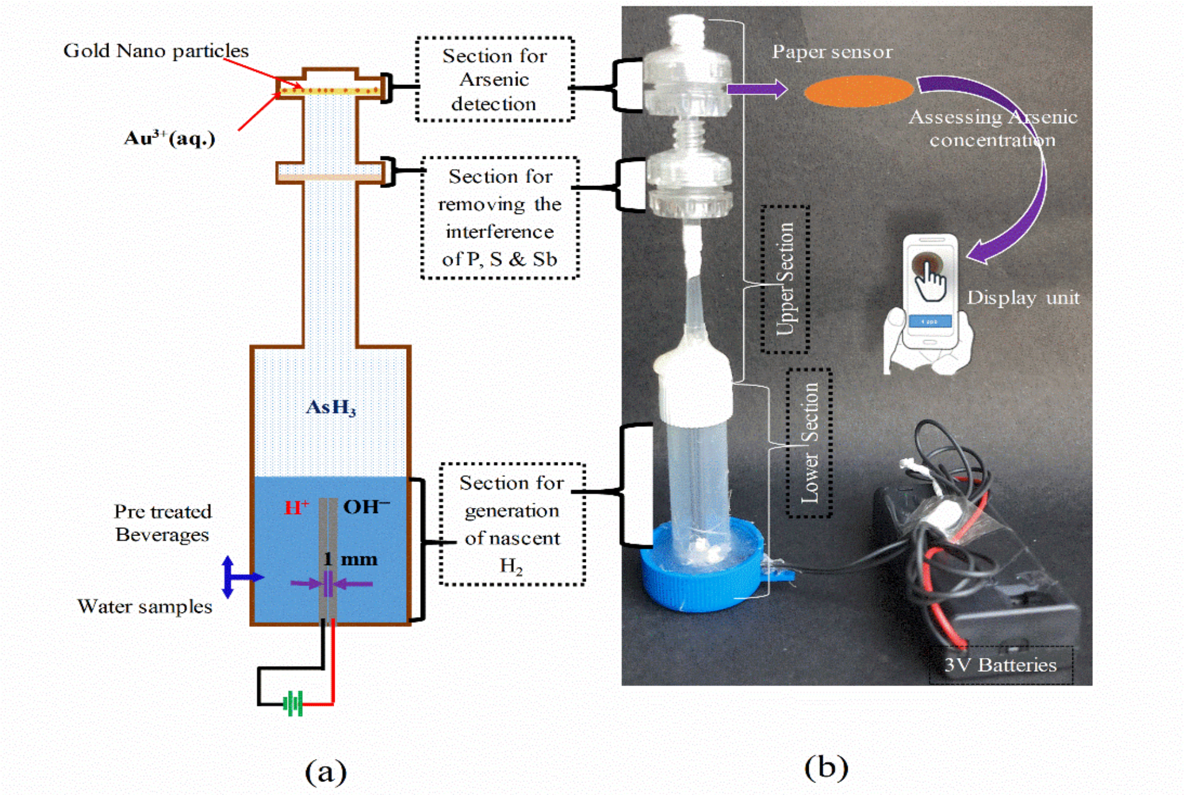


Fig. 3.2: Different components of the sensor prototype, (a) schematic diagram, (b) photograph of the fabricated prototype.

3.2.6 Experimental procedure

3.2.6.1 Operating the hybrid prototype and indigenous mobile App

The experiments were carried out in the prototype (*Fig. 3.2*) with a capacity of 5 ml. Initially, the water sample of 2 ml was taken into the reactor. Then 50 µL of 32% Hydrochloric acid of Molarity 10.2 M was added to the water sample to attain the acidic condition of the mixture. Immediately, the upper section of the prototype was connected with its bottom section, and a 3 V supply was given to initiate the conversion of arsenic into arsine (AsH_3) gas. In this process, some other gases, namely SbH_3, H_2S, and PH_3, may generate in the reaction due to the presence of the interfering elements Sb, S, and P. Those gases flowed upward through the column and passed across the copper chloride-soaked cotton bed which was kept on the lower syringe filter holder. The bed was kept to arrest the interfering gases to nullify the false responses. Then the interference free arsine gas directly reacted with a yellowish gold chloride paper strip and acted as a sensor.

Finally, the color changes of the sensor confirmed the presence of arsenic in the water sample. The color of the sensor's response was changed from grey-red to purple-red depending upon the amount of arsenic present in the sample. The concentration of arsenic in the sample was estimated from the color intensity of the paper sesnor's response. The experiments were repeated thrice to ensure reproducibility. These colored paper sensors were further analyzed with the indigenous mobile App to quantify the arsenic concentration. The entire procedure has been described in *Fig. 3.3* given below. The analysis were also repeated for different arsenic concentrations of 0, 4, 6, 8, 10, 20,50, and 100 µg/L. Similar procedure was followed to measure arsenic concentration in the several beverages' samples. The energy dispersive X-ray (EDX) analysis (Make: Zeiss, Model: Sigma) was performed to check the conversion of gold Au^{3+} to Au^0. This conversion brought the color changes of the paper sensor due to the formation of gold nanoparticles (AuNPs) inside the paper strip.

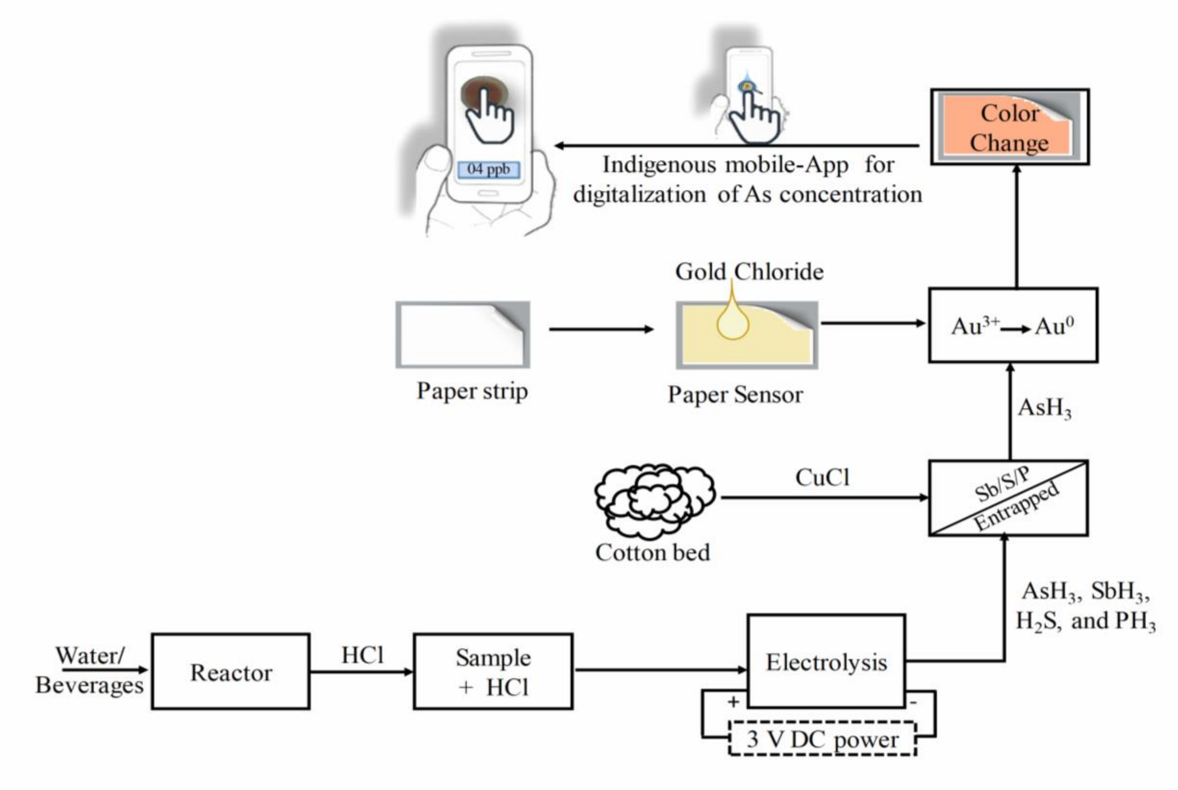


Fig. 3.3: Schematic representation of the Step of Operation (SOP) of the hybrid sensor.

3.3 Results and discussion

3.3.1 Electrochemically reduction of arsenic compounds into arsine gas and performance of the sensor prototype

The proposed arsenic sensor responds depending on the arsine gas concentration as this gas reacts with the paper sensor and changes the sensor's color, which measures the concentration of arsenic. Here, the main challenges recline within the concentration of arsine gas- less is the concentration, more is the confrontations. That is why converting trace amounts of arsenic compounds into arsine gas is critical for the sensor. The conversion takes place as per the following chemical reaction,

$As^{3+} + 3H^{+} \rightarrow AsH_3 (\uparrow)$………………………………… (1)

$As (O_4)^{3-} + 11H^{+} \rightarrow AsH_3 (\uparrow) + 4H_2O$………………... (2)

In this pathway, hydrogen reduces both the compound As^{3+} and As^{5+} to As^{3-} and produces AsH_3. For this, very active or atomic hydrogen is recommended at a very low concentration of the arsenic compound. So, water splitting by electrolysis technique is integrated with the process to empower the reduction process, which successfully transforms the arsenic compounds into arsine gas. In this study, two graphite rods are used as electrodes in the electrolytic cell because graphite's structure enables it to be an excellent conductor. The high number of delocalized electrons allows electricity to pass through graphite rapidly. Moreover, little amount of HCl (0.024 V/V%) is also added to increase the conductivity of the aqueous sample. HCl is a strong acid that almost entirely dissociates upon mixing with water. This means Hydrogen and Chlorine ions will be free-flowing in the water (electrolyte), allowing the water to conduct better. This work may be considered environmentally friendly or green technology based development due to minimal waste production and negligible acid usage in the lock & key reaction.

The POCT prototype's performance has been investigated in various arsenic concentrations (4-100) µg/L. The prototype's paper sensor gives a specific color intensity depending on the amount of arsenic present in the sample. For the qualitative study, a color chart (*Fig. 3.4*) is prepared based on the responses of the paper sensor. The initial color of the paper sensor is a slite yellowish color, shown in the very first slot. The second slot on the chart is for the blank test, in which the color of the paper sensor strip remains unchanged due to the absence of arsenic in the sample.

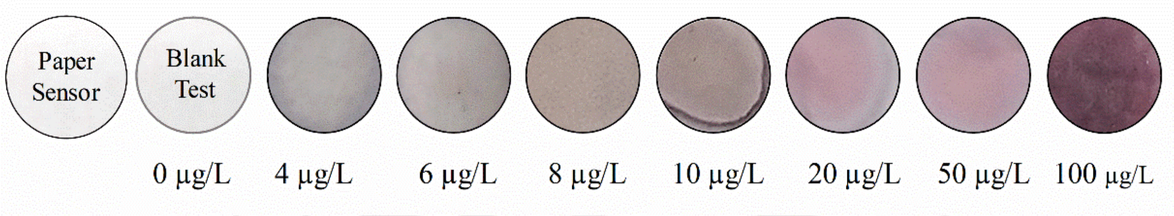


Fig. 3.4: A Color chart for measuring the arsenic Concentration of a sample.

Further, as shown in *Fig. 3.4*, the successive changes in color intensity from Gary-red to purple-red color depict the arsenic concentration from 4-100 µg/L. The different colors that appeared on the paper sensor's responses strip are due to the formation of the AuNPs on the paper strip, according to Eq. (3) given below.

$AsH_3 + 2Au^{3+} \rightarrow As^{3+} + 2Au\ (\downarrow)$ …………………………………… (3)

During the reaction, trivalent gold (Au^{3+}) of gold chloride is reduced to zerovalent gold (Au^0). The formation of metallic gold (Au^0) was conformed through the Energy Dispersive X-ray (EDX) analysis. For this, the paper sensor strips were analyzed before and after the reaction. And are displayed in *Fig. 3.5*a and *Fig. 3.5*b, respectively. The weight percentages of the compounds present in the paper sensor were shown in the insert table of *Fig. 3.5*a and *Fig. 3.5*b. Compositions are Au 51.3% and Cl 48.7% for the before reaction and Au 66.5%, As 18.8% and Cl 14.7% for after reaction, respectively. From *Fig. 3.5*b, the percentage of chloride reduces, gold increases as a result of their chemical transformation, which provides a strong sign of metallic gold formation during the reaction, and the arsenic percentage indicates some amount of arsine gas was arrested in the paper sensor. The color contrasts, from gray-red to dark red in *Fig. 3.4*, arise due to the variation of the size of AuNPs' , a concise summary is provided in below section 3.3.2.

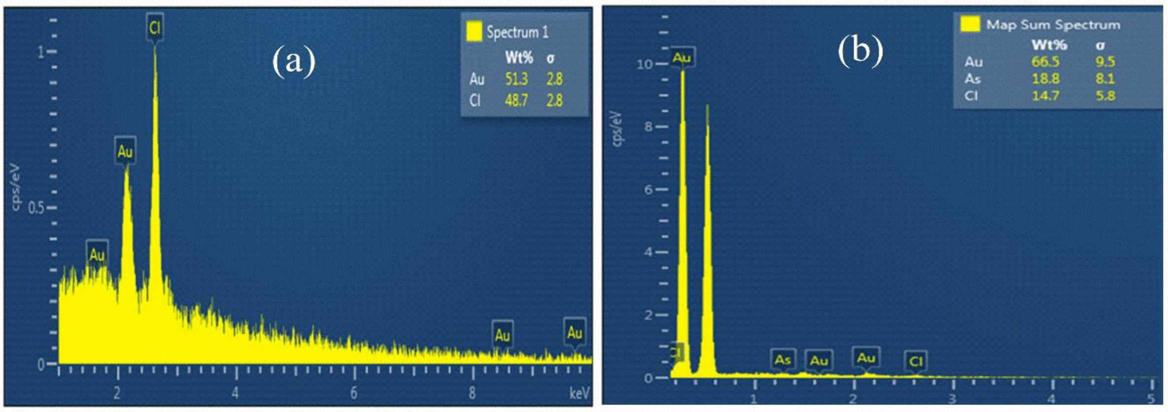


Fig. 3.5: Confirmation of metallic gold formation by EDX while arsine gas reacts with gold chloride paper sensor. Images (a) and (b) illustrate the EDX analysis before and after the reaction of the paper strip.

3.3.2 TEM analysis for measuring the AuNPs size on paper sensor's responses

The color contrasts, from gray-red to dark red, are due to the size variation of AuNPs. To reveal the connectivity between color intensity and particle sizes (i.e. AuNPs), we have presented TEM analysis results in *Fig. 3.6*, for four different arsenic concentrations in µg/L unit (4, 10, and 50). The figure specifies that particle size changes with concentrations of arsenic. The size of gold nanoparticles increases with concentration. Also, there is a strong tendency of agglomeration in higher concentrations due large size and number of AuNPs. In summary, the TEM images (*Fig. 3.6*) reveal that the morphologies of the AuNPs are different at different concentrations of arsenic on the paper strip, and the morphology of the AuNPs (~17 nm) gradually changed from mono-dispersion to aggregation with increasing concentrations of arsenic. The degree of aggregation depends on the arsenic concentration.

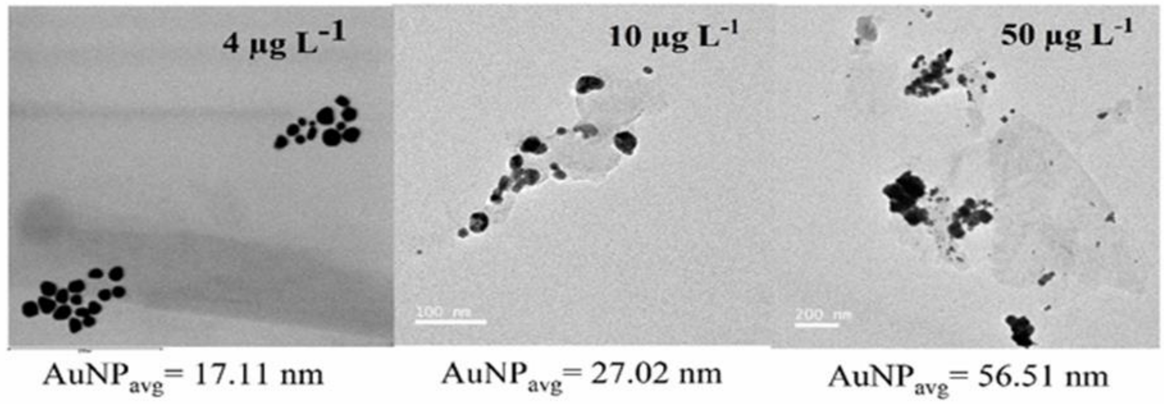


Fig. 3.6: TEM images illustrated formation of AuNPs with different amounts of arsenic, such as 4, 10, and 50 µg/L. (AuNPavg - average size of AuNPs)

The color chart proposed in the present study is extremely effective for the visual estimation of arsenic concentration in the portable water and field samples. The sensor is suitable for estimating arsenic up to 100 µg/L since, beyond this range, it gives a dark red color. This makes it difficult to distinguish them visually between the concentration ranges. Measuring the higher arsenic concentration of the samples may be done by the dilution process. The actual concentration of the sample can be obtained from the product of the dilution factor and the estimated value after dilution. This process is prone to the interference of sulfur, phosphorus, and antimony. They can develop the same color on the paper sensor by converting gold to metallic gold. Sufficient persuasions should be taken to eliminate their interfering effects. For this, all these elements/compounds are transformed into either hydrogen sulfide, or phosphine, or stibine gases depending upon the impurity present there. A cotton bed soaked in cuprous chloride (CuCl) is placed in the lower spring filter holder to ensure elimination. It will act as a barrier against interfering elements and allow only arsine gas. The detection of low-level arsenic is possible in this study. The minimum detection limit is 4 µg/L. The response time was 5 min for up to 20 µg/L, and it will take 3 min for 30 -100 µg/L. Due to minimal waste production, negligible acid usage, and cost-effectiveness, the sensor developed in this work is reflected in green technology.

3.3.3 Exploration of arsenic concentration quantitatively using ImageJ software and corroboration with AAS

The recommended color chart in the present study is extremely effective for qualitatively (visually) measuring arsenic concentration in the portable water and field samples. To neglect the eye error, it is necessary to make a study for a quantitative estimation to know the accuracy values of unknown samples. For that determination, ImageJ software was used to calibrate the colored sensor paper strip. This method can give the color intensity of the sensor paper strip by discriminating, reading, and measuring it. The uploaded paper sensor strip center spot area was selected during the analysis. Then it will read RGB values of the color image, and based on those values; it will generate grayscale values. In the current study, gray values for the arsenic concentration of 4, 6, 8, 10, 20, 50, and 100 µg/L were extracted along with the 4th statistical moment and mentioned in *Table 3.1*. The obtained data plotted a graph between the gray value vs concentration. It gives the best fitting by non-linear fit with exponential decay (Model ExpDec2) seen in *Fig. 3.7*, its regression coefficient (R^2) is ~1 (0.999). The best-fitting equation is provided below and can be used to directly estimate the concentrations of an unknown sample.

$$y = 156.69 \times \exp[-x/57.34] + 246841.23 \times \exp[-x/10.89] - 2.96 \ldots\ldots\ldots\ldots\ldots (A.1)$$

Where, y = Arsenic concentration, and

x = Gray value of the paper sensor concentrations of an unknown sample.

Table 3.1: Gray values (Color intensity) with respect to arsenic concentration.

Gray Value (color Intensity)	Arsenic Concentration (µg/L)
231	0
171	4
164	6
157	8
147	10
120	20
100	50
89	100

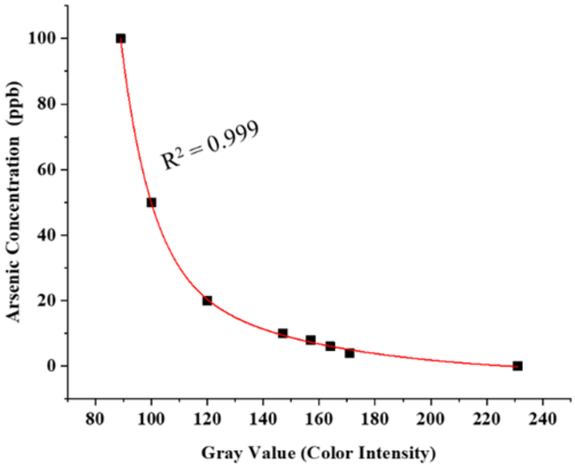


Fig. 3.7: Calibration curve for measuring the arsenic concentration quantitatively based on ImageJ analysis. (Gray value vs. concentration (µg/L))

Current results have been corroboration with AAS analysis data to attain a better confidence level on the performance of the suggested field kit since it is one of the most reliable approaches for precisely measuring arsenic content. To operate the AAS, a standard protocol is followed, and each and every sample was analyzed three times to ensure repeatability. The average values and their standard deviation values are provided in *Table 3.2*. The tabular data has also been displayed graphically (*Fig. 3.8*), resulting in a straight line with an R^2 value of 0.997. The data show a slight variation in the whole range with an average percentage deviation of about 3.51 percent. Visually, the present kit is capable of diagnosing the arsenic concentration up to 100 µg/L. The above-range arsenic concentration is impossible to detect due to the formation of dark color on the paper sensor. It will give dark red, making it more difficult to distinguish between the two successive paper sensors. That is why we have shown the results up to 100 µg/L in *Table 3.2* and *Fig. 3.8*. However, a higher concentration sample could be diluted properly and later multiplied by the dilution factor to get as close as the actual concentration.

Table 3.2: Comparison of the arsenic concentration results of the proposed kit with that obtained by AAS.

By Present Kit (µg/L)	By AAS (µg/L)
0	0
NA	2.00 ± 0.03
4	3.99 ± 0.17
6	6.21 ± 0.21
8	8.33 ± 0.28
10	10.37± 0.37
20	22.05 ± 0.52
50	50.69± 1.37
100	105.58± 3.08

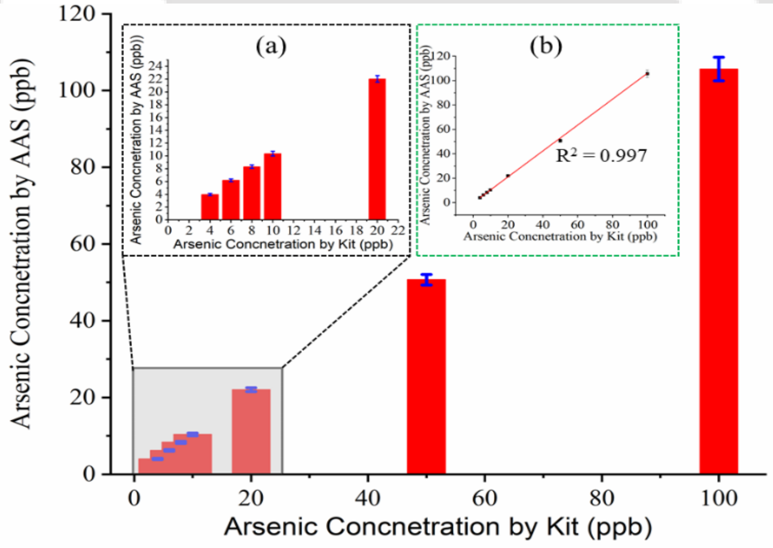


Fig. 3.8: Enactment of prototype with respect to AAS results. (a) inflated view for 2-20 µg/L, (b) linear fitting of the data.

3.3.4 Development of an indigenous Java-based mobile App and digitization of field samples' arsenic concentration

The RGB values of the paper sensor's response, obtained from ImageJ analysis, were used to write a Java-based code for designing and developing a mobile App, which would show the arsenic concentration digitally. In this development, the first requirement is the RGB values of the color of the paper sensor. For this, color images of the sensor's responses at various concentrations ranging from 0-100 µg/L were captured and uploaded in the ImageJ software for getting RGB values and their corresponding gray values, as described in Ravula et al. [45], shown in *Table 3.3*. The graphical representation of those RGB values is shown in *Fig. 3.9*. These values were used to make a Java-based code that can read the color intensity of any paper sensor. The algorithm was executed by correlating the RGB values with the Gray values, which digitally read the arsenic concentration by decoding the equation mentioned below.

$$y = 156.69 \times \exp[-x/57.34] + 246841.23 \times \exp[-x/10.89] - 2.96 \ldots\ldots\ldots\ldots\ldots (1)$$

Where, x = Gray values of the paper sensor's responses,
y = Arsenic concentration

The entire code has been uploaded to Android Studio to create an Android support application (App) in APK file format. After the App was designed, it was installed on the mobile device. The App was prosecuted in the following ways: (i) opening the App on your phone, (ii) selecting the option for uploading the response image of the paper sensor, either from the files/photos folder or by shooting a new picture with the camera. (iii) uploading the image to the application, and (iv) touching the colored area of the uploaded paper sensor's response image that you uploaded. The Java-based code was then processed automatically and showed the sample's RGB values and digital values of corresponding arsenic concentration right away on the screen. The light effect is always a great issue for an optical sensor as the quality of the image differs with surrounding light sources. So, this issue could heavily affect the aforementioned qualitative and quantitative studies. For example, the much and less intense light could change the appearance of the color paler and more intense, respectively. The effect of illusion can be minimized by implementing a controlled environment, such as image capturing within a dark chamber. Despite this limitation, optical sensor technology is widely used across the world because of its simplicity, portability, rapidity, and accuracy, like UV-Vis spectrophotometers [47]. Moreover, it can easily be integrated with the smartphone, as done in the present work.

Table 3.3: Mean RGB values with the corresponding intensity of the paper sensor’s responses for the range of 0-100 µg/L.

Concentration (µg/L)	R	G	B	Gray values
0	231	232	230	231
4	175	170	168	171
6	170	162	160	164
8	171	155	147	157
10	159	143	139	147
20	152	103	106	120
50	145	83	99	100
100	111	77	91	89

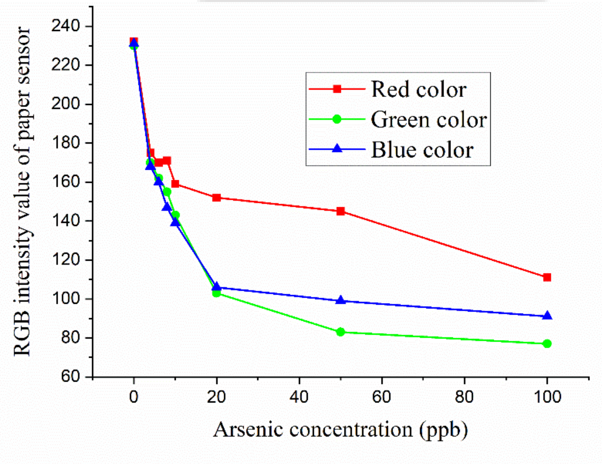


Fig. 3.9: Graph illustrating the mean RGB values for paper sensor’s responses in the range of 0-100 µg/L.

Further, the field samples are analyzed to check the performance of the App developed in the present work. For that, BRW (Brahmaputra River Water) samples were collected and centrifuged to separate the solid impurities. Initially, the supernatant was checked under AAS to measure the arsenic concentration, and no arsenic was found. Then the supernatant was spiked with arsenic at various concentrations of 4, 6, 8, 10, 50, and 100 µg/L, and the spiked samples were used to test the efficiency of the indigenous Kit and App. The sensor responses were compared with the color chart, which gave tentative values of arsenic concentration shown in the 2nd column of *Table 3.4*. For more accurate measurements, concentration was estimated using ImageJ software as provided in the 4th column of *Table 3.4*. Again concentration was determined by operating the indigenous App mentioned in column 3 of *Table 3.4*. The corresponding AAS readings were placed in the last column to compare all these three results with standard measurements. The same results are also shown graphically in *Fig. 3.10*.

The results endorse that the indigenous App is highly efficient in measuring the unknown samples' arsenic concentration. It shows an insignificant average absolute deviation of 2.96% compared to the AAS interpretation. On the other hand, a color chart-based measurement obtained from the indigenous Kit shows an average absolute error of 5.28%, which is a little higher than the indigenous App. Moreover, ImageJ software produces the least average absolute deviation of 1.51% due to its higher view factor than the App. During the measurement, the App focuses locally, i.e., a local area touched upon by the end user's finger, whereas ImageJ reads the entire paper strip. The execution of the App may be further improved by upgrading the background code that can read a larger area like ImageJ software.

Table 3.4: Performance comparison of the indigenous Kit and App with ImageJ and ASS. (mean arsenic concentrations are tabulated).

Sample	Color chart	By App	By ImageJ	By AAS
BRW	0	0	0	0
	04	04.32	04.15	4.43
	06	06.38	06.23	06.65
	08	08.65	08.67	08.92
	10	10.54	10.67	09.89
	50	50.96	51.35	50.68
	100	101.93	101.55	100.81

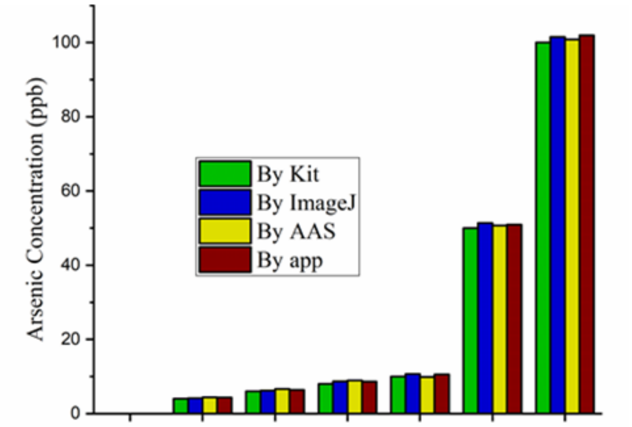


Fig. 3.10: Comparison of the performance of color chart obtained from the indigenous Kit, indigenous App Integrated Kit, and ImageJ with AAS.

3.3.5 Assessment of kit response through field samples

The aforementioned study's success inspires us to test the efficacy of our newly developed POCT kit in detecting arsenic in field samples. We collected water samples from three different sources for this study: The Brahmaputra river water (BRW) from North Amingaon (26°11'09.7"N 91°43'17.4"E and 26°10'55.2"N 91°41'50.3"E), Guwahati, Assam, Assam groundwater (AGW) taken from Ghoramara (26°11'23.6"N 91°42'32.3"E), North Guwahati, Assam, and deep tube-well (DTW) from North 24 Parganas (22° 56′ 4.28″ N, 88° 43′ 50.71″ E), Gaighata Region, West Bengal. Out of these three samples, BRW exhibits more turbidity due to the presence of various contaminants such as heavy metals (Al, Co, Cd, Cu, Cr, Hg, Mn, Ni, Pb, Zn, etc.) and total dissolved solids (TDS). The turbidity behavior of this BRW sample also varies seasonally as well. Hence, we collected BRW samples in three seasons: Sample 1 from the Summer season (June month), sample 2 from the Monsoon season (September month), and sample 3 from the Winter season (December month), and centrifuged all collections of samples before proceeding with further investigation. Later, the arsenic concertation of all raw samples was then evaluated by AAS. We simulated field samples using the spiking technique because the arsenic concentration in all raw samples was zero. Then, to simulate arsenic-contaminated field samples, we mixed varying quantities of arsenic with AGW and BRW. Finally, we tested the performance of our newly established POCT kit using these spiked samples. With the use of the ImageJ analysis technique, we were able to quantify the visual findings. Again the AAS technique was used to determine the actual quantity of arsenic in the samples. *Table 3.5* summarizes the results of the entire analysis, and tabulated data were contrasted using the bar chart in *Fig. 3.11* to understand the findings in a better way. *Table 3.5* demonstrates that the developed paper-based POCT kit effectively detects arsenic in field samples and found this device is also quite effective regarding field samples.

Regarding AAS measurement, there was an absolute variance of 5.08% and 6.84% for visually seen present kit and ImageJ analysis, respectively. Due to the camera's limitations and the situation of available ambient light intensity during the imaging of the paper sensors, this deviation was slightly higher in the ImageJ analysis. Both the table and the map demonstrate the suggested technique's excellent performance.

Table 3.5: Measured arsenic concentration (μg/L) from different field water samples analysis.

Sample	Arsenic present	Arsenic added	Arsenic found by kit	Arsenic found by AAS	Arsenic found by ImageJ analysis
Distilled water	Nil	0	0	0	0
	Nil	04	04	03.92	4.12
	Nil	08	08	08.23	08.18
	Nil	10	10	10.45	10.15
Brahmaputra river water Sample 1	Nil	0	0	0	0
	Nil	04	04	03.72	4.44
	Nil	08	08	07.85	7.56
	Nil	10	10	09.65	10.95
Brahmaputra river water Sample 2	Nil	0	0	0	0
	Nil	04	04	04.15	4.43
	Nil	08	08	08.67	08.92
	Nil	10	10	10.67	9.71
Brahmaputra river water Sample 3	2	0	0	2.01	NA
	2	04	06	05.97	5.97
	2	08	10	09.91	9.44
	2	10	10	12.04	11.24
WBTW	0	0	0	0	0
Groundwater	Nil	4	4	04.34	4.64

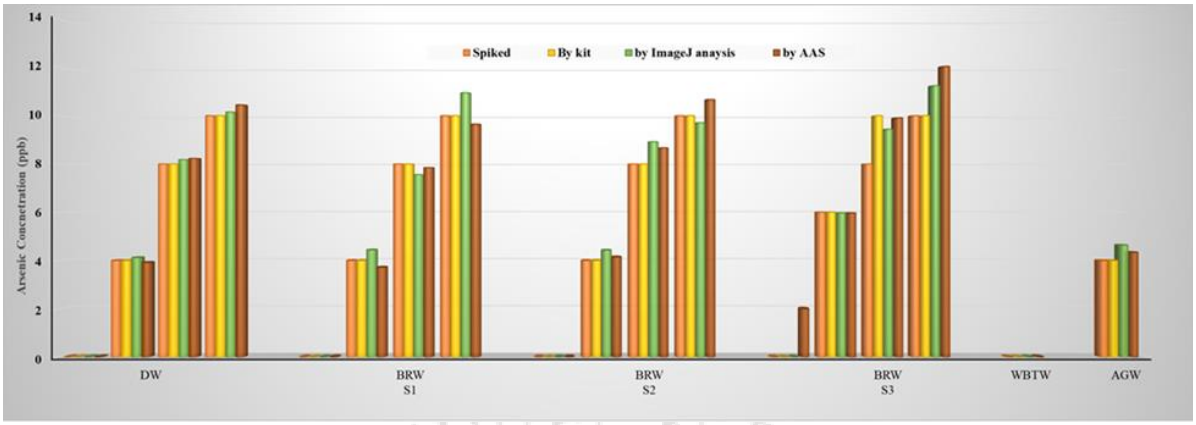


Fig. 3.11: Performance evaluation of the current prototype using ImageJ and AAS analysis. (The abbreviations denote samples acquired from a variety of sources. DW -distilled water; BRW S1 -Brahmaputra River water collected during the summer; BRW S2 -Brahmaputra River water collected during the monsoon; BRW S3 -Brahmaputra River water collected during the winter; AGW -Assam groundwater; WBTW -West Bengal deep tube well water).

3.3.6 Reckoning of hybrid digital sensor's performance on various beverages

After getting success in the various water samples, the Kit's performance was tested with different beverages. All beverage samples were pretreated according to Wallace's (2001) method, as mentioned in Section 3.2.3, before being analyzed in the hybrid digital sensor, and the availability of arsenic was examined under AAS. It was found that all beverages contained zero arsenic concentration. So, the beverage samples were spiked with an arsenic concentration of 100 µg/L before its pretreatment, and then samples were prepared for analysis. The arsenic concentration of pretreated samples was then measured with AAS, as tabulated in column 2 of Table 3. The AAS results showed a lower value than the added one. It is due to the loss of some amount of arsenic during the pretreatment. All the samples were also analyzed with the currently developed Kit. Finally, arsenic Concentration was evaluated in three ways, - using i) a color chart, ii) ImageJ software, and iii) an indigenous mobile App. The obtained results are mentioned in *Table 3.6*, and also a graphical representation is shown in *Fig. 3.12*. The table shows the gradual improvement in prediction from a color chart to an indigenous mobile App. The average absolute deviations in the reading of color chart, ImageJ, and indigenous mobile App are 4.11%, 1.46%, and 2.36%, respectively, compared with AAS results. These results authenticate that the App is highly efficient in detecting arsenic concentration from the different beverage samples.

Table 3.6: Appraised arsenic concentration (µg/L) from different beverage samples.

Samples	AAS	Color chart	ImageJ softwere	Indigenous Mobile App
Thumbs Up	85.131	80	84.055	83.746
Sprite	89.173	90	89.023	88.447
Coca-Cola	94.137	90	93.304	92.868
7up	90.431	90	89.859	89.569
Pepsi	86.275	80	85.913	85.603
Miranda	90.512	90	87.441	86.354
Fanta	84.559	80	82.865	79.865
Apple	94.143	90	91.504	90.521
Pineapple	93.959	90	91.912	91.251
Orange	94.481	90	93.973	93.338
Grapes	96.593	90	94.893	94.311

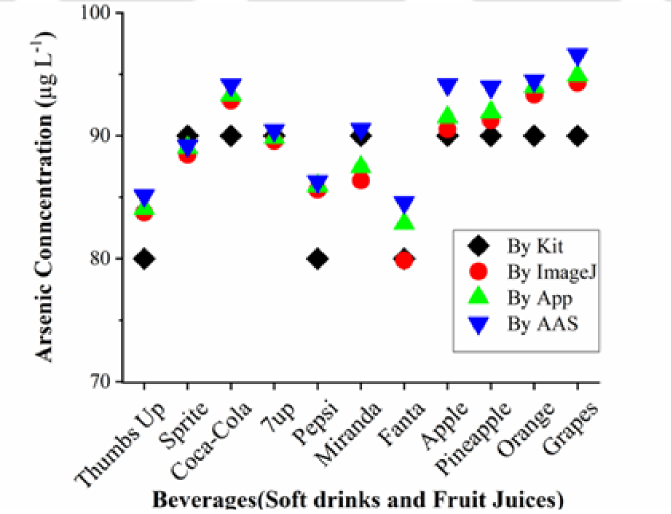


Fig. 3.12: Performance of the indigenous mobile App with respect to the color chart, ImageJ, and AAS analysis for beverages.

3.4 Conclusion

This study has successfully developed a highly sensitive hybrid digital sensor for room temperature detection and quantification of arsenic from the different samples of water and beverage. The present POCT Kit is advantageous in terms of low detection time, easy operation, and the use of trace amounts of chemical reagents compared to the available test Kits. The present prototype may be considered to be a green sensor because of its little waste generation and minimum utilization of hazardous acid in the process. arsenic compounds have been converted to AsH_3 gas through the electrolysis technique to minimize the acid utilization and make the process zinc (Zn) free. The requirement of a 2 ml reagent volume makes the sensor less hazardous and more environmentally friendly. Response time of the sensor is 5 min and 3 min for the arsenic concentration range of (4-20) µg/L and (30-100) µg/L, respectively. This study has made both qualitative and quantitative estimations of arsenic from unknown samples of natural water and beverages. The results have also been validated with AAS readings. The color chart gives the average absolute deviation of 5.28% and 4.11% from water and beverages, respectively. The ImageJ software produces the average absolute deviation of 1.51% and 1.46% from water and beverages, respectively. On the other hand, mobile App confirms the minimum error of 2.96% and 2.36% for water and beverages, respectively. The mobile App-based digital sensor has been developed to minimize human efforts and negate measurement errors. The App has been generated using the java based code using RGB values of the sensor. This digital sensor accurately measures the arsenic concentration from the field samples. With this prototype, a miniaturized device can be fabricated, which can be used as a portable, eco-friendly, and user-friendly hybrid digital sensor for rapid detection of arsenic on the sites.

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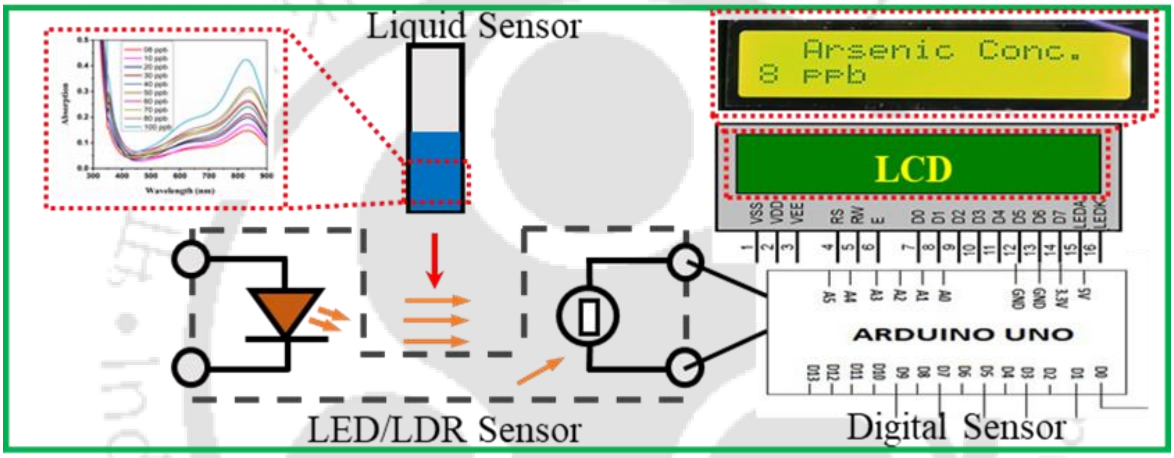
CHAPTER-4

A Photoresistor-based Point-of-Care Digital Sensor for Rapid Colorimetric Detection of Arsenic

Chapter 4 : A Photoresistor-based Point-of-Care Digital Sensor for Rapid Colorimetric Detection of Arsenic

Graphical Abstract



Abstract:

The contamination of water sources with arsenic from natural and human activities is one of the most critical global issues today. Now more than ever, it is essential to conduct continuous real-time assessments of water quality to identify the presence of arsenic pollutants and address potential threats. However, the majority of existing laboratory and portable techniques are either expensive or time-consuming due to the expert-driven complexity of their analysis processes. This study presents colorimetric sensors for the on-site quantitative liquid-phase detection of arsenic in water, vegetables, and rice. These sensors electronically measure the change in the color intensities of different arsenic concentrations. The sensor was converted to a point-of-care testing (POCT) device prototype and calibrated with known samples before testing with the real samples. The calibration plot showed that the intensity of the color change was proportional to the change in the resistance of the LDR. The prototype was further translated into a Portable device. The device can be used to monitor arsenic in real samples. This device is specific, stable, and user-friendly, allowing for the rapid detection of the arsenic contaminant in water and vegetables, and can detect arsenic in the range of 8-100 µg/L.

Keywords: Arsenic, Colorimetric, LDR sensor, Portable digital sensor, POCT device

4.1 Introduction

The increasing arsenic concentration of groundwater is a significant problem in many areas of the world [1], [2]. The recent discovery of groundwater in West Bengal, Bangladesh, and different places has led to a reassessment of the factors that regulate the distribution of arsenic in the natural environment and how it accumulates [3]–[5]. The distribution of arsenic in the region's aquifers is highly random [6], [7]. The potential sources of arsenic are volcanoes, arsenic-containing minerals, industrial processes like chromate copper arsenate for smelting, wood treatment, solid residues from mining extraction, and agrochemicals etc. [8], [9]. Due to this aquatic contamination, eatable and drinkable items such as vegetables, food items, and soft drinks are getting enriched with arsenic and becoming health alarming to the human being. Exposure to arsenic can trigger toxic effects on millions of people. The poisonous impact may cause cancer and other diseases like hypertension, thickening and discoloration of the skin, and diabetes [10], [11]. So it is essential to ensure the arsenic concentration is below the allowable limit in potable water, edible and drinkable items. So, there is an outmost need of real-time detection on monitoring of arsenic. For accurate measurements of arsenic, the widely used laboratory-based methods are atomic absorption spectrophotometry (AAS) hydride generation AAS (HG-AAS), Inductively coupled plasma mass spectrometry (ICP-MS), HG-induced plasma- ICP (HGIP-ICP), graphite furnace AAS (GF-AAS). However, these are expensive, and facilities are not always available. Similarly, arsenic detection has proven less costly and more accurate to the lower limit in the electrochemical method. It still depends on the lab setting with the bulk electrodes in the electrochemical cell and suffers from other co-deposition interference metals [12]–[16].

It is more desirable to measure the concentration directly in the field. So, there is a high demand for simple, sensitive, inexpensive, fast, and on-site detection methods for aqueous inorganic arsenic [17]. The colorimetric method can fulfill this requirement, as one of the reliable approaches is based either on the 'arsenomolybdenum blue' formation [18] or arsine (AsH_3) generation [19] [20]. These analytical methods can be used to determine arsenic at the micro-levels or less. However, arsine gas suffered from safety and environmental issues due to toxic arsine gas produced during the operation [2]. Despite this disadvantage, this colorimetric technique has been widely used worldwide because of its ability and simplicity of on-site use.

As per the literature [21], this colorimetric molybdenum blue method was proposed by Osmond J. D. in 1964, and then Johnson and Pilson [22] first proposed an elegant modification scheme of this method for measuring arsenic and phosphate separately in an aqueous solution [12]. The modification involves with selective reduction of arsenate to arsenite. This scheme gives the measurement of phosphates independently and determines the total inorganic arsenic in the solution. The use of thiosulfate ($Na_2S_2O_3$) in this process is the weakest aspect, which produces SO_2 and readily escapes from the acidic solution [23]. It leads to the formation of colloidal sulfur, which precipitates immediately. It slows down the reaction rate and reduces the effectiveness, particularly for field measurements. It takes about 1 hr to complete the reaction at $Na_2S_2O_3$ concentrations below 1 mol/L. In the past 10 years, a number of reducing agents have been tested [8], [24]– [26], but no one fulfills the requirements of the scheme originally described by Johnson and Pilson. In a nutshell, dithionite has been suggested [9] to address the Johnson and Pilson scheme requirement. Side by side, the oxidation scheme was also grown to get the total arsenic of the sample. For calculating the total arsenic in water, the various authors [24] [25] used different oxidizing agents like H_2O_2, KIO_3, $KClO_n$, KIO_n (where $1 \leq n \leq 4$), $KMnO_4$, and Fe^{2+}/Fe^{3+} to convert the arsenite to arsenate. Among those, KIO_3 was the most commonly used oxidant for arsenite oxidation.

The existing literature has shown that the molybdenum blue method has undergone various modifications[1], [24], [26]–[29]. Many researchers used the established technique, but it requires trained personnel to prepare reagents containing ammonium molybdate (AM), potassium antimony tartrate (PAT), sulfuric acid, and L-ascorbic acid as this reagent mixture becomes generate color while mixing, will take time to form the complex and unstable after 24 hr. Another more convenient procedure for the on-site application of the molybdenum blue method is described in the ISO norm [4]. In contrast to the standard approach, the reagent mixture requires two solutions for the ISO norm: one mixture containing AM, PAT, and sulfuric acid and a second containing L-ascorbic acid solution. Lenoble et al. [24] optimized the reagents concentrations of those solutions and the required amount of solutions for the taken sample. In this analysis, solutions are added to the water sample successively, and this procedure takes almost 1 hr to detect 10 µg/L of H_3AsO_4 at room temperature or 10 min at 50 ^{0}C [24]. In this case, the low concentration of AM in the final solution slowers down the rate of color development. Increasing the concentration of the AM in a mixture can enhance the rate of color development, potentially eliminating the need for a heating step. However, it is crucial to optimize the concentrations of other components in the mixture to maintain the desired reaction and product outcomes [29], [30]. Metelka et al. [31] studied the potentiometric titration method to determine the arsenate by converting it to molybdoarenstae hetero-poly anions.

Affordable field test kits are essential for accessibility to the common people. Some of the above literatures have focused on developing field test kits, and most of them are not digital. In this direction, Joyati Das et al.'s [32] work is one of the visible attempts. They developed a hydrogel-based colorimetric paper sensor to develop the arsenomoyldbdate complex on it. For this study limit of detection was low (10 µg/L), but it isn't easy to distinguish two conjugative sensor responses.

The molybdenum blue method's significant disadvantage in arsenic detection from water is the interfering of phosphate with arsenate. Arsenate and phosphate's physical and chemical properties are similar and usually exist together in the water. Therefore, they form molybdenum complexes, namely molybdophosphate and molybdoarsenate, respectively, with similar color band spectra and molar absorptivity (ε_{max} ~20,000 l mol^{-1} cm^{-1}) in the 500–950 nm range [24], [25].

According to the aforementioned investigations, it is clear that there are numerous colorimetric methods for the detection of arsenic. Each methodology has its own set of merits and demerits. However, some of the above techniques can be implemented in the digital platform, which may help to develop the portable arsenic sensor for monitoring the water quality. So, the present work attempts to design a digital point-of-care testing (POCT) device based on the optical sensor's principle. This can give fast analysis and accurate diagnostics. For this, the Molybdenum Blue method has been modified to achieve rapid and accurate measurements of total arsenic in an aqueous and edible sample, below 10 µg/L concentrations. For this, reagent concertation and reagent mixing scheme have been optimized before the sensor development. The present optimized scheme performs well in the presence of phosphate interference up to 1 mg/L . The reaction generates blue color, which indicates the formation of aresnomolybdate complex. The color intensity changes with arsenic concentration, and it is measured using the principle of an optical sensor. A prototype of the optical sensor has been fabricated with the help of LED, LDR, and Arduino-based microprocessors. Sensor's responses have been calibrated with the known arsenic concentration. This prototype can display the arsenic concentration at µg/L level on an LCD screen. The sensor works fine for aqueous and edible samples, and performance is comparable with AAS results. It is easy to handle for unskilled peoples and the study's detection limit was 8 µg/L which is below the world health organization's guideline value i.e., 10 µg/L.

4.2 Materials and methods

4.2.1 Materials

Sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$), Ammonium molybdate tetrahydrate, L-ascorbic acid, and Sodium dihydrogen phosphate were procured from Sigma-Aldrich, India. The chemicals above were of analytical grade and used without further purification. All the stock solutions and dilutions are prepared systematically. The Milli-Q grade water was used in all the experiments unless stated otherwise. The Arduino Uno R3 development board, Breadboard, liquid crystal display (LCD), white light emitting diode (LED) resistors, light dependent resistors (LDR), and Multimeter were procured from Rhydo Labz (India). UV cuvette from AXIVA private limited, India. Jumper wires (M&M) supplied by Robotics Embedded Education India.

4.2.2 Preparation of Reagents

Arsenic solutions were prepared by dissolving 0.0173 g of sodium arsenite in 1000 ml of deionized water to make 1 mg/L of stock solution. 100 µg/L and 1 µg/L solutions were prepared by diluting the 1 mg/L solution. The ammonium molybdate solution was prepared by adding 8.9 g of ammonium molybdate heptahydrate to 100 ml of water. The $KMnO_4$ solution was prepared by dissolving 0.8 g of $KMnO_4$ in 100 ml of water to convert As(III) to As(V). The ascorbic acid solution was made by dissolving 11.1 g of ascorbic acid in 100 ml of water, and different concentrations (2M, 3M, 4M, 4.5M, 5M, and 6M) of acid solutions were prepared by diluting concentrated HCl and H_2SO_4 acids.

4.2.3 Optimization of reagent's mixing scheme for molybdenum blue method

The molybdenum blue method is one of the confirmatory tests for liquid phase detection of arsenic (As (v)). The formation of the molybdenum blue complex is the key point of this route.

In the molybdenum blue method, the four fundamental reagents, namely $KMnO_4$, ammonium molybdate, HCl (or H_2SO_4), and ascorbic acid, are necessary to form the color solution of the arsenomolybdate complex. $KMnO_4$ is used to convert As(III) to As(V), ammonium molybdate heptahydrate is used to form the molybdenum blue complex, and HCl or H_2SO_4 is used to provide the necessary pH of the reaction mixture. Ascorbic acid acts as an initiator of the reaction, i.e. formation of arsenomolybdate complex from As (V). The color changes observed in this reaction is used to determine the concentration of arsenic in the sample. It is important to note that a particular order/sequence of mixing of reagents and optimum reagent concentrations can give an accurate result. So, this section aims to find out the optimum concentration and mixing sequence of the reagents. The following three mixing sequence is possible as shown in *Fig. 4.1*. The only sequence, shown in *Fig. 4.1*c produces the blue color, which is the indication of formation of arsenomolybdate complex. The formed arsenomolybdate blue complex acts as liquid sensor for arsenic.

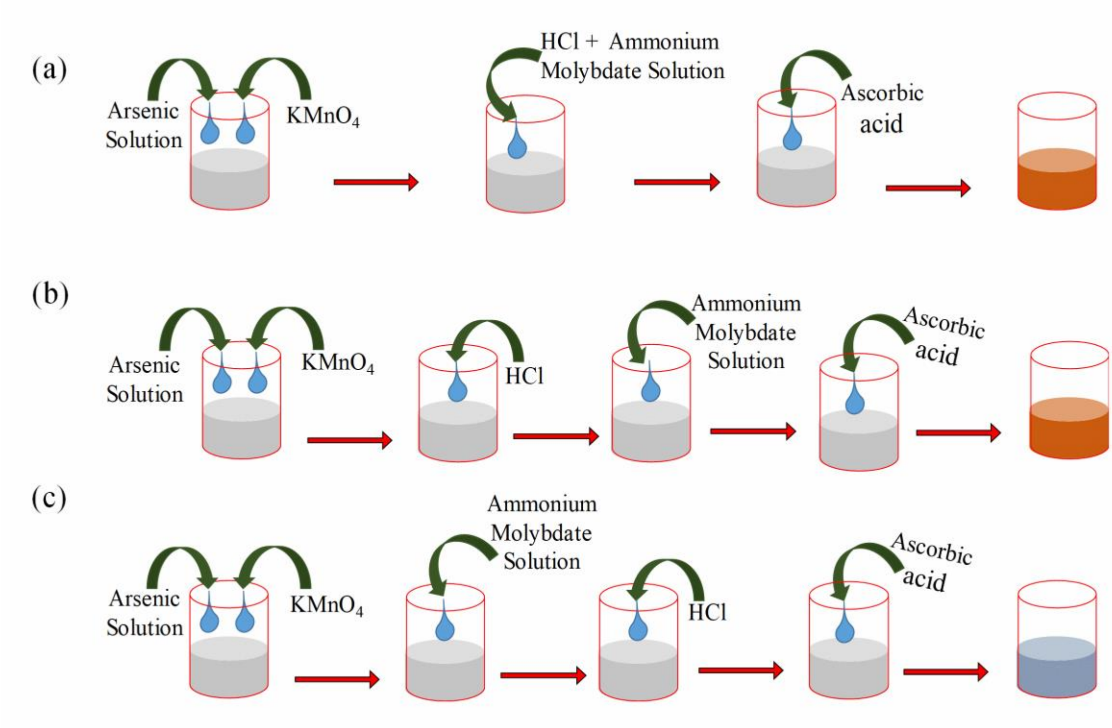


Fig. 4.1: Various mixing sequences for the molybdenum blue complex-based liquid sensor. (a) & (b), the mixing order does not produce an arsenomolybdate complex. (c) the right mixing order forms the arsenomolybdate blue complex. (blue-colored solution).

The formation of the complex was confirmed by UV-Vis spectroscopy. The quantification of this color intensity may directly relate to the arsenic concentration of the sample. The other two sequences, *Fig. 4.1*a and *Fig. 4.1*b, do not produce a blue color.

4.2.4 Design and fabrication of the digital sensor

The design aimed to create a Light-Emitting Diode (LED)/ Light Dependent Resistor (LDR) based digital sensor for quantifying the color intensity of liquid sensor arsenomolybdate complex, which may lead to a calibration plot of arsenic concertation vs absorbance. The circuit consists of a white LED, an LDR, an Arduino microprocessor, resistors, and a liquid crystal display (LCD). All the components are connected through a breadboard and jumper wires (M&M), as shown in *Fig. 4.2*d. *Fig. 4.2*b illustrates the working principle of an LED/LDR optical sensor, where the LED and LDR are placed opposite to each other across a sensing chamber. Here, a cuvette is working as a sensing chamber, where a molybdenum blue test will be carried out. *Fig. 4.2*b shows the actual circuit of the optical sensor developed indigenously. It displays the digital output of the LDR in terms of resistance, which can be used to develop a calibration curve for measuring the actual arsenic concentration. Here, a Multimeter is used to test the response of the fabricated sensor. Finally, the Multimeter has been replaced with an Arduino Uno development board and an LCD, as shown in *Fig. 4.2*c. In this, the LDR's output is converted into digital concentration using calibration data. The resulting prototype (*Fig. 4.2*d) is a portable digital sensor convenient for measuring the concentration of arsenic in liquid samples.

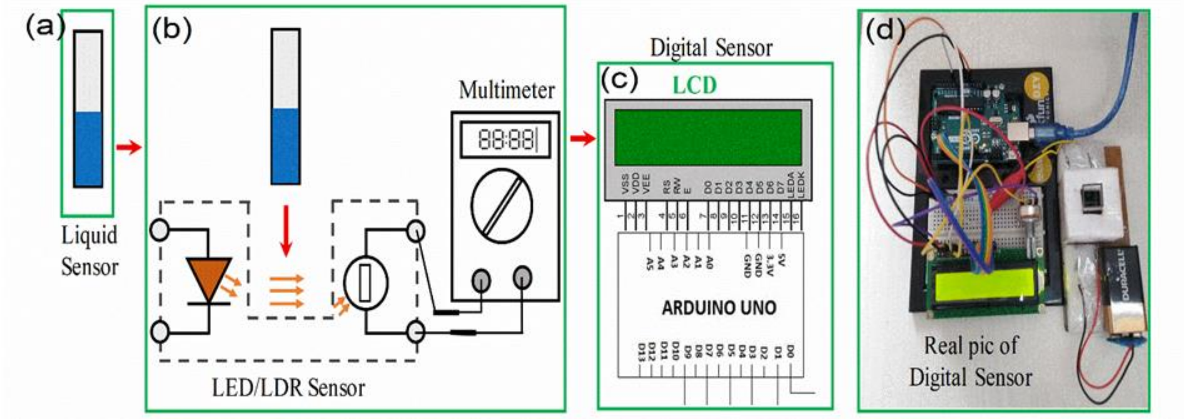


Fig. 4.2: Illustrates the prototype fabrication steps of the POCT arsenic sensor. (a) The liquid sensor in the sensing chamber, (b) LED/LDR sensor circuit, (c) Arduino Uno integrated LCD for digitalizing the arsenic concentration (µg/L), (d) photograph of the prototype.

4.2.5 Experimental methodology

The experiments were performed based on the scheme shown in Fig. 1c. Initially, one ml of aqueous sample was taken in the UV cuvette and mixed with 50 µL of $KMnO_4$ solution. Then 200 µL of ammoniummolybdate and 50 µL of HCl were added with the mixture in a sequence as mention in *Fig. 4.1*c. Finally, 20 µL of ascorbic acid was added to initiate the α to β keggin transformation, which confirmed the formation of arsenomolybdate complex by its characteristics blue color (*Fig. 4.2*a). Now, the color intensity of the aresnomolybdate complex solution (liquid sensor) was measured unsing indegeneous LED/LDR digital sensor as shown in *Fig. 4.2*b and corelated with the Arenic concentration through a calibration curve. The working principle and various components of the sensor have been described in *section 4.2.4*. Finally, the indigenous digital sensor will display the digital value in terms of concentration (µg/L) on the screen.

4.3 Results and discussion

4.3.1 Optimum mixing scheme and reagent's concentrations

The blue color formation in *Fig. 4.1*c is an indication of the formation of an arsenomolybdate complex, which will be used to develop an optical sensor for liquid-phase measurement of arsenic concentration in a sample. The formation of the complex was confirmed by UV-Vis spectroscopy, and the maximum absorbance was observed between 810-850 nm, as shown in *Fig. 4.3*a. The plots (*Fig. 4.3*a) show the UV absorbance for the concentration range of 08-100 µg/L arsenic. The figure indicates that the intensity of absorbance increases as the concentration of the solution increases.

The formation of blue colored arsenomolybdate complex is the heart of the sensor. It forms in two eteps,- at first formation of α-Keggin in the presence of HCl or H_2SO_4, and then conversion of α-Keggin to β-Kiggin in the presence of ascorbic acid, which produces the blue color during the conversation. However, similar color may be observed due to the interference of phosphate. So, the optimum concentration of acids and phosphate is essential for this kinetics at which phosphate will not interfere, but arsenic will form the complex. For this purpose, reaction kinetics has been studied with the help of UV-Vis spectroscopy, following the reaction scheme mentioned in *Fig. 4.1*c. reaction was performed in a cuvette with different concentrations of HCl and H_2SO_4 ranging from 2-7 M. The time-dependent kinetics of the formation of the arsenomolybdate complex was observed under UV-Vis spectroscopy within the wavelength ranging from 820-850 nm. The detailed analysis has been shown in *Fig. 4.2*b and *Fig. 4.2*c for HCl and H_2SO_4, respectively. Both figures show that absorbance initially changes rapidly with time and then becomes flat. The intersecting point gives the reaction completion time. The reaction completion time decreases with increasing acid concentration. A higher concentration of acids leads to a completion time of around 25 minutes, while a lower concentration may lead to a formation time of more than 3 hrs. The time-dependent stability of the complex was also studied at moderated acid concentrations (4.5-6 M), as shown in *Fig. 4.2*d and *Fig. 4.2*e. The figures indicate that the absorption peak of the arsenomolybdate complex shifts below 800 after 4 to 5 hours at higher acid concentrations, leading to a slight change in color intensity and turbidity. This shows that the higher concentration of acid decreases the stability of the complex. So, this analysis gives an optimum acid concentration of around 4.5 M and 5 M for

H_2SO_4 and HCl, respectively. Twenty-five minutes is considered the sensor's response time at these optimized acid concentrations.

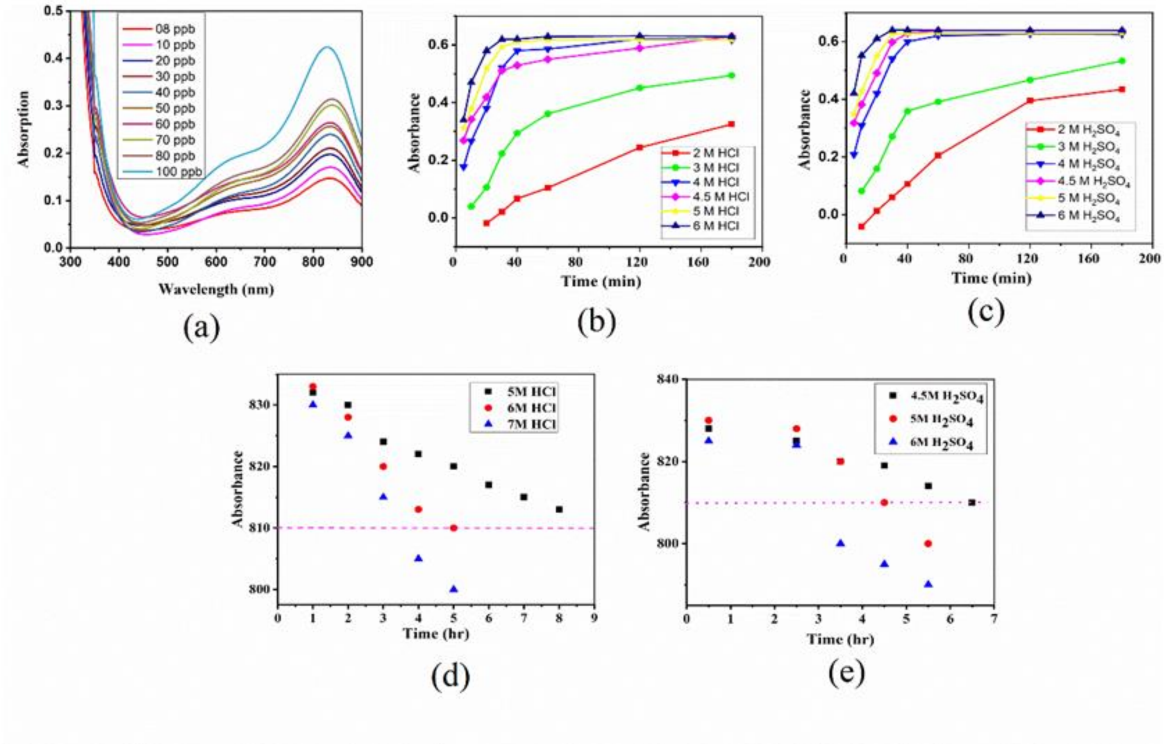


Fig. 4.3: (a) shows the UV-Vis spectra for the formation of an arsenomolybdate blue complex (liquid sensor). (b) and (c) show the absorption kinetics of the arsenomolybdate blue complex with time and various acid strengths. (d) and (e) show the time-dependent stability of the liquid sensor.

The role of ascorbic acid is crucial in both the kinetics- the transformation of α-Keggin to β-Kiggin, and in preventing the formation of the phosphomolybdate complex. The greenish-blue phosphomolybdate complex will interfere with the reaction and mislead the sensor’s performance. So, ascorbic acid concentration was also optimized to eliminate the phosphate interference and get optimum sensor responses. For this, a range of 0.2-0.5 (% wt./wt.) ascorbic acid concentrations were tested, and a concentration of 0.4% wt./wt. was found optimum for detecting arsenic. This ascorbic acid concentration can prevent phosphate interference up to 200 µg/L. The detailed process was described in Annexure II.

4.3.2 Selecting a suitable unknown resistance for a digital sensor

The selecting a suitable resistor in a digital sensor is a critical step in ensuring accurate and reliable measurements based on multimeter readings. Selecting a suitable resistance in a digital sensor is a critical task. For this, after confirming the formation of the Keggin complex using UV-Vis spectroscopy, the color intensities of all samples were measured in terms of resistance using an LDR sensor and a multimeter initially. As the concentration of arsenic increased, the resistance reading on the Multimeter also increased, indicating a proportional relationship between the concentration and the resistance to light. A simple voltage divider circuit was used to calculate the resistance in the digital sensor. The unknown resistance of the arsenic sample was determined out of 1 kΩ, 3 kΩ, 5 kΩ, and 10 kΩ resistors and comparing the results with the readings obtained from the Multimeter as shown in *Fig. 4.4*. It shows that 3 kΩ resistor and Multimeter produce almost same results. So, this 3 kΩ resistor was selected as suitable resistor for a digital sensor.

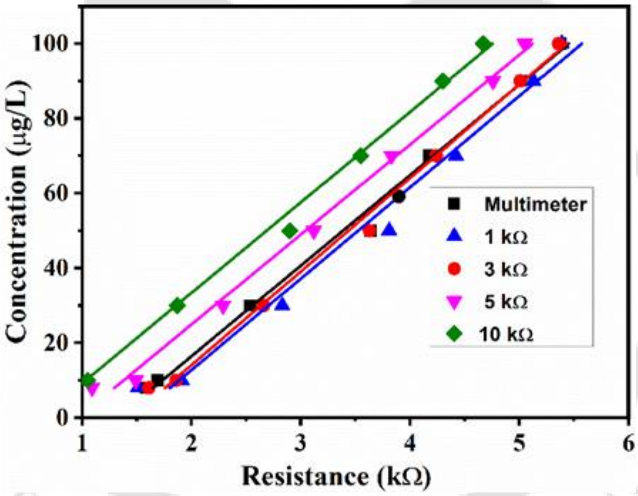


Fig. 4.4: Illustrates the resistor plots with respect to concentrations to select a suitable unknown resistance for a digital sensor.

4.3.3 Sensing Mechanism

Before dispensing the sample, the empty cuvette was illuminated by a light source, i.e., a LED light, and the initial resistance of the liquid sensor was recorded using the LDR. The resistance readings of the water sample and blank test samples were then measured, giving the same values and considered as R_0. The sensors' final resistances (R_i) were recorded after dispensing various concentrations of arsenic samples, ranging from 0-100 µg/L. It was observed that the color intensities of the sensor increased with increasing the arsenic concentration. The intensity decreases due to a decrease in light transmission to the LDR and an increase in R_i. A calibration plot was drawn using these R_i values and the optimized resistor value to calculate the unknown sample concentration (in *Fig. 4.5*a). It yields the following linear equation (1) with a high regression coefficient (R^2) value of 0.994,

$y = 24.88\,x - 35.47$……………………………..(1)

y = Arsenic concentration of the sample

x = resistance of sample drawn from LDR sensor

The above equation was incorporated into the interface algorithm between the microprocessor and the display unit. The digital sensor was completed so that the LCD could display the concentration of any unknown arsenic sample, as shown in *Fig. 4.5*c.

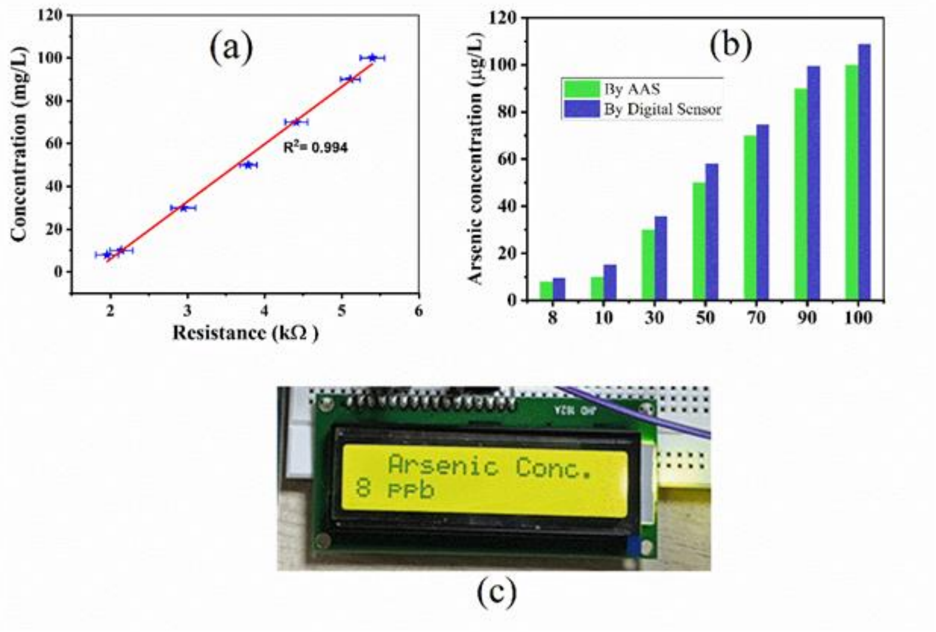


Fig. 4.5: (a) shows the calibration plot (Resistance vs. Concentration). (b) Assessment of digital sensor response through field samples, (c) Photograph of the digital response of the prototype.

4.3.3.1 Performance of sensor prototype on field water samples

The study aimed to assess the efficacy of a digital sensor in detecting arsenic in field samples collected from the Brahmaputra river in Guwahati, Assam. The Brahmaputra river water in this area exhibits more turbidity due to the presence of various contaminants such as heavy metals, total dissolved solids, etc. The samples were centrifuged to remove any particles before proceeding with further investigation. The arsenic concentration of the raw water samples was evaluated using AAS and was found to be zero. The spiking technique was used to simulate arsenic-contaminated field samples. The samples were then analyzed with both the digital sensor and AAS. The results of the study are shown graphically in *Fig. 4.5*c, and it was found that there was an absolute standard deviation of 0.78% compared to the AAS measurement. The study confirmed the excellent performance of the digital sensor in detecting arsenic in field samples, which may help to ensure the safety of drinking water in arsenic-contaminated areas.

4.3.4 Assessment of arsenic in vegetable and rice samples

The performance of the above-described indigenous optical sensor was thoroughly tested with various real-life samples like vegetable and food samples. The vegetable and food samples were collected from a local market, IIT Guwahati, Assam, India. Rice-1, Carrot, beetroot, potato, potato skin, tomato, green peas, maggi, and dried grapes were collected from a local market, IIT Guwahati, Assam, India. At the same time, Rice-2 was collected from Habra, Wes Bengal, India. The vegetables were cleaned with water to eliminate dust and soil particles and then ringed with deionized. Samples were chopped into small pieces with a ceramic knife and kept in clean polyethylene bags before analysis. To measure the arsenic of every sanple, all samples were dried in an oven at 80 °C for 48 hr, followed by cooling in a desiccator. After being cooled, the samples were ground into powder using a laboratory grinder (Butterfly Smart Mixer Grinder, 750 W, India) and kept at room temperature until digestion. The primary digestion process was employed to extract arsenic from processed vegetables or rice samples. The experimental method was nearly similar to earlier research [33]–[36]. In brief, subsamples (0.5 g) were put into quartz tubes that were digested in 5 ml of concentrated nitric acid (HNO_3) that were cleaned and dried before 5 ml of HNO_3 (Fluka TraceSELECT, purity greater than 69.0%) was added. Digestion of the samples took place at room temperature overnight inside the fume hood. On the following day, samples were heated in a water bath set to 80 °C for 2-3 hours until the volume of extract had been reduced to 1–2 ml, and fumes were no longer being produced. After that, the samples were allowed to return back to room temperature, then 10 ml of distilled water was added directly into the quartz tubes to dilute the concentrated extract. The diluted extract was filtered using Whatman filter paper No. 42 and diluted further with distilled water to a final volume of 25 ml. The final volume was utilized for AAS analysis to measure the arsenic of the sample. From the AAS results, it was found that all the samples were arsenic-free. Due to this reason, the spiking technique was planned for the raw vegetables and rice with the known amount of arsenic through cooked and soaking. For this, 500 µg/L and 1000 µg/L of arsenic solution were used for cooked and soaking. Cooking was done for 30 minutes, and socking was allowed for 48 hours. Synthesized arsenic enriched samples were collected and followed the same produce i.e., Bhatti et al. (2013) method as mentioned above to get the extract. The extract was analyzed with AAS and an indigenously developed digital optical sensor to estimate the arsenic concentration. The obtained results are shown graphically in *Fig. 4.6*. According to the findings, the current study provides the optimum response for measuring arsenic concentrations in vegetables and food items. And that it has a good level of concordance with AAS. The average standard deviation for boiling was 0.68, while the average standard deviation for soaking was 0.51.

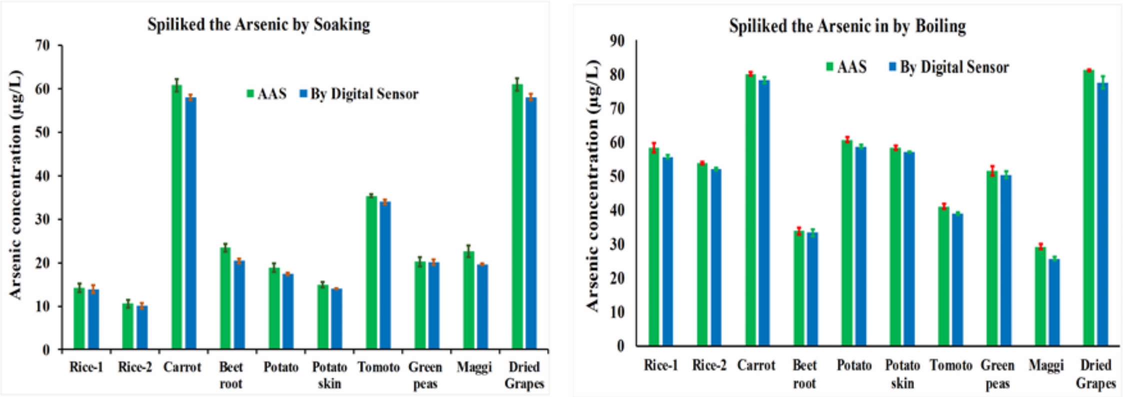


Fig. 4.6: Compares the performance of the fabricated digital sensor with AAS results for the real samples of vegetables and rice. (a) Arsenic spiked by soaking (b) Arsenic spiking by boiling.

4.4 Conclusion

The current study describes the design and development of a LED/LDR based optical sensor for liquid-phase detection of arsenic in water, vegetables, and rice. The colorimetric reaction - forming a blue-colored arsenomolybdate complex, plays a key role in this sensor. The color intensity of the arsenomolybdate complex changes with the amount of arsenic present in the sample. The formation of the complex was confirmed by UV-Vis spectroscopy. A simple LED/LDR sensor was developed to measure the color intensities of the liquid sensor. Light from the illuminated LED passes through the sensing chamber, and an LDR captures the transmitted rays opposite the LED. The calibration plot shows the monotonic variation of LDR resistance with arsenic concentration. The LED/LDR sensor unit is integrated with an LCD screen via an Arduino circuit to obtain the digital response of the sensor. The device's performance has been tested against various spiked samples of water, vegetables, and rice. The device shows good responses within the concentration range of 8-100 µg/L and a response time of about twenty-five min. It can be used as a POC testing kit due to its specificity, portability, and user-friendliness. The device has the potential for real-time detection of arsenic in water, vegetables, and rice in the field, which might be helpful in maintaining the quality of the food chain in arsenic-contaminated areas.

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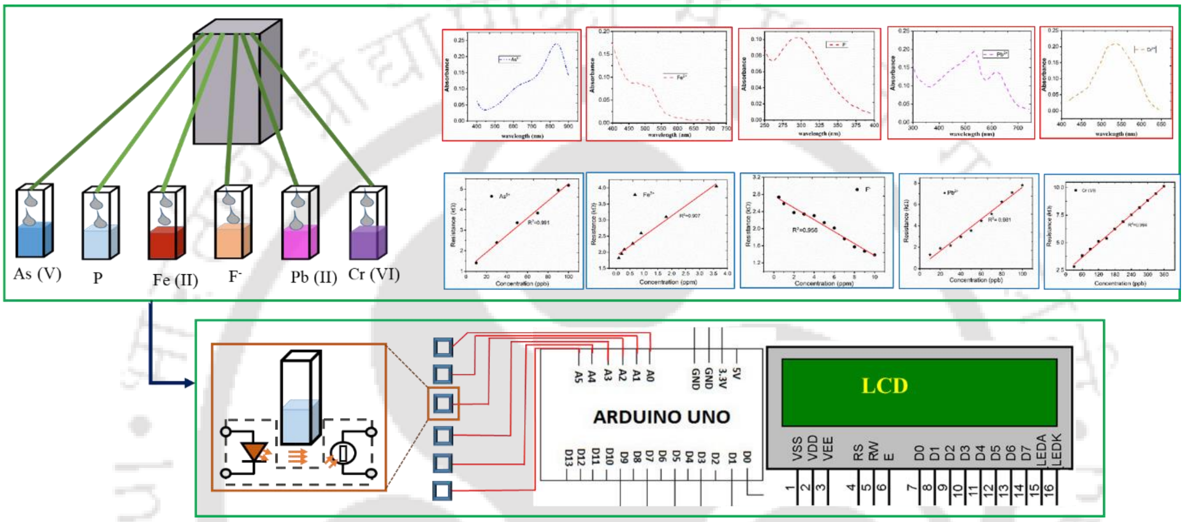
CHAPTER-5

Colorimetric Optical Analysis for Simultaneously Determining the Presence of Multiple Pollutants in Water

Chapter 5 : Colorimetric Optical Analysis for Simultaneously Determining the Presence of Multiple Pollutants in Water

Graphical Abstract



Abstract

In recent years, the contamination of water bodies by natural and manmade pollution are the most pressing global problems. Regular on-site monitoring of water quality has been mandated, in which several kinds of substances, such as organic, inorganic, heavy metals, and biological wastes, are discovered using defined techniques. However, the majority of present laboratory techniques are either expensive or time-consuming due to the process including expert-driven, advanced analysis techniques. The present study has developed the E-Eye for the simultaneous detection of heavy metals in samples. The sensor can do the quantitative on-site measurement of Arsenic (As), Iron (Fe), Chromium (Cr), Fluoride (F), Lead (Pb2+), and Phosphate levels in drinking water. The point-of-care-testing (POCT) device was used to measure the fluctuations in color intensities of liquid sensors by transforming them into electrical signals in response to variations in the concentrations of targeted pollutants. Prior to successful validations with actual samples, the sensors were calibrated using known amounts of contaminants. The sensors were then illuminated with a light-emitting diode from one side, while the transmitted rays were recorded by a light-dependent resistor (LDR) from the opposite side. The fluctuation in color intensity of the liquid sensor as a function of pollutant loading was found to vary linearly with the LDR's resistance. The sensor was subsequently adapted into a particular, stable, and user-friendly point-of-care device (POCT) that allowed for the affordable detection of contaminants in aquatic samples on the spot.

Key Words: E-Eye, optical sensors, water pollution, multiple pollutants, multiplexing

5.1 Introduction

Measuring harmful environmental pollutants in real-time has become a crucial area of research in recent years, given its significant impact on reducing long-term health risks[1], [2]. Point-of-care-testing (POCT) devices, which are small, inexpensive, and user-friendly, are expected to play a vital role in achieving this goal [3]–[5]. Among the many contaminants in various water sources, the detection of these pollutants through POC testing has become a primary focus of attention, owing to their extensive global health implications [6]–[8]. Commercially available POCT devices are now capable of detecting a range of contaminants, including soluble and insoluble inorganic [9] and organic [9] pollutants, heavy metal ions, biological (BOD) and chemical (COD) [10]–[12] oxygen demands, and pathogens [13]. Despite significant advancements in this field over the past few decades [14], there remain several issues that require resolution.

An urgent requirement exists for a POCT device that can specifically detect targeted pollutants such as Arsenic (As), Iron (Fe), Chromium (Cr), Fluoride (F), Lead (Pb), and Phosphate in drinking water. The World Health Organization (WHO) states that the maximum permissible level of arsenic in drinking water is 10 μg/L. Currently, the recommended limit for iron in water is 0.3 mg/L, according to the Department of Natural Resources (DNR). Iron is classified as a secondary or "aesthetic" contaminant. Chromium concentrations in surface and drinking water may exceed the World Health Organization's maximum allowable concentration of 0.05 mg/L. The highest allowable amount of F in drinking water is 1.5 mg/L , while the maximum allowable amount of Pb^{2+} is 0.05 mg/L [9].

For instance, there is an immediate need for a POCT device that can find targeted pollutants in drinking water. The World Health Organization (WHO) says that the maximum permissible contaminant level of arsenic in drinking water is 10 μg/L. As per the Department of Natural Resources (DNR) 0.3 mg/L is the current recommended limit for iron in water. Iron is classified as a secondary or "aesthetic" contaminant. As a result, chromium concentrations in surface and drinking water can exceed the World Health Organization’s maximum allowable concentration of 0.05 mg/L. the most amount of F that can be in drinking water is 1.5 mg/L, while the most amount of Pb^{2+} is 0.05 mg/L [15]. Chromium concentrations in both surface and drinking water have the potential to surpass the World Health Organization's maximum allowable concentration of 0.05 mg/L [16].

Arsenicosis, Iron posing, chromium posing, Fluorosis, lead poisoning, and phosphate poising can be caused by more Arsenic (As), Iron (Fe), Chromium (Cr), Fluoride (F), Lead (Pb), and Phosphate in the water [17]. Also, too much of those metals in drinking water can cause other long-term problems with skin lesions and hard patches on the palms of hands and soles of feet; skin and internal organ cancers; diseases of blood vessels in the legs; and also diabetes, high blood pressure, and reproductive disorders. [18]–[20] the skin, teeth, and bones, the brain, the lungs, and the heart [21]–[25]. For Iron diabetes, hemochromatosis, stomach problems, and nausea. It can also damage the liver, pancreas, and heart. For Cr diarrhoea, stomach and intestinal bleedings, cramps, and liver and kidney damage. phosphates hypocalcaemia, hypotension and tachycardia. Human health can benefit from Cr III at appropriate levels, as it is involved in lipid and glucose metabolism, while Cr VI is toxic [26], [27]. Exposure to Cr VI can lead to health issues such as skin rashes, kidney and liver damage, internal hemorrhage, teeth abnormalities, and respiratory ailments, including lung cancer [28]–[31].

Absolutely, there is a significant need for an E-Eye and POCT device that can provide rapid information on the levels of targeted pollutants in drinking water. It is worth noting that optical [32], [33] or electrochemical [34], [35] techniques are commonly used in conventional methods for determining these elements in water. However, many of these methods are either costly or time-consuming and necessitate specialized testing and analysis [36]. In this context, recent advances in micro or nanoscale technologies have shown promise for the development of inexpensive, specific, stable, portable, rapid, and user-friendly POCT devices for detecting these contaminants. For instance, the utilization of various nanomaterials [37], [38], nanostructures [13], and carbon allotropes [39] has enabled the measurement of dissolved targeted pollutants in water. Nevertheless, these sensors have yet to be integrated into POCT devices due to their high fabrication cost, the requirement for specialist handling, and longer detection time.

In this study, we have developed colorimetric sensors based on liquid sensors that are capable of measuring the levels of targeted pollutants in drinking water. The concept of using liquid sensors to create colorimetric sensors was inspired by recent noteworthy contributions [40], [41]. These sensors work by detecting color changes resulting from specific chemical reactions: (a) ammonium molybdate and As(IV) for arsenic sensing, (b) 1,10-Phenanthroline and Fe^{2+} for Iron sensing, (c) 1,5-diphenylcarbazide and Cr(VI) for chromium sensing, (d) Fe(III) thiocyanate and F^- for fluoride sensing, (e) 1,5-Diphenylthiocarbazone (dithizone) and Pb(II) for lead sensing, and (f) ammonium molybdate and P for phosphate sensing.

In summary, the study developed liquid-based colorimetric sensors that can detect targeted pollutants in drinking water. The sensors work by detecting changes in color caused by specific chemical reactions. To measure these color fluctuations, an LED and a light-dependent resistor were used, which proved to be sensitive and able to detect unknown quantities of pollutants in water samples. The sensors were then incorporated into an Arduino platform to create a portable and user-friendly POCT device for on-site, low-cost, and immediate monitoring of water quality. The performance of the device shows promise in providing an efficient and accessible platform for monitoring water quality in real-time.

5.2 Materials and methods

5.2.1 Materials

Sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$), Ammonium molybdate tetrahydrate, L-ascorbic acid, Sodium dihydrogen phosphate were procured Sigma-Aldrich, India. Iron (iii) Chloride, Ammonium thiocyanate, Bromothymol blue, 1,5-Diphenylthiocarbazone (Dithizone), Sodium Fluoride, Lead (ii) Chloride, Potassium sodium tartrate, Dimethyl formamide (DMF), Carbon tetra chloride (CCl_4), Sodium hydroxide, Hydrochloric acid, 125 mm filter paper (grade 1), were obtained from Merck (India). The chemicals above were of analytical grade and used without further purification. The Milli-Q grade water was used in all the experiments unless stated otherwise. The Arduino Uno R3 development board, Liquid crystal display (LCD), resistors were procured from Rhydo Labz (India).

5.2.2 Experimental procedure

5.2.2.1 Background of Calorimetry based detection of targeted pollutants

Colorimetric-based detection techniques for water pollutants involve the use of chemical reactions that produce a change in color when a target pollutant is present in a water sample. The intensity of the color change is proportional to the concentration of the contaminant. These techniques are often simple, inexpensive, and require minimal equipment, making them ideal for field testing. For this study, initially targeted pollutant are detected individually based on colorimetric detection technique. For that, 1,5-Diphenylthiocarbazone (Dithizone) used for the detection of lead (Pb^{2+}) in water samples. Dithizone reacts with Pb^{2+} to form a red-colored complex, which can be quantified using a spectrophotometer. 1,10-Phenanthroline monohydrate used for the detection of iron (Fe) in water samples. The addition of 1,10-Phenanthroline monohydrate to a water sample containing Fe results in the formation of a reddish-orange complex, which can be detected using a colorimeter. Fluoride (F) in water can be detected using the iron (III) thiocyanate solution, where fluoride ions react and result the formation of a colorless complex. Chromium (Cr) in water can be detected using the diphenylcarbazide method, where diphenylcarbazide is used to react with Cr (VI) ions to form a purple-colored complex, which can be measured using a colorimeter. These are the targeted pollutants of colorimetric-based detection techniques for water pollutants. All those methods are used after optimizing the reactants. All the specific chemical reactions are written bellow.

$$As + 12MoO_4^{2-} + 24\,NH^{4+} + 3H_20 \rightarrow (NH_4)_3AsMo_{12}O_{40} + 24\,NH^{4+} + 12H^+ \ldots.\ldots (1)$$

$$(NH_4)_2MoO_4 + 18\,H_3PO_4 \rightarrow (NH_4)_3PO_4.12MoO_3 + 42\,H_2O \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$Fe^{2+} + 3\,C_{12}H_8N_2H^+ \rightarrow [Fe(C_{12}H_8N_2)_3]^{2+} + 3H^+ \ldots\ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Fe\,(SCN)_3 + 6NaF \rightarrow (FeF_6)^{3-} + 6Na^+ + 3(SCN)^- \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(4)$$

$$PbCl_2 + 2\,C_{13}H_{12}N_4S \rightarrow Pb\,(C_{13}H_{12}N_4S)_2 + 2Cl \ldots\ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(5)$$

$$H_2CrO_4 + 2HOC_6H_4NHN = CPh_2 \rightarrow Cr(C_6H_4NHN = CPh_2)_2 + 2H_2O + 2H^+ \ldots\ldots.\ldots (6)$$

5.2.2.2 Test for detecting multiple pollutants in water

5.2.2.2.1 Arsenic test:

The molybdenum blue test is a commonly used colorimetric method for detecting the presence of arsenic in water samples. In this method, arsenic is first oxidized to arsenate using $KMnO_4$. Then, ammonium molybdate is added to the sample, which reacts with the arsenate to form a complex. This complex is then reduced using ascorbic acid, which produces a blue color. The intensity of the blue color is proportional to the concentration of arsenic in the sample. Phosphate is a common interfering element in the molybdenum blue test because it can also react with ammonium molybdate to form a complex that produces a blue color. Therefore, it is important to optimize the reactants such that they can find out the maximum phosphate interfering permitted.

In order to test for the presence of arsenic in a water sample contaminated with multiple metals, a solution of ammonium molybdate and an acid mixture was held in a porous media to prevent any reactions with contaminants or atmospheric conditions. Premixing of reactants will mislead the test and not allow the formation of arsenomolybdate complex after adding the arsenic to the solution. For the As colorimetric sensor, a 10 µg of hydrogel was initially placed in a cuvette. Then the optimal composition of reagents for the Molybdenum blue test, 250 µL of ammonium molybdate solution, and 250 µL of a mixture containing 0.4 wt% L-ascorbic acid were added to the hydrogel sequentially, and it was absorbed in hydrogel completely [42]. Next, 250 µL of the prepared arsenate stock solution was added to the hydrogel, as shown in *Fig. 5.1a*.

Checking of Phosphate interference: Similar produce was followed with 0.3 wt% L-ascorbic acid for checking the phosphate interference to understand whether phosphate is present in the sample. The positive results will give the light blue color as shown in *Fig. 5.1b*

5.2.2.3 Iron test

To optimize the response of 1,10-phenanthroline, the reactions were carried out using a 1:1:2 weight ratio of 1,10-phenanthroline, ascorbic acid, and trisodium citrate. The process involved mixing the three materials in a mortar at room temperature for 10-15 minutes and then keeping the obtained product in an airtight vial. It was found that 2.5 mg was sufficient for 0.25 mL of contaminated water. In Fe colorimetric sensor, 2.5 mg of the material was initially taken in a cuvette, then 250 µL of iron-containing water was injected into the cuvette. The color changes were observed, and the color intensity depended on the iron concentration. Initial trials were conducted using pure water ranging from 0- 4 mg/L iron-containing water, and the orange-red color appeared within 4 minutes. As shown in *Fig. 5.1c*, the color intensity of the solution developed as the iron concentration increased.

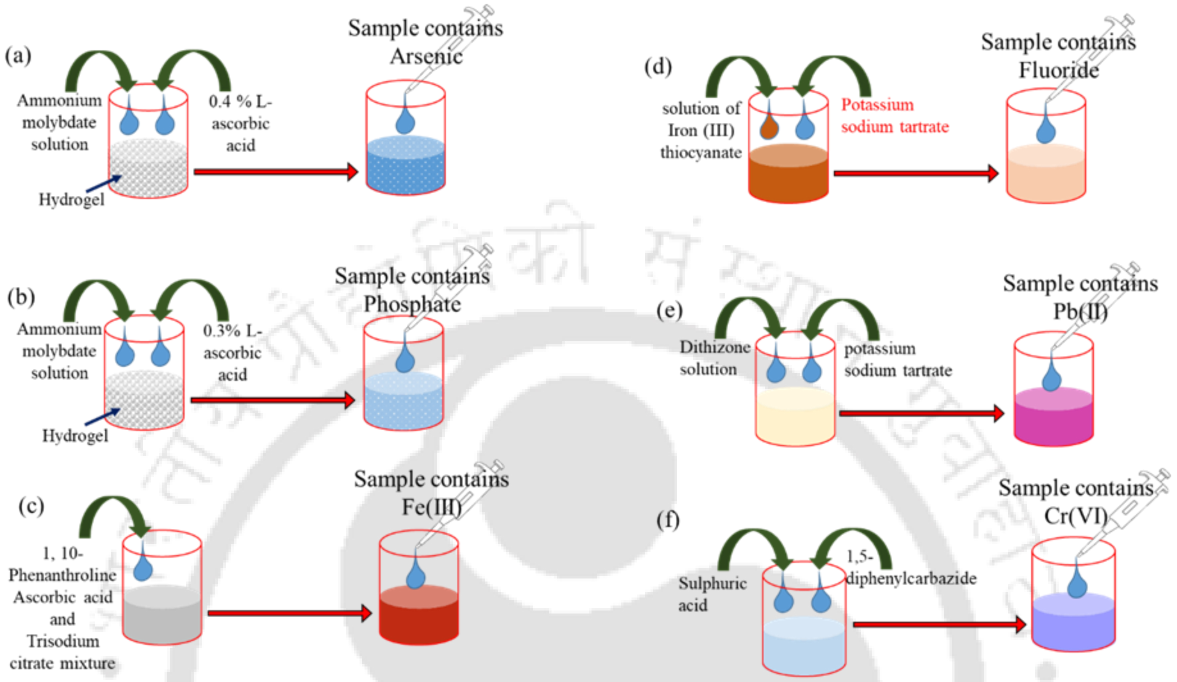


Fig. 5.1: Systematic procedure for colorimetric based measurement of different pollutants, Arsenic (As), Phosphate, Iron (Fe), Fluoride (F), Lead (Pb), and Chromium (Cr).

5.2.2.4 Fluoride test

For the F colorimetric sensor, 0.1 M of ammonium thiocyanate solution (w/v) and 0.1 M of iron (III) chloride solution (w/v) were mixed in equal amounts to make a red brick iron (III) thiocyanate solution (v/v). Then, 10 µl potassium sodium tartrate was added to 100 µl of a red brick iron (III) thiocyanate solution. Now 500 µl of sample volume i.e. fluoride-containing water sample was added to the synthesized mixture *Fig. 5.1d*. The intensity of the red brick color solution became red to a colorless solution.

5.2.2.5 Lead test

For the Pb^{2+} sensor, 60 µl of a 0.05% dithizone solution in CCl_4 was mixed with 30 µl of a saturated potassium sodium tartrate solution. It generated a light pink color as shown in *Fig. 5.1e*.

5.2.2.6 Chromium test

0.2 M Sulphuric Acid and 0.5 % (w/v) 1, 5-diphenylcarbazide were added, and the mixture was gently shaken and let for 4 min. The mixture turned to purple color as shown in *Fig. 5.1f*. All the above-mentioned reaction was conformed with a UV-vis spectroscope.

5.2.3 E-Eye for the detection of heavy metals

To identify multiple metals at a time, the study developed a system as shown in *Fig. 5.2*. It consists of a 10 ml sample vial and six 3 ml cuvettes, as depicted in the figure. The cuvettes were utilized as reaction chambers for targeted pollutants. The corresponding reagent mixtures were first transferred into the cuvettes and then connected to the 10 ml sample vial via 45^0 angles capillary tubes. Upon filling the contaminated water sample into the center vial, the water immediately flowed into the different reaction chambers through capillary tubes due to the self-siphoning technique. The specific chemical reactions occurred at different cuvettes and corresponding characteristic color was formed. Each color represented the presence of a specific metal in the water.

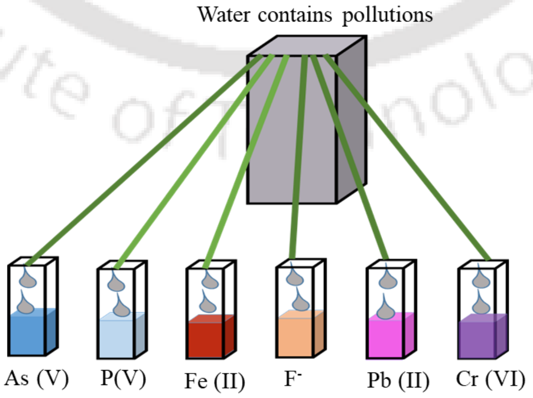


Fig. 5.2: Schematically illustrate the colorimetric detection for targeted pollutants in a single drop of water and their color change.

5.2.4 Design and fabrication of a multi-fluxing digital sensor

The digital sensor was designed and fabricated to quantify the color intensities of the diffident liquid-based sensors for measuring the concentration of targeted pollutants. The sensor detected the metal pollutants through a colorimetric method. It consisted of a series of Light-Emitting Diode (LED) and a Light Dependent Resistor (LDR) sensor. Those would enable the development of a calibration plot of concentration vs. absorbance for individual samples. The circuit comprises a white LED's, an LDR's, an Arduino microprocessor, resistors, and a liquid crystal display (LCD), connected through a breadboard and jumper wires (M&M). *Fig. 5.3*a illustrates the working principle of the multiplexed LED/LDR optical sensors. The LED and LDR are placed opposite to each other across a sensing chamber. *Fig. 5.3a* is a schematic representation of the multiplexed circuit of the optical sensor developed in-house, which displays the digital output of the LDR in terms of resistance. This is used to form the calibration curve for measuring the actual concentrations of specific pollutants. A Multimeter was used to check the response of the multiplexed sensors. Later on replaced it was replaced with an Arduino Uno development board and an LCD, as shown in *Fig. 5.3b*. In this configuration, the LDR's output is converted into digital value for all the pollutants using calibration data. The resulting prototype is a portable digital sensor that is convenient for measuring the concentration of all the targeted pollutants in liquid samples.

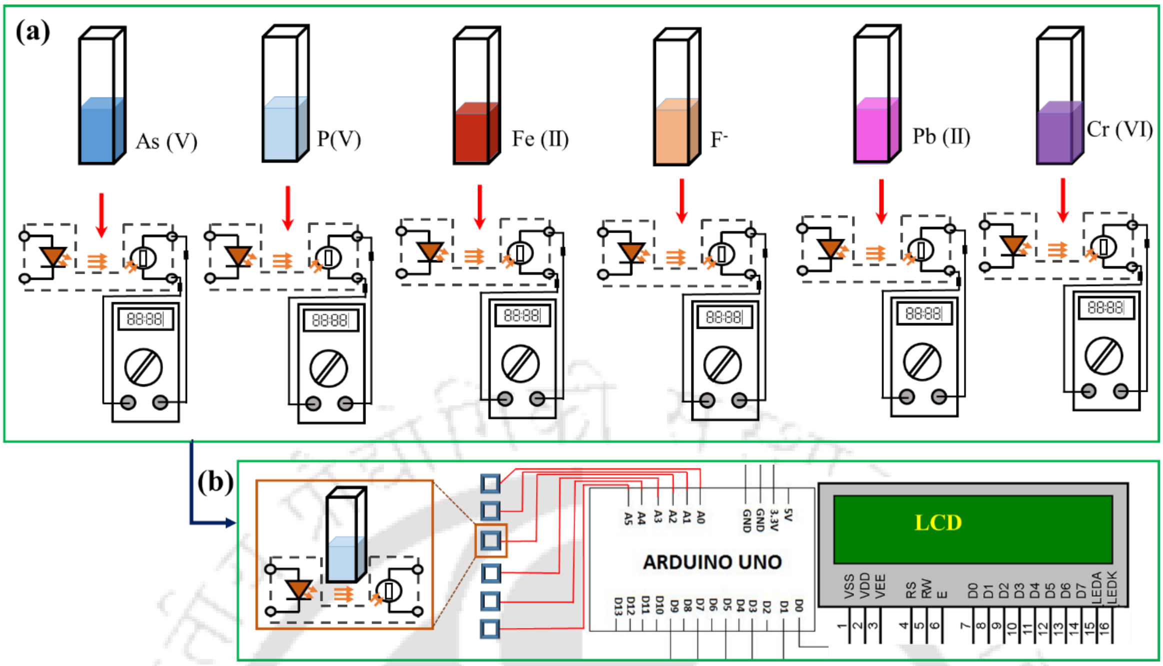


Fig. 5.3: Illustrates the prototype fabrication steps of the POCT Multiplexing sensor. (a) LED/LDR sensor circuits, (b) Arduino Uno integrated LCD for digitalizing the Multiple pollutants concentration.

5.3 Results and detection

The work focuses on developing some reliable sensors for liquid-phase detection of targeted pollutants. This will rely on interfering components and reactants concentrations. For that, in the As and P colorimetric sensor a range (0.2-0.5) % wt./wt. of ascorbic acid concentrations was tested and a concentration of 0.4% wt./wt. was found to provide the best results for arsenic in samples with up to 0.2 mg/L of phosphate. The detailed process for testing the weight percentages of ascorbic acid for arsenic concentration was described in Annexure II.

The blue color formation in *Fig. 5.1a* is an indication of the formation of an arsenomolybdate complex, which will be used to develop an optical sensor for liquid-phase measurement of arsenic concentration in a sample. The formation of the complex was confirmed by UV-Vis spectroscopy, and the maximum absorbance was observed between 810-850 nm, as shown in *Fig. 5.4a*. It was observed that the color intensity of the sensor increases as the concentration of arsenic increases. The formation of blue colored arsenomolybdate complex is the heart of the sensor. It forms in two steps, - at first, the formation of α-Keggin, and then the conversion of α-Keggin to β-Kiggin in the presence of ascorbic acid, which produces the blue color during the conversation. For this reaction, up to 200 μg/L of phosphate concentration does not interfere in the reaction. The sensor for the phosphate contains 0.3% of ascorbic acid in the hydrogel. The color's generation indicates the presence of phosphate ≥ 300 μg/L. In that situation, the reading is not accurate for arsenic.

Similarly, the other targeted pollutants were also confirmed through UV-Vis spectroscopy of their specific colored compound. In the case of the Iron sensor, as indicated in *Fig. 5.4b*, the minimum absorption wavelength was determined at 510 nm. The color change was caused by the development of a 1,10-phenanthroline-iron complex (II), which was formed by a rapid combination of iron (II) and 1,10-phenanthroline. The complex is visible to the naked eye. The color changes from colorless to red depending on the concentration of Iron. The response time is about 4 min. For the Fluoride sensor, as shown in *Fig. 5.4c*, the UV-Visible spectra of the complex formed from the interaction between Iron (III) thiocyanate and fluoride are depicted with a maximum absorption peak occurring at 290 nm [43]. In the case of the Lead sensor, Fig. 5.4d depicts the UV-Visible spectra of the interaction between dithizone and Pb^{2+}. It represents the pinkish Pb^{2+} (II) dithizone with an absorption peak at 533 nm [44],[45]. Finally, the Chromium sensor gives a λ_{max} at 545 nm, as shown in *Fig. 5.4e*. It produces a purple color.

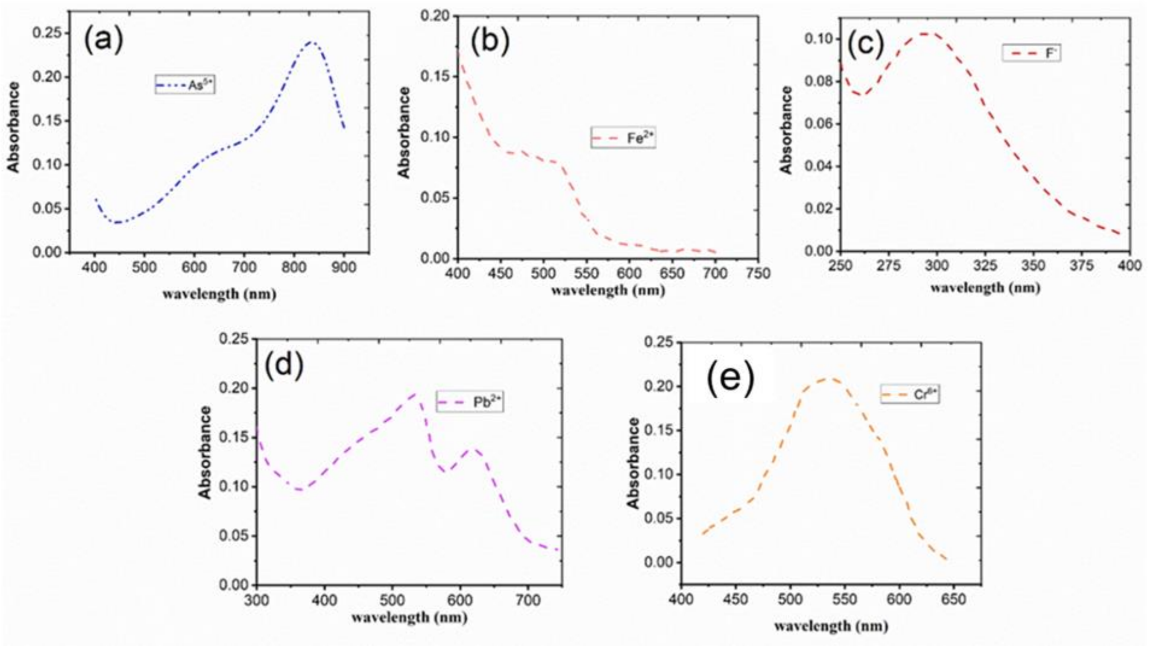


Fig. 5.4: Characteristic wavelengths of absorbance intensities for targeted pollutants determined using UV-vis spectroscopy.

It's important to note that all the above mentions test is a qualitative test’s, meaning that it can detect the presence of targeted pollutants but cannot provide information on the exact concentration of the sample. The quantitative estimation was carried out using the developed calibration curve discussed in the following section.

Before dispensing the samples, the empty cuvette was illuminated by a light source, i.e., a LED light, and the initial resistance of the liquid sensor was recorded using the LDR. The resistance readings of the water sample and blank test samples were then measured and considered as R_{i0}, where “i” represents As, Fe, F, Pb, and Cr, respectively. The sensors' final resistances (R_i) were recorded after dispensing various concentrations of targeted pollutants’ samples. It was observed that the color intensities of the sensor increased by increasing the concentration for Arsenic, Lead, Iron, and Chromium. On the other hand, the intensity decreased for the Fluoride. The light intensity decreases/increases due to higher/lower absorption of the transmitted light. It causes a higher/lower resistance value (R_i) of the LDR.The calibration plots were drawn using these Ri and concentration values (Fig. 5.5). Those yield the following linear equations (1-5) with a high regression coefficient (R^2) value of 0.91, 0.907, 0.956, 0.98, and 0.994 for As, Fe, F, Pb, and Cr, respectively as shown in *Fig. 5.5*.

$$y_1 = 0.042\, x_1 + 1.09 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$y_2 = 0.65\, x_2 + 1.86 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$y_3 = -0.15\, x_3 + 2.77 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$y_4 = 0.078\, x_4 - 0.071 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$y_5 = 0.021\, x_5 + 2.38 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

y_1 to y_5 corresponds to the concentrations of the As, Fe, F, Pb, Cr samples respectively and x_1 to x_5 represents respective resistance of sample drawn from LDR sensors. The above equations were incorporated into the interface algorithm between the microprocessor and the display unit. The digital sensor was completed so that the LCD could display the concentration of any unknown arsenic sample, as shown in Fig. 5.6.

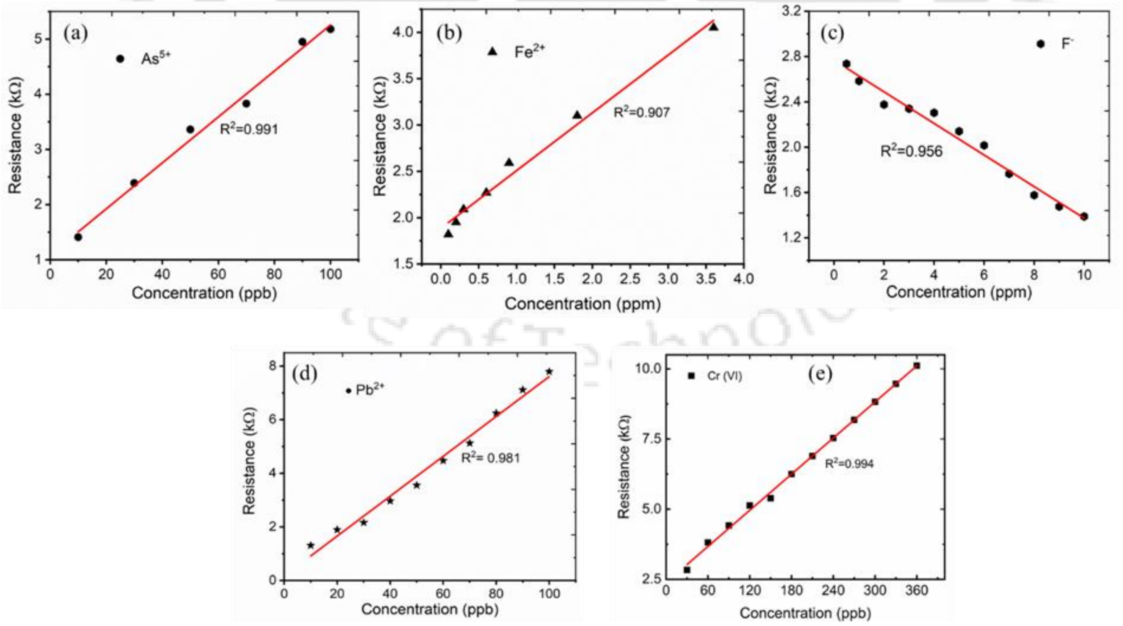


Fig. 5.5: Calibration plots for As(V), Fe(II), F^-, Pb(II), and Cr(VI). (Concentration Vs Resistance).

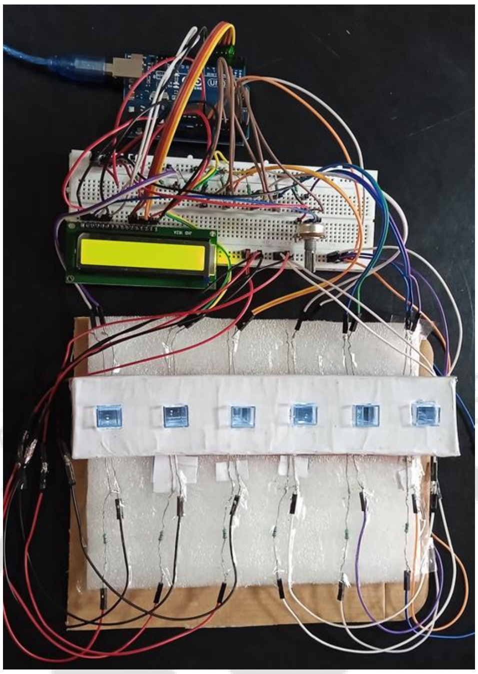


Fig. 5.6: Photography of the multiplexing digital sensor of colorimetric optical analysis for simultaneously determining the presence of Multiple pollutants in water.

The study also aimed to assess the efficacy of a digital sensor by detecting targeted pollutants in spiked water samples. The samples were spiked with known quantities of targeted pollutants before proceeding with further investigation. The samples were then analyzed with the digital sensor. The results of the study are shown in *Table 5.1*. The study confirmed the excellent performance of the digital sensor in the simultaneous detection of multiple targeted pollutants, which may help to ensure the drinking water's quality in contaminated areas.

Table 5.1: Results of spiked sample analysis by the digital sensor.

Pollutants	Spiked quantity	Detected quantity	Average Deviation
Arsenic	10	12.4	1.2
(µg/L)	70	75.5	2.75
Iron	200	206.5	3.25
(µg/L)	900	923.5	11.75
Fluoride	2	2.63	0.315
(mg/L)	8	8.47	0.235
Lead	20	25.1	2.55
(µg/L)	70	76.8	3.4
Chromium	30	32.3	1.15
(µg/L)	210	220.6	5.3

5.4 Conclusion

The current study describes the design and development of a colorimetric based digital POCT device to detect water pollutants simultaneously. Initially, colorimetric based liquid sensors were generated with optimized reactants for the targeted pollutants. The color intensity of the liquid sensors changes with the amount of targeted pollutants present in the sample. The formation of specific compounds was confirmed through UV-Vis spectroscopy. And further analyzed all the liquid sensors quantitatively under LDR sensor. The calibration plot shows the monotonic variation of LDR resistance with pollutants concentration. The LED/LDR sensor units are integrated with an LCD screen via an Arduino circuit to obtain the digital response of the sensor. The device's performance has been tested against various spiked samples of water. The device shows good response time. It can be used as a POC testing kit due to its specificity, portability, and user-friendliness. The device has the potential for real-time detection of arsenic and other pollutants in water in the field, which might help to maintain the quality of the water in contaminated areas.

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CHAPTER-6

Conclusion and Future Work

Chapter 6 : Conclusion and Future Work

6.1 Conclusion

Chapter 6 addresses various conclusions drawn in the thesis along with possible scope for future research. The thesis focuses on the design and development of POCTs for on-site detection of pollutants in water, beverages, vegetables and rice. The important conclusions of the thesis are as follows:

In the first technical chapter i.e. Chapter -2, a user-friendly POCT device was developed to detect arsenic in potable water. The device's response time is nearly 4 min and 2 min for the arsenic concentration range of (10-20) µg/L and (20-500) µg/L, respectively. A color chart was developed for qualitative estimation of arsenic. It helps to assess arsenic concentration visually. It was further quantified using ImageJ software. The quantitative data were also validated with AAS results. In the case of field samples, this device works at a higher efficiency with an average deviation of 9.13 %. It can be used as a portable, eco-friendly, and user-friendly POCT device for rapid detection of arsenic in the fields.

In the second technical chapter i.e. Chapter -3, a highly sensitive hybrid digital sensor was developed for room temperature detection of arsenic in the different samples of water and beverages. The device response time is 5 min and 3 min for the range of (4-20) µg/L and (30-100) µg/L of arsenic concentration, respectively. The mobile App-based digital sensor was developed to negate human effort and errors in measuring arsenic concentration by analyzing paper sensors. This digital sensor gives a high efficiency in terms of field samples. In the comparison of AAS measurement, there was an absolute deviation of 2.96 % for water samples and 1.46 % for beverages. It can be used as a portable, eco-friendly, and user-friendly hybrid digital sensor for rapid detection of arsenic in the fields.

In the third technical chapter i.e. Chapter -4, a photoresistor-based arsenic-specific Digital sensor was developed. It detects arsenic in the liquid-phase. Initially, a LED/LDR sensor was designed to measure the variations in color intensities of the liquid sensor, and then LED/LDR combination was integrated with an Arduino circuit and developed a prototype. It can detect arsenic in potable water, vegetables and rice.

In the last technical chapter i.e. Chapter -5, a prototype of multiplexing of the optical sensor was developed. It can simultaneously monitor the multiple contaminants of water, namely As (V), Fe(II), F(-I), Pb(II) and Cr(VI). The indigenously developed multiplexed sensor can estimate the concentration of As (V), Fe(II), F(-I), Pb(II) and Cr(VI) in the range of (10-100 μg/L), (0.1-3.6 μg/L), (0.5-10 mg/L), (10-100 μg/L) and (30-360 μg/L) respectively, which covers the allowable limit of the respective contaminants.

6.2 Future Work

There are certain scopes for improving and uplifting performance of the sensor and quality monitoring activities. The work can also be extended to other fields, bacteria, viruses, biomarkers for various diseases, etc. A few such points are highlighted here.

a) To check the performance, the devices under the actual filed and industrial effluent samples, which are a little different from the spiked samples

b) To develop POCT devices and Digital sensors for other heavy metals

c) Further, a digital device can be made based on the results obtained from this non-digital prototype.

d) Knowledge gained from this work can be extended for the detection of biomarkers from biological samples.

e) An attempt can be made to detect harmful bacteria and viruses in aquatic samples.

Annexure I

List of publications

In Journals

1. **Ravula Rajasekhar**, Krishna Pada Bhabak, and Tapas K. Mandal. "User-friendly point of care test device for detection of arsenic in potable water: Prototype, design, and artifact." **Asia-Pacific Journal of Chemical Engineering** 17, no. 5 (2022): e2815.
2. **Ravula Rajasekhar**, Dalia Dasgupta Mandal, and Tapas K. Mandal. "A highly sensitive hybrid digital sensor for room temperature arsenic detection." **Journal of Environmental Chemical Engineering** 11, no. 5 (2023):110381.
3. **Ravula Rajasekhar**, Tapas K. Mandal.; A Photoresistor-based Arsenic-specific Digital Sensor for Rapid and Affordable Colorimetric Detection at the Point-of-Care *(Manuscript Communicated).*
4. **Ravula Rajasekhar**, Tapas K. Mandal, Colorimetric optical analysis for determining the presence of Multiple metals in a single drop of water *(Manuscript is under preparation).*

In Patents

1. Tapas K. Mandal.; **Ravula Rajasekhar**.; Android based App to measure the arsenic concentration using a paper sensor *(**Patent submitted**; Application with Reference Number 2022101420000101)*

In Conferences

1. **Ravula Rajasekhar**.; Shubham Maurya.; Krishna Pada Bhabak.; Tapas K. Mandal. An Electrolytic Modification of the Gutzeit Method, for the Determination of Arsenite in Drinking Water, TEQIP-III Sponsored 1st **National Student Conference on Advances in Chemical Engineering**, Assam Engineering college, 2019.
2. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Development of an eco-friendly field test kit for detection of arsenic in contaminated water, **CHEMCON 2019**, 15th - 19th December 2019, IIT Delhi.
3. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Current Status of Arsenic Detection Via Gutzeit Method. **RECYCLE 2020**, 13th -14th February 2020, IIT Guwahati.
4. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Development of a green synthesis technique of iron oxide nanoparticles for efficient separation of arsenate from water, **WATER 2020**, 23rd -25th January 2020, IIT Guwahati.
5. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal.; Green Synthesis Iron Oxide Nanoparticles its Application in Remediation of Contaminated Water. **Workshop cum Symposium**. 12th -13th February 2020, Center for the Environment, IIT Guwahati.
6. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Participated in the one-day symposium on '**Recent Advancements in Environmental Research (RAER 2017)**, organized by the Center for the Environment at IIT Guwhati on 5th June 2017.
7. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Participated in "3rd National workshop on **MEMS/NEMS and Theranostic Devices**" organized by Center for the Excellence in Research and Development of Nanoelectronic Theranostic Devices under the aegis of center for Nanotechnology, IIT Guwahati, during 21st to 23rd March 2017.
8. **Ravula Rajasekhar**.; Krishna Pada Bhabak.; Tapas K. Mandal. Participated at the Workshop on "**Advances in Environmental Protection and Sustainability (AEPS 2018)**" organized by center for the Environment ai IIT Guwhati on 2nd June 2018.

In Webinars

9. **Ravula Rajasekhar**.; Attended AICTE Sponsored STTP-II on “**Industrial Pollution & Control Strategies**” organized by Department of Chemical Engineering, Anurag Group of Institutions, Ghatkesar (M), Medchal (D), Telangana held during 5th -10th October 2020.
10. **Ravula Rajasekhar**.; Participated in TEQIP-III sponsored one-day National Webinar on’ **RECENT PRACTICES IN ENVIRONMENTAL ENGINEEING (RPEE-2020)**’ Organized by Department of Civil Engineering, Government College of Engineering Kalahandi Bhawanipatna in collaboration with Government College of Engineering jalgaon, Maharastra on 18th October, 2020.
11. **Ravula Rajasekhar**.; Participated in the 3-Day Online FDP on “**Environmental Pollution – Sustainable Technologies for its Control (EP-STC**)” organized by Chemistry Division of Department of Basic Sciences and Humanities, GMR Institute of Technology, Rajam during 19th - 21st October, 2020.

Outside thesis

Publications

1. Sinha, Rupam, Nirmal Roy, **Ravula Rajasekhar**, Aabhas Karnawat, and Tapas K. Mandal. "N-doped carbon dot from cigarette-tobacco: Picric acid sensing in real water sample and synthesis of CD-MWCNT nano-composite for UV-photodetection." **Journal of Environmental Chemical Engineering** 9, no. 1 (2021): 104971.
2. Sinha, Rupam, Anil P. Bidkar, **Ravula Rajasekhar**, Siddhartha S. Ghosh, and Tapas K. Mandal. "A facile synthesis of nontoxic luminescent carbon dots for detection of chromium and iron in real water sample and bio-imaging." **The Canadian Journal of Chemical Engineering** 98, no. 1 (2020): 194-204.

Conference proceedings

3. Rupam Sinha, **Ravula Rajasekhar**, and Tapas K. Mandal, Carbon dots photoluminescence technique to detect total Chromium in industrial wastewater. **The International Nanotech & Nanoscience Conference and Exhibition Nanotech France** 2019. DOI: https://doi.org/10.26799/cp-nanotechfrance2019.
4. **Ravula Rajasekhar**, Thirukumaran Kandasamy, Siddhartha S. Ghosh and Tapas Kumar Mandal, Design and development of a prototype of POCT device for estimation of protein concentration in a biological sample. **CUChE Alumni Symposium 2022 On "Circular Economy on Sustainable Basis: The Role of Chemical Engineers**." 1, no. 2 (2022): 48-52, ISBN: 978-81-954649-1-3.

Annexure II

Optimizing Ascorbic Acid Concentration to Eliminate Phosphate Interference in Experiment/ Analysis:

In this study, the optimization of ascorbic acid and phosphate concentrations in the solutions was described for the detection of arsenic presence in water using the molybdenum blue test. The process is outlined below.

Intillay, a blank test was performed by treating ascorbic acid of varying concentrations with ammonium molybdate in an aqueous solution to observe any color change. The colors were noted for each concentration to determine a positive or negative result for the blank test. A negative result indicates that no color change was observed when ascorbic acid was added to the ammonium molybdate solution. As molybdate changes color at higher concentrations of ascorbic acid, this test was conducted to determine the optimal concentration of ascorbic acid.

Table A1: Optimization of ascorbic acid concentration (wt/wt %) based on blank test.

Ascorbic acid (wt/wt %)	Blank test color (positive/negative)
100	Positive
50	Positive
10	Positive
0.6	Positive
0.55	Positive
0.5	Negative
0.4	Negative
0.3	Negative
0.2	Negative
0.1	Negative

A phosphate test was conducted by testing different concentrations of phosphate with varying concentrations of ammonium molybdate to detect any interference. The presence of phosphate was confirmed by a greenish-blue color. The concentration of ascorbic acid with a lower limit of phosphate interference was considered optimal.

Table A2: Optimization of Ascorbic acid (wt/wt %) based on phosphate interference.

Phosphate (mg/L)	Ascorbic acid concentration (wt/wt %)			
	0.5	0.4	0.3	0.2
1	Positive	Positive	Positive	Negative
0.5	Positive	Positive	Negative	Negative
0.4	Positive	Positive	Negative	Negative
0.3	Positive	Positive	Negative	Negative
0.2	Positive	Negative	Negative	Negative
0.1	Negative	Negative	Negative	Negative
Blank test	Negative	Negative	Negative	Negative

Based on these observations, it was determined that in order to make the test more flexible (i.e., to allow for greater phosphate concentration interference), lower weight percent ascorbic acid, specifically 0.3 or 0.2 wt%, was the most effective option, followed by 0.4 wt% ascorbic acid and then 0.5 wt% ascorbic acid. "More flexibility" in this context refers to the test's ability to tolerate a larger amount of phosphate interference without impacting the detection of arsenic.

Arsenic test: After selecting the weight percentages of ascorbic acid, they were then tested for arsenic concentration using the molybdenum blue test.

Table A3: Optimization of Ascorbic acid (wt/wt %) for arsenic detection.

Arsenic (mg/L)	Ascorbic acid concentration (wt/wt %)			
	0.5	0.4	0.3	0.2
0.01	Positive	Positive	Negative	Negative
0.001	Negative	Negative	Negative	Negative

It is evident from this study that in order to detect arsenic up to the WHO limit, the optimized concentration of ascorbic acid was determined to be 0.4 wt%. This concentration allows for a phosphate concentration interference allowance of up to 200 μg/L .