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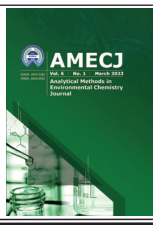

A review of colorimetric and fluorometric detection of arsenic: arsenate and arsenite

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ABSTRACT
Arsenic is a highly toxic metalloid that forms different chemical states in nature, including arsenate and arsenite, as common inorganic forms. Exposure to arsenic may cause adverse effects on human health and the environment. Therefore, the detection of arsenic is critical. Exploring new approaches with low detection ranges and high sensitivity is crucial. This review paper consists of optical methods, including colorimetric and fluorometric methods, which detect arsenite and arsenate (μg L^{-1}). Initially proposed colorimetric approaches such as the Gutzeit and molybdenum blue method can easily to use. However, the production of toxic substances limits their applications. Later, structurally modified molecules, nanoparticle-based assays, and their modifications are used for arsenic detection. Fluorometric methods also have noticeable attention to arsenic detection. Fluorescent approaches reported in this paper are based on semiconductor nanomaterials, other nanomaterials, and their modifications, etc. In addition, arsenate's catalytic and inhibitory activity on enzyme activity can be used to detect arsenic through colorimetric and fluorometric methods. This review highlighted the advantages, disadvantages, comparisons, and uses of colorimetric and fluorometric methods in detecting arsenite and arsenate.

1. Introduction

Arsenic is the 20th most abundant and widely distributed element in the earth's crust. It is categorized as a metal and as a metalloid [1]. Arsenic occurs in several chemical oxidation states in nature, such as As (V), As (III), As (0), and As (-III) [2]. Also, it can exist in both organic and inorganic forms. Inorganic arsenic exists as arsenate [As (V)] and arsenite [As (III)], while organic forms are monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA), dithiol arsenate (DTA), etc. [3]. Even the trace concentration of arsenic shows higher toxicity than most other heavy metals. Both inorganic forms of arsenate and arsenite are hazardous and toxic. Among them, arsenite is considered the most toxic form [4]. Contaminated groundwater, wastewater, and drinking water are the main sources of arsenic that enter the environment through industrial operations, agricultural activities, etc. [4-6]. Exposure to arsenic in the long term can cause adverse effects on human health, such as skin cancers, skin lesions, neurotoxicity, cardiovascular disease, diabetes, etc. [5]. Therefore, the detection and removal of arsenic are critical to human health. The World Health Organization (WHO) and US Environmental Protection Agency (USEPA) have stipulated some guidelines with a 0.01 mg L^{-1} 10 μg

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L^{-1} permissible limit of arsenic for drinking water [6]. Therefore, a series of techniques are developed to monitor the arsenic concentration. Numerous laboratory techniques, including Atomic Absorption Spectroscopy (AAS) [7], Atomic Fluorescence Spectroscopy (AFS) [8], Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) [9], Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) [9,10], Graphite Furnace Atomic Absorption (GFAA), Hydride Generation Atomic Adsorption (HGAA) and Neutron Activation analysis [11] are some of the methods which currently being used. They provide accurate detection of arsenic even at trace concentrations. High expensive instrumentation, the requirement for sophisticated laboratory setup, significant maintenance and operation expertise, and producing highly toxic chemicals during the process are the drawbacks of these methods. Also, the inability to be used in the field is one of the significant practical limitations [12]. Therefore, various chromatographic methods, electrochemical methods [13, 14], and optical methods are developed to overcome these limitations [15]. Among them, optical methods are essential due to their high sensitivity, selectivity, simplicity in operation, fast response, cost-effectiveness, and offer field monitoring. Therefore, optical methods are considered as promising techniques for arsenic detection [14,16,17,18]. This review presents current optical methods including colorimetric and fluorometric methods for detecting arsenate and arsenite.

2. Experimental

The different technologies based on colorimetric and fluorometric methods were used to detect and analyse arsenic (arsenite and arsenate) in various matrixes.

2.1. Colorimetric detection of arsenite and arsenate

2.1.1.Advancement of colorimetric methods and detection based on structurally modified molecules.

Colorimetric methods are based on the Gutzeit reaction, which detects arsenic quantitatively. This method relies on the reaction of arsenic gas with hydrogen ions to form a yellow stain on mercuric chloride paper in the presence of reducing agents. However, certain limitations are followed when analyzing the groundwater samples, and it generates highly toxic arsine gas and byproducts of mercuric compounds. Therefore, protective equipment is required for the analysis procedure [19,20]. In addition, traditional methods such as molybdenum blue [21-25], ethyl violet, and gallocyanin can be used for sensing arsenic, yet, the interferences intervened with the results, and low sensitivity hinders its applicant the resulting [26]. Therefore, scientists have continuously tried to remove the generation of toxic chemicals and interferences in the detection. Some arsenic detection methods are involved with structurally modified molecules. For example, norbornene-based rhodamine monomers (Nor-Rh) and their polymer form (PNor-Rh) are used for very selective detection of arsenite colorimetrically and fluorometrically in the presence of potassium iodate and hydrochloric acid. They used an open form of rhodamine which is pink and highly luminescent. When arsenite was present in the sample, the reaction led to the oxidation of As (III) to As (V) by iodate reduction reaction to iodine. As a result, the solution changed its pink color to brown colorimetrically and observed green color fluorometrically. With the increase in arsenite concentration, the intensity of the brown color increased. As a result, they developed a simple and easy practical device based on a polymer-coated paper strip for field analysis. Also, they synthesized a thiol-based norbornene monomer (Nor-Th) and its polymer form (PNor-Th) for arsenite removal from water [27]. Aptamers are single-strand RNA or DNA oligonucleotides that can bind with target ions or molecules. Recently aptamers are used as a recognition element for developing arsenic detection methods [28-33]. For example, Zhou and colleagues [33] proposed a method for colorimetric detection of arsenite based on regulating hemin peroxidase catalytic activity using arsenic binding aptamers. They used

an arsenic-binding DNA aptamer called Ars-3. Hemin acts as a catalyst that can catalyze many oxidation reactions. But the catalytic activity of hemin is very slow in the aqueous medium. Catalytic activity can be improved by hemin binding onto the surface of nanosheets or covered with guanine-rich oligonucleotide by forming an active form of the G-quadruplex structure. Arsenic binding aptamers can inhibit hemin catalytic activity temporarily. In the presence of arsenite, As (III) binds to Ars-3 and forms an aptamer-As (III) complex. Therefore, an oxidation reaction occurs in TMB molecules to generate yellow diamine products. While the absence of arsenite, Ars-3 aptamers complex with pyrrole rings of hemin. As a result, the catalytic activity of hemin is decreased and generates blue products of cation radicals. Therefore, this method is susceptible and selective for arsenite. Figure 1 shows the schematic description of the As (III) detection by arsenic binding aptamers.

Traditional methods are easy to perform and inexpensive, yet restrained by the generation of toxic products. In addition, the difficulty increases due to complex redox reactions and complex separations. Therefore, other anions and cations can interfere with the analysis. The same assay can be used for both colorimetric and fluorometric detections. Also, redox reactions are specific to the analyte. Therefore, the involvement of other ions can be negligible. These assays/polymer-coated paper strips can be used for field analysis [33,34].

2.1.2. Usage of metal nanoparticles

Researchers enhanced the involvement of novel methods with non-toxic product generation. Recently, they have used metal nanoparticles and their modifications to detect heavy metals, including arsenic, to obtain a visual color change in the analysis [35]. It is based on the excellent optical properties of nanoparticles, such as high sensitivity, selectivity, and high extinction coefficient in visible regions. Gold nanoparticles [36-40] and silver nanoparticles [41-49] are mainly used for analysis procedures. Color change occurs due to the aggregation of nanoparticles with the target analyte and size-dependent Surface Plasmon resonance (SPR) properties of nanoparticles [48,49].

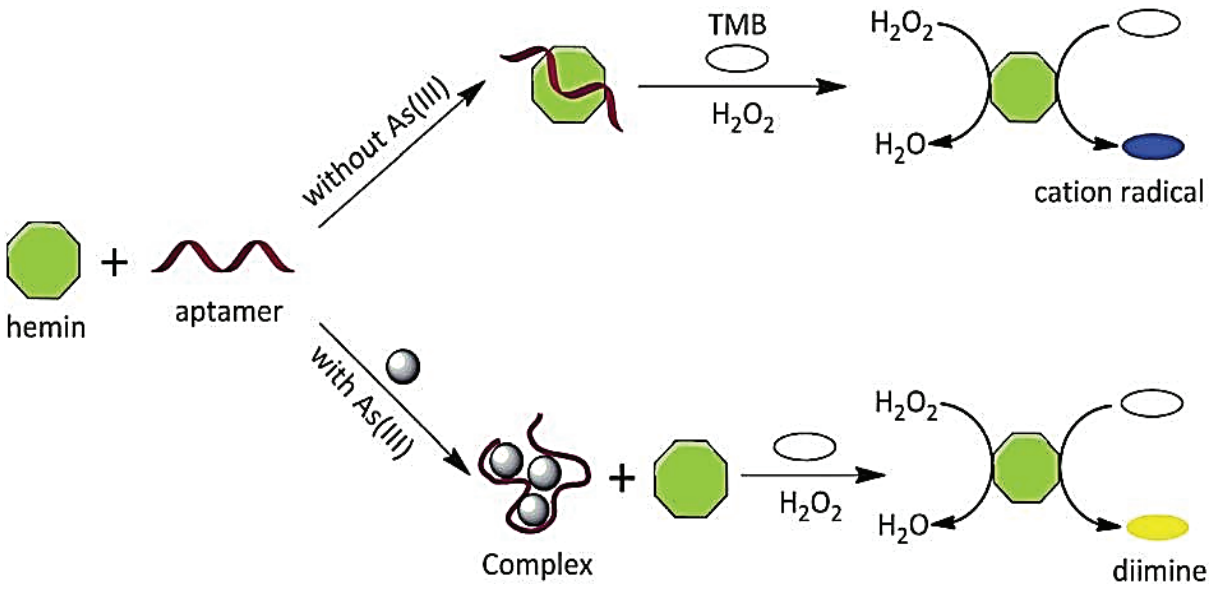

Fig. 1. Schematic description of the colorimetric detection of As (III) based on the inhibition of hemin peroxidase activity by arsenic-binding aptamers [33]

2.1.2.1. Detection based on the modified silver nanoparticles.

Recently, nanotechnology has been commonly used in optical detection due to the uniqueness of its chemical, biological, and physical properties. Different types of nanoparticles show different functions by providing different optical, fluorescent, and magnetic properties. And their modifications enhance their detection ability [50]. Thiol-based ligands are commonly used because they have an excellent capacity to bind with arsenic [51,52]. As an example, a multiligands assay was synthesized for the detection of arsenite based on silver nanoparticles (AgNPs) which were modified by multiligands containing glutathione (GSH), dithiothreitol (DTT) and asparagine (Asn). Thiol-based ligands bind to the surface of AgNPs, which can vastly enhance the selectivity toward the arsenite. Aggregation occurs due to the formation of As-O and As-S linkages between GSH/DTT/Asn-AgNPs and As (III). Color change occurs from yellow to light pink and purple with increased arsenite concentration. Figure 2 shows the GSH/DTT/Asn-AgNPs complex formation and aggregation with arsenite. This method is promising for the colorimetric detection of arsenite with high sensitivity, low cost, and facility for rapid onsite detection [52].

In addition, arsenic adsorbents such as iron (III) oxide are widely used for detection procedures. Siangproh and his group [53,54] developed silver nanoplates (AgNPls) to detect arsenite and arsenate. Although, the matrix effect interferes with the detection. Therefore, they synthesized and applied ferrihydrite-coated silica gel (SiO_2-Fh) modified by the Eric Arifin method to adsorb arsenic (arsenate and arsenite). SiO_2-Fh has a high affinity to selectively adsorb arsenic and avoid the matrix effect. When arsenic adsorbs onto the SiO_2-Fh, the dark blue color of AgNPls was changed to purple, pink, orange, and yellow respective to the concentrations of arsenic present in the sample. Both arsenate and arsenite show similar behavior on the color changes of AgNPls. Therefore, this method is suitable for the determination of total inorganic arsenic.

2.1.2.2. Detection based on the modified gold nanoparticles

Gold nanoparticles (AuNPs) have numerous applications in the optical detection of heavy metals, including arsenate, arsenite, and aromatic compounds [54,55]. Most of the AuNPs based assays rely on modifications with specific binding ligands. Priyadarshani *et al.* [55] synthesized gold nanorod (GNR) based sensor GNR-PEG-DMSA. It is highly sensitive, specific, and cost-effective for rapidly detecting arsenite and arsenate. GNR-PEG-DMSA sensor is synthesized through conjugation of GNR with poly (ethylene glycol) methyl ether thiol (mPEG-SH) followed by the addition of meso-2,3-dimercaptosuccinic acid. CTAB is

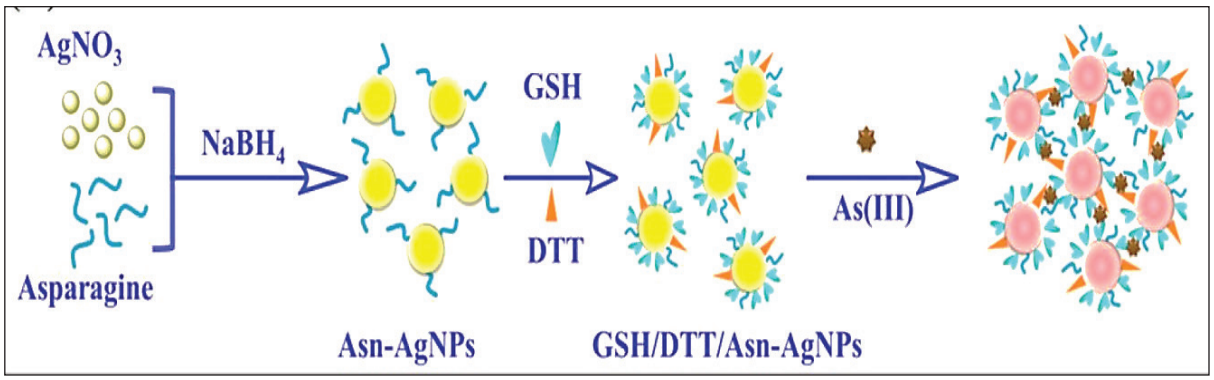

Fig. 2. Illustration of synthesis of GSH/DTT/Asn-AgNPs used as a colorimetric probe for As (III) detection [52]

used as a surfactant which is capped with GNR, and the stability of the sensor is maintained by PEG. DMSA is a ligand that covalently binds to GNR and has a high affinity to arsenic. Also, the arsenite and arsenate bind with free –SH groups of DMSA, which are present on the surface of GNR. Aggregation initiates with the formation of As-thiolate complexes between nanorods. The Colour of the solution changes from dark bluish-purple to almost colorless. This sensor has a regenerating ability using strong chelating agents like EDTA. Also, it can be used to quantitatively determine the total arsenic in a sample using a small amount of sensor materials. Figure 3 shows the synthesis of the GNR-PEG-DMSA sensor and its interactions with As (III) and As (V).

The same group proposed Europium functionalized single gold nanoparticle-based new sensor GNP-MMT@Eu for colorimetric detection of trace concentration of both As (III) and As (V). Nanosensor is synthesized by chemical conjugation of GNPs with 2-mercapto-4-methyl-5-thiazoleacetic acid (MMT) followed by fluorescent europium chloride [Eu (III)]. Aggregation occurs in the presence of arsenite or arsenate by binding to the surface of GNP-MMT@Eu. It occurs through coordinated Eu-OH groups consisting on the surface of GNP-MMT@Eu via electrostatic attraction and covalent-type interactions. Afterward, it forms the GNP-MMT@Eu-As (III)/As (V) complex. Arsenate shows rapid and more sensitive color changes for the nanosensor than the arsenite. The initial color of the sensor gradually changes from red to blue. Sensor-based paper strips can detect total arsenic content in field applications, and most importantly, the sensors can be regenerated. Here, Figure 4 shows the synthesis of gold nanosensors and its interaction with As (III) and As (V) [56]. Zhang and co-workers [57] proposed an arsenate detection method based on the inhibitory effect of arsenate on acid phosphatase (ACP) bioactivity using citrate-capped AuNPs as the optical reporter [57-59]. They used adenosine 5'-monophosphate (AMP) as the substrate and prevented AuNPs from aggregation. The activity of ACP hydrolyses the charged nucleotide into the uncharged nucleoside. The presence of ACP dephosphorylation of AMP to adenosine and resulting adenosine leads to the aggregation by nucleoside binds to AuNPs through metal-ligand interaction by replacing weakly bound citrate. As a result, the color change occurred from red to purple to blue. But in the presence of As (V), the color of the solution reversed from blue through purple to the initial red color due to the inhibitory

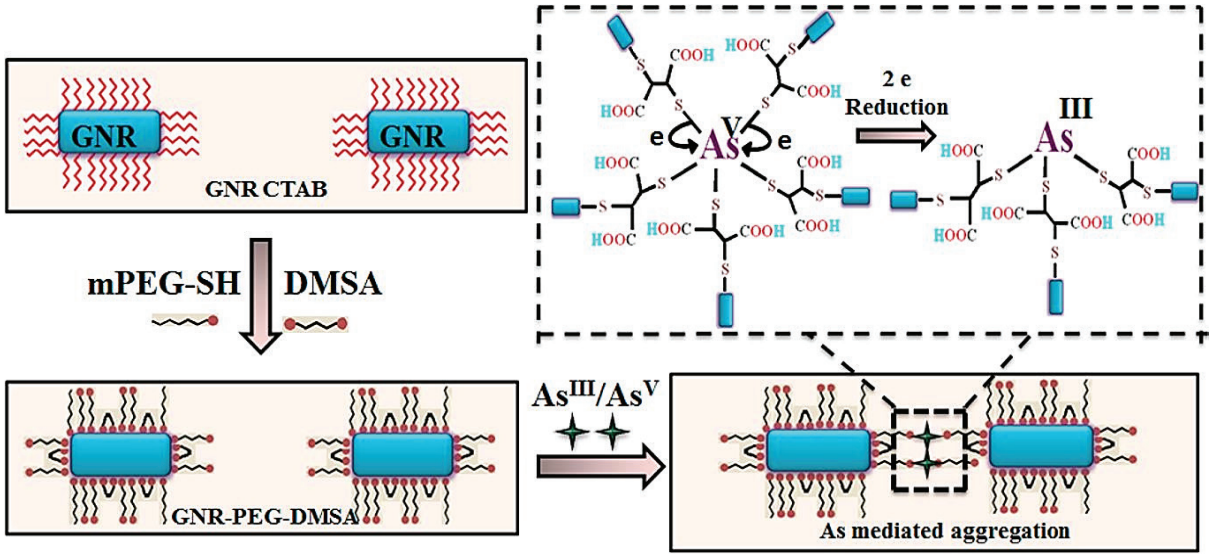

Fig. 3. Illustration of the fabrication of GNR-PEG-DMSA sensor and its interactions with As (III) and As (V) [55]

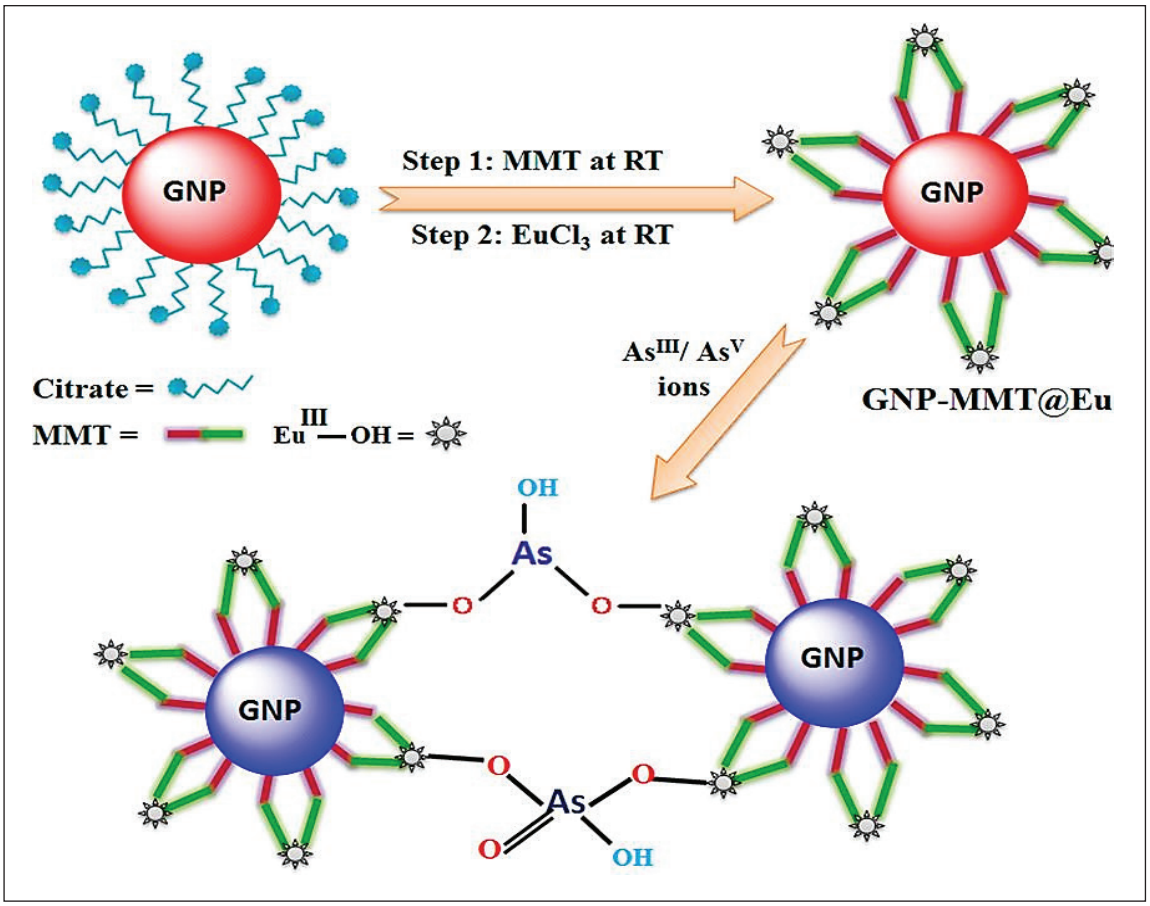

Fig. 4. Schematic of the synthesis of the gold nanosensor, GNP-MMT@Eu, and its aggregation with arsenate and arsenite [56]

effect of As (V) on ACP activity by competing with AMP in the enzymatic reaction. The color change occurred respectively with the As (V) concentration increment. This sensor has a remarkable sensitivity towards As (V). However, there are some limitations in this assay. Their visible detection limit is a bit higher than the standard limit of WHO, and certain concentrations of Cu^{2+}, F^-, and $H_2PO_4^-$ can interfere with arsenate detection. Figure 5 shows the catalytic activity of ACP on AMP with As (V) and without As (V) and color changes in AuNPs.

A simple paper-based microfluidic device was developed to rapidly detect arsenite in low concentrations with a low detection range. The microfluidic device is based on modified gold nanoparticles (Au-TA-TG), that can rapidly interact with arsenite to produce a visible dark bluish-black precipitate at the interfacial zone. Figure 6 depicts the schematic diagram of the microfluidic device, which is made up of "Y"-shaped Whatman filter paper and two arms that are immersed in modified gold nanoparticle solution and arsenic sample separately. Due to the capillary action of the "Y"-shaped device, fluids start flowing into the channel. When Au-TA-TG solution meets arsenite ions, a bluish-black precipitate can be observed quickly. Thioctic acid (TA) and thioguanine (TG) are used to modify gold nanoparticles that have a specific ability to bind and interact with arsenite. The color change occurs due to the aggregation of gold nanoparticles and arsenite. The portable, power-free microfluidic device is cost-effective and safe, which makes it suitable for environmental analysis [60].

Tetradecyl (tri hexyl) phosphonium chloride has a strong interaction with arsenite. This ionic liquid is modified with gold nanoparticles to produce highly

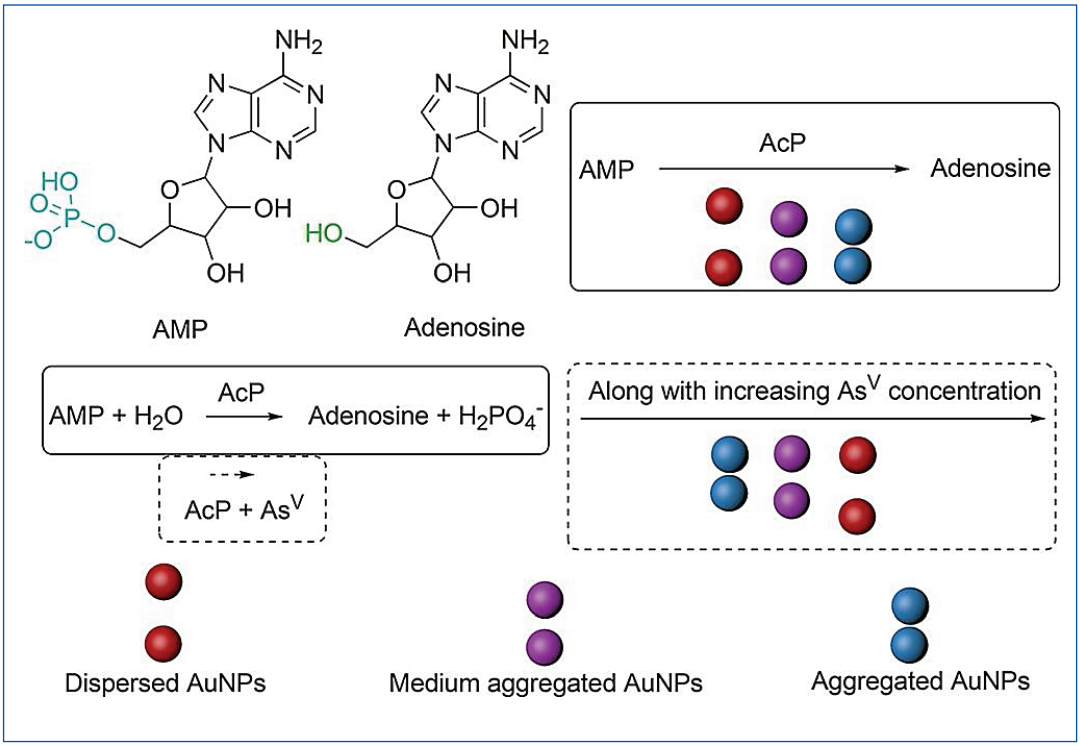

Fig. 5. Illustration of bioactivity of ACP on AMP with arsenate and without arsenate and color changes in AuNPs with an increment of arsenate concentration [57]

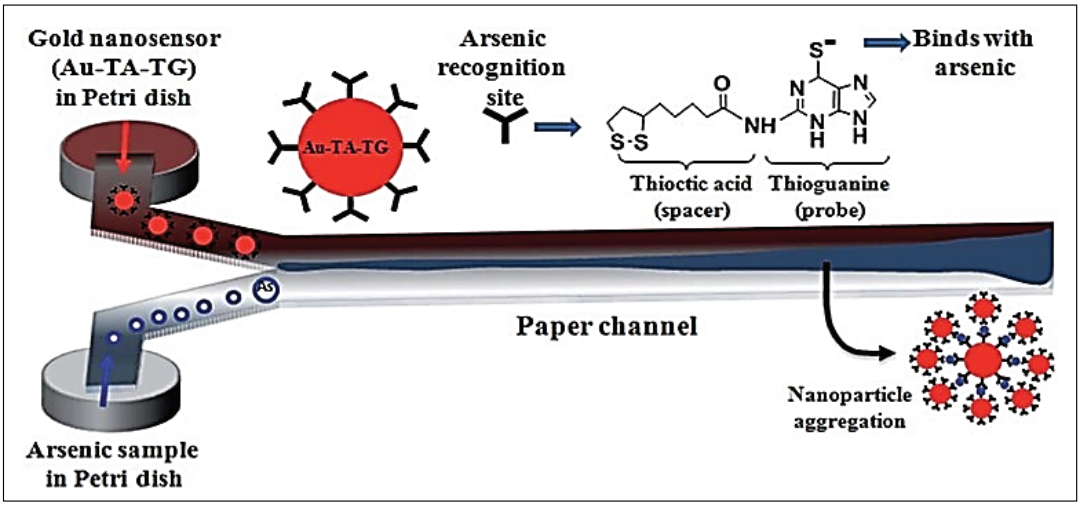

Fig. 6. Illustration of a paper-based microfluidic device for the detection of arsenite using modified gold nanoparticles (Au-TA-TG) [60]

sensitive visual observations for arsenite detection, with the probe color changing from red to blue in the presence of arsenite. The total amount of inorganic arsenic can be determined using this probe. The low cost and high tolerance to common ions make the probe suitable for field analysis. Figure 7 shows the behavior of the probe in the presence of arsenite and arsenate [61].

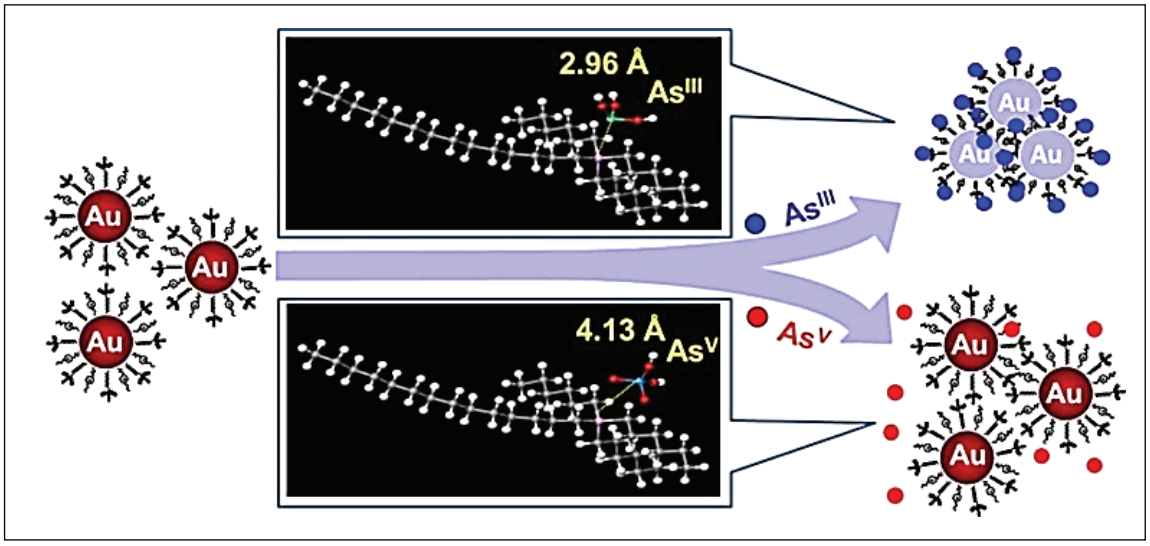

Fig. 7. Illustration of gold nanoparticle probe and its behavior with the presence of arsenic [61]

The unique properties of nanoparticles offer many advantages including simple, low cost, less time-consuming, nontoxic, and ease of data interpretation. Modifications of nanomaterials increase selectivity and sensitivity. Moreover, some sensors can regenerate and are reusable. However, there are some disadvantages such as complicated preparation processes, and long reaction times. Also, the external environment can be affected by nanoparticles' stability, and continuous temperature maintenance is essential [55-61].

2.1.2.3. Detection based on unmodified gold nanoparticles

Gold nanoparticle-based colorimetric assays consist of ligand modifications that can be synthesized through expensive processes. Therefore, scientists were encouraged to develop unmodified metal nanoparticles conveniently and cost-effectively [62,63]. For example, a simpler and more economical assay that relied on different adsorption properties on AuNPs between random coil G-/T-rich ssDNA and folded DNA bound to arsenite was synthesized. While arsenite can easily attach to the G-/T-rich ssDNA via hydrogen bonds, G-/T-rich ssDNA can efficiently adsorb onto AuNPs that prevent the salt-induced aggregation by enhancing the electrostatic repulsion between ssDNA-adsorbed AuNPs and maintain the stability of AuNPs. Therefore, adding enough salt leads to the collection, and the color of unmodified AuNPs changes from red to blue resulting in ssDNA becoming compact and folded DNA. Figure 8 represents the colorimetric strategy of arsenite detection. Visual inspection can be used for semi-quantitative detection of arsenite, while UV/Vis absorbance spectroscopy technique can be used for quantitative detection [63].

Peptide ligands are promising materials for desired target analytes, including metals, biomolecules, and drugs [64,65]. Yang et al. [65] have specifically As (III)-binding heptapeptide sequences of T-Q-S-Y-K-H-G through phage display peptide library techniques using a biopanning process. The sensing system contains a unique peptide sequence for target recognition and unmodified AuNPs as the sensing probe. Due to slow aggregation and the prerequisite of high concentration of heptapeptide, cysteine residue (C terminal) is conjugated to the end of the heptapeptide sequence resulting in an octapeptide sequence of T-Q-S-Y-K-H-G-C which induce the aggregation of AuNPs more effectively. In the absence of As (III), the octapeptide can bridge with AuNPs, and the color of unmodified AuNPs changes from wine red to blue. Nitrogen-containing functional groups (-NH, -N=), –OH groups, and sulfur-containing groups (-SH), which are present in the peptide sequence, can bind

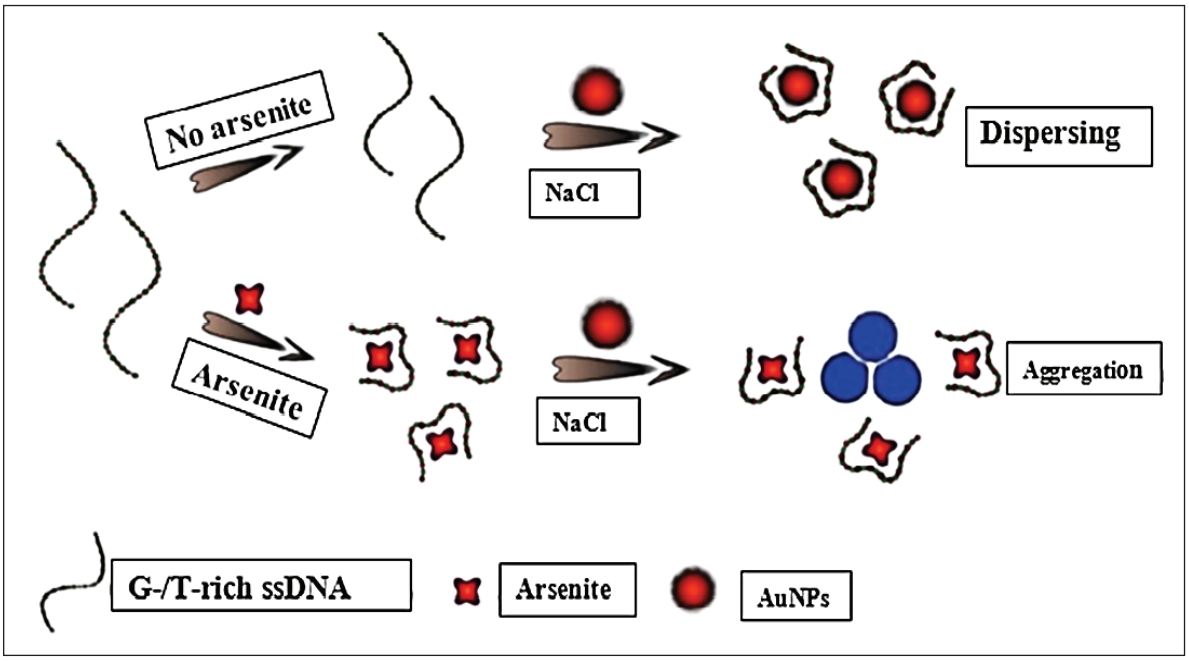

Fig. 8. AuNPs-based colorimetric strategy for arsenite detection [63]

with As (III) via strong hydrogen bonds. With As (III) presence, octapeptide binds to As (III) and prevents the peptide binding with AuNPs; the color remains red. UV/Vis spectroscopic technique can determine As (III) concentration. As (V) and other metal ions do not have significant affection on As (III) detection. Therefore, this method is unique for the determination of arsenite. Operation is easier and more convenient than other complicated methods based on modified AuNPs, aptamers, and aggregation inducers. Unmodified nanoparticles can be used for naked-eye detection without complicated instrumentation and much knowledge. They do not require chemical modifications. Therefore, they are more economical, convenient, simple, sensitive, selective, reliable, and cost-effective. Other competitive ions do not interfere or slightly interfere with the detection [62-65]

2.1.2.4. Detection based on arsenate adsorption on nanozymes

Some nanomaterials, such as metal oxide nanoparticles, noble metal nanoparticles, carbon-based nanomaterials, and two-dimensional nanomaterials, have a natural enzyme-mimicking activity called nanozymes. Among them, two-dimensional nanomaterials show excellent enzyme-mimicking activity. Therefore, researchers are interested in using nanozymes due to their ease of mass production with low cost, robustness, high stability, etc. For example, cobalt oxyhydroxide (CoOOH) nanoflakes, a promising material used in catalysis material for dual-mode assay of arsenate detection, rely on its peroxidase-like activity. CoOOH nanoflakes are synthesized by a one-pot ultrasonic process used for "signal off" colorimetric detection of arsenate. This excellent peroxidase-like activity of CoOOH nanoflakes can effectively catalyze chromogenic substrate (ABTS) into its green color oxidized product ($ABTS_{OX}$). The presence of arsenate, As (V), adsorbs onto the CoOOH surface, and interaction occurs between nanoflakes and As (V). It occurs through electrostatic and covalent interaction (As-O) to attenuate the peroxidase-like activity of CoOOH. As a result, catalytic activity decreased, and the green color solution changed to very pale green or close to colorless. Sensitivity can increase significantly by modifying the electrode surface. The assay is highly selective for arsenate and can detect total inorganic arsenic. The advantages are that it can be used for handy, onsite, sensitive,

selective, and signal-off assay for As (V). Also, CoOOH nanoflakes can be further developed to detect trace As (V) with obviously improved sensitivity and detection limit. Figure 9 shows the dual-mode assay for arsenate detection based on the peroxidase-like activity of CoOOH nanoflakes [66].

2.1.2.5. Detection of dual heavy metal ions together, including arsenite

Researchers developed simple chemical sensors to detect toxic heavy metals, including arsenic, cyanide, and mercury, due to their adverse effects on humans and the environment [67-69]. A simple Schiff's base colorimetric chemosensors have been designed for the naked eye detection of Hg^{2+} and As^{3+} using a simple step reaction. They used Isatin with 3,3-dihydroxybenzidine to obtain binding sites in the chemosensors that bind mercuric ions and arsenite. Three chemosensors, CS1, CS2, and CS3 were synthesized for different color changes. The color change of CS1 and CS3 changes from orange to colorless in the presence of Hg^{2+} and from orange to aqua-blue for As^{3+}. CS1 shows specific selectivity for Hg^{2+} and As^{3+}. However, it does not show significant color change with adding other cations. CS2 changes its color from yellow to pink with the addition of Hg^{2+}. Figure 10 shows the binding mechanism of CS1, CS2, and CS3 with Hg^{2+} and AsO_2^-. These sensors can monitor the mercury and arsenic level in the environment [68]. Neety Yadav and colleagues [69] developed a single chemical sensor capable of detecting both arsenite and cyanide ions, with detection limits in the micro- and nano-range. The probe was synthesized by the reaction of thiosemicarbazide dissolved in ethanol with 2-hydroxy-1-naphthaldehyde (Fig. 11). This probe has two acidic protons that are deprotonated through the interaction of arsenite and cyanide ions, which are indicated by the color change that occurs in this sensor. In the presence of arsenite and cyanide ions, the color of the probe changes from light yellow to dark yellow due to deprotonation and strong hydrogen bonding between the probe

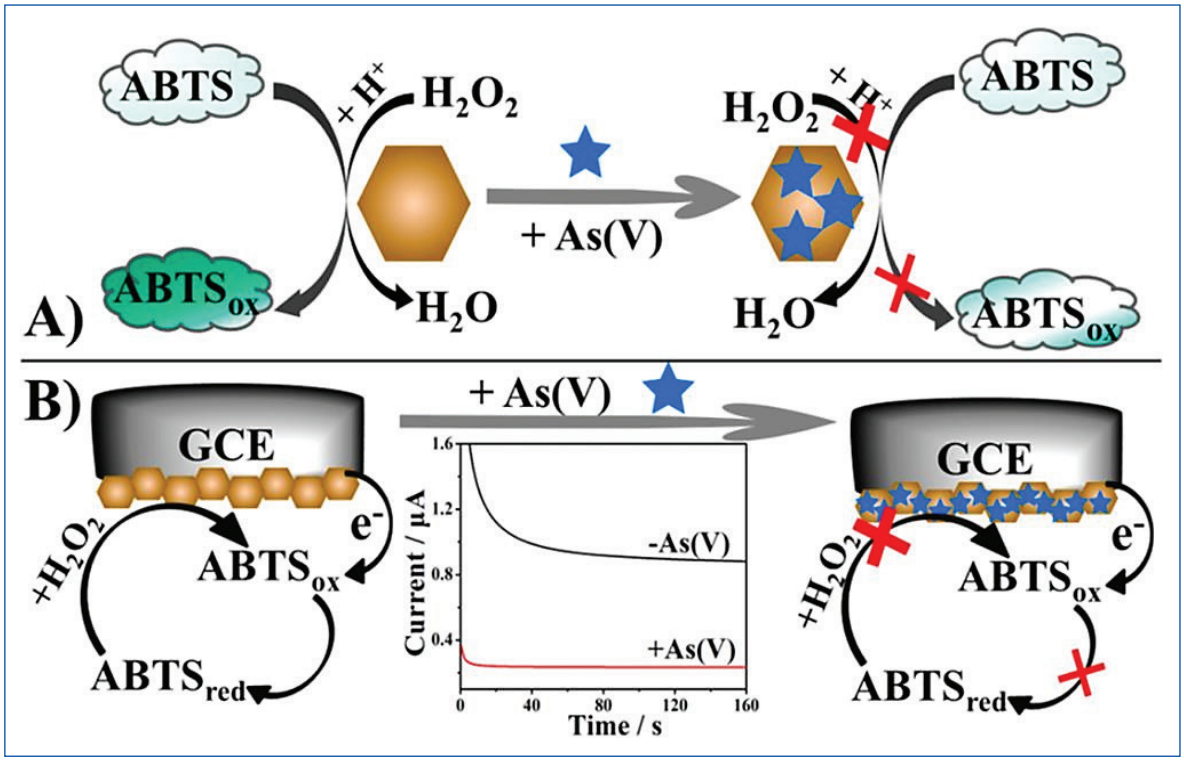

Fig. 9. Schematic illustration of the colorimetric probe of CoOOH nanoflakes for arsenate detection (A) and illustration of electrochemical assay of CoOOH nanoflakes modified electrode for arsenate detection (B) [66]

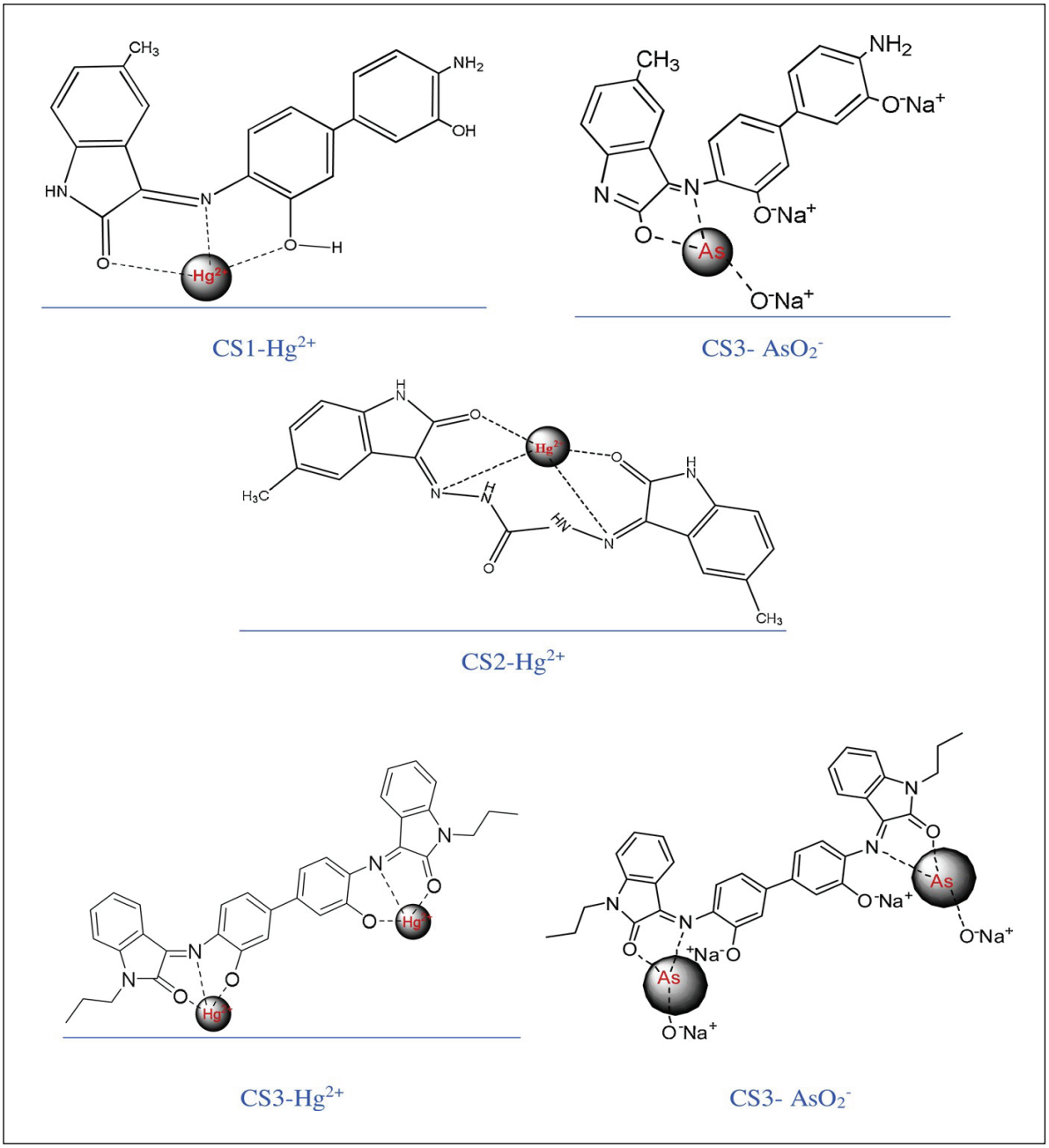

Fig. 10. Schematic representation of the binding mechanism of CS1, CS2 and CS3 with Hg^{2+} and AsO_2^- [68]

and anions. The fluorometric analysis is conducted using the same probe, utilizing the increase in fluorescence emission due to deprotonation and hydrogen bonding. The probe has the advantage of detecting the high toxicity of two anions, simply and cost-effectively.

When considering the above methods, most are shown low detection limits. Also, sensitivity and selectivity towards arsenate, arsenite or both forms are very high. It is especially high in nanomaterials compared to traditional methods. Table 1 shows the overview of above mentioned colorimetric methods, which offer low detection limits below the permissible limit of WHO. Many ways discussed in the text are used for the detection of As (III) due to the excellent oxidation property of As (III) which oxidizes into As (V). Therefore, total inorganic arsenic concentration can be detected using these methods.

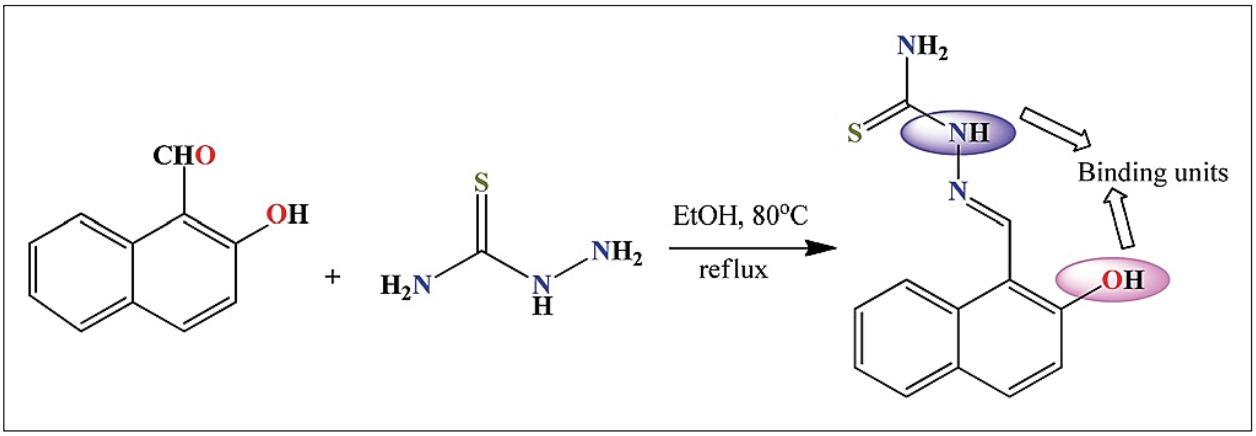

Fig. 11. Diagram of the synthesis of the probe for the detect arsenite and cyanide ions [69]

Table 1. Comparison of colorimetric methods discussed in this review based on structural modifications of molecules and usage of metal nanoparticles.

Materials	Arsenic	LR (µg L^{-1})	LOD (µg L^{-1})	References
Aptamer/ Hemin-H_2O_2 system	As (III)	-	6.00	[33]
GSH/DTT/Asn- AgNPs	As (III)	0.4-20	0.36	[52]
AgNPls/SiO_2-Fh	As (III) & As (V)	500-30000	0.50	[54]
GNR-PEG-DMSA	As (III) & As (V)	0.001-10	1.00	[55]
GNP-MMT@Eu	As (III) & As (V)	1-1000	1.00	[56]
AuNPs/ACP/AMP	As (V)	7.5-7520	7.50	[57]
AuNPs/G-T- rich DNA	As (III)	5-2000	2.00	[63]
Heptapeptide/ AuNPs	As (III)	-	4.00	[65]
CoOOH nanoflakes	As (V)	4-500	3.72	[66]

LOD: Detection limit
LR: Linear range

2.2. Fluorometric determination of arsenite

Fluorometric methods have tremendous attention for detecting arsenic due to their simplicity, less expensiveness, ease of operation, non-destructive, and fast response with low detection limits. Therefore, it is a promising technique for the detection of arsenic and it is very useful for environmental analysis [70-73].

2.2.1.Detection based on arsenite interacts with thiolated nanostructures.

Recently, quantum dots (QDs) have been used as one of the promising materials to detect arsenic due to their low toxicity and unique optical properties. QDs properties include high quantum yield, size-tunable spectral properties, long fluorescence lifetime, and narrow symmetrical emission peaks. Also, the preparation of QDs is very simple, straightforward, and does not require toxic precursors and organic solvents [73-77]. For example, environmentally friendly dithiothreitol (DTT) functionalized water-soluble carbon quantum dots (CQDs) were synthesized by microwave pyrolysis of citric acid and cysteamine to produce a fluorophore for

turn-on detection of arsenite. DTT exhibits on the surface of CQDs using S-S bonds to impart –SH functionalities on their surface formed DTT-CQDs complex. In the presence of As (III), arsenite binds with the sulfur group of CQDs through the –SH group of DDT ligand to form a stable As (III)-DDT-CQDs complex, and as a result, CQDs exhibit blue fluorescence. Figure 12 shows the synthesis of functionalized CQDs and arsenite binding processes on CQDs. Compared with metal-based semiconductor QDs such as CdS-QDs, ZnS-QDs, and CdTe-QDs, the mentioned approach is useful for real-world applications, including environmental analysis. Because metal-based semiconductor QDs influence the environment and human health due to their elemental composition and toxicity [77]

When considering a single colorful fluorescent probe, they only exhibit the change of fluorescence brightness with limited quantitative capability. Instead of the brightness changes, the detection of color variation is the most important factor for accurate quantification of the analyte. Therefore, pH and fluorescent test papers are widely used with fluorescent materials printed onto the paper. These analyses are low-cost, easy to operate, and portable. For example, a color multiplexing-based fluorescent test paper was developed for dosage-sensitive detection of As (III) with clear color visualization using fluorescent red and cyan probes, which can achieve a wide color variation from red to cyan. They have synthesized cyan CDs and red CdTe QDs-based cyan and red probes by hydrothermal and classical methods, respectively. Also, CdTe QDs are modified by GSH and DTT ligands, enhancing the aqueous solubility and strong binding affinity for As (III). As a result of the modification, many free–SH groups are on the surface of QDs. As-S bonds are formed byAs (III) addition and trigger the aggregation of GSH/DTT-QDs resulting in the fluorescence color change from red to cyan. A wide range of color variations

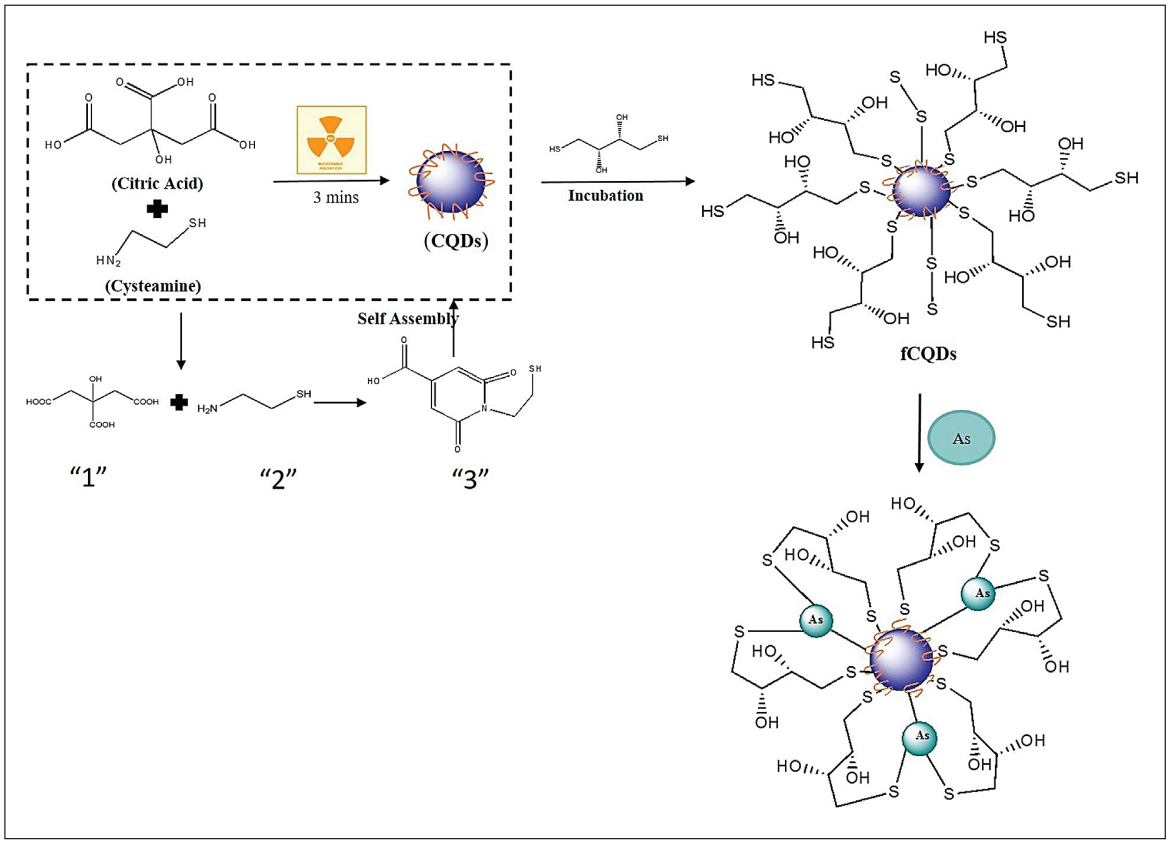

Fig. 12. Schematic representation of the synthesized DTT functionalized CQDs for detection of arsenite and binding mechanism of arsenite [77]

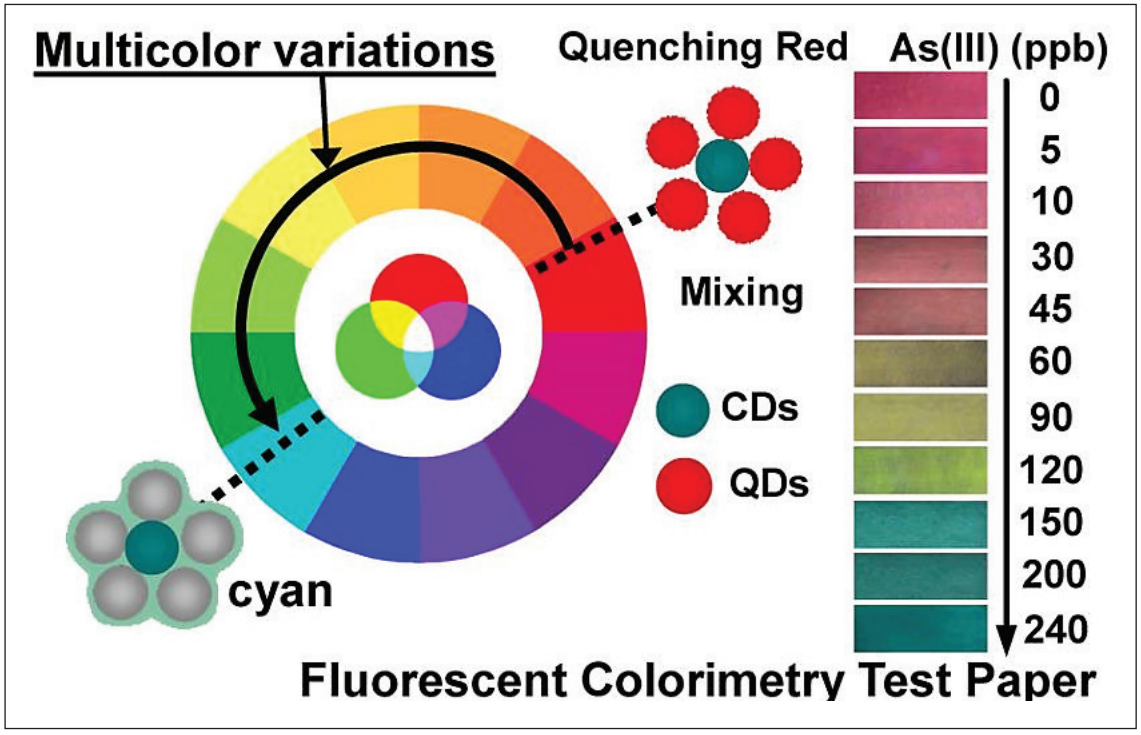

Fig. 13. Fluorescent colorimetry test paper for obtaining various color variations from red to cyan using CDs and QDs dual probe [78].

can be observed with As (III) concentration. They realized dosage-sensitive visualization of arsenite detection by the ink of sensory solution printed on the test paper. Figure 13 represents the fabricated fluorescent colorimetry test paper's various color variations from red to cyan for arsenite detection [78].

Nanoclusters containing fewer atoms show a high quantum yield and can be used to sense toxic metal ions. Gold nanoclusters and silver nanoclusters are commonly used to develop fluorometric sensing approaches. Quantum yield can be increased by adding capping ligands such as thiol-based and dipeptide ligands. Compared with other capping materials such as glutathione, dipeptide ligand-capped gold clusters show significantly high fluorescence yield. As an example, dipeptide (L-cysteinyl-Lcsteine) capped water-soluble fluorescent few-atom gold nanoclusters were synthesized using a core etching pathway through a "Top-down" mechanism for the detection of arsenite (Fig. 14). Synthesized dicysteine capped fluorescent gold cluster sensor is highly efficient, selective and extremely sensitive for arsenite without any extra modifications. With the addition of arsenite, fluorescence intensity enhances gradually. The reason may be due to the positive charge of As (III) interacting with the negative charge thiolated gold cluster and due to the electrons in the gold cluster that flow toward electron poor As (III) ions. Meanwhile, in the presence of arsenite, the radiative decay rate of the gold cluster is increased, and the fluorescence decay time is decreased. These fluorescent sensors can detect As (III) at low concentrations with high specific responses. Also, fluorescent sensors can be reused by adding succinic acid to chelates As (III) through complexation [79].

Selectively sensitive silver-doped hollow CdS/ZnS bi-layer nanoparticles (Ag-h-CdS/ZnS) are synthesized using the sacrificial core method to detect arsenite. AgBr nanoparticles were used as the core to synthesize Ag-h-CdS/ZnS nanoparticles. Also, L-cysteine is used to functionalize the nanoparticles. In the presence of arsenite, cysteine interacts with arenite, resulting in fluorescence

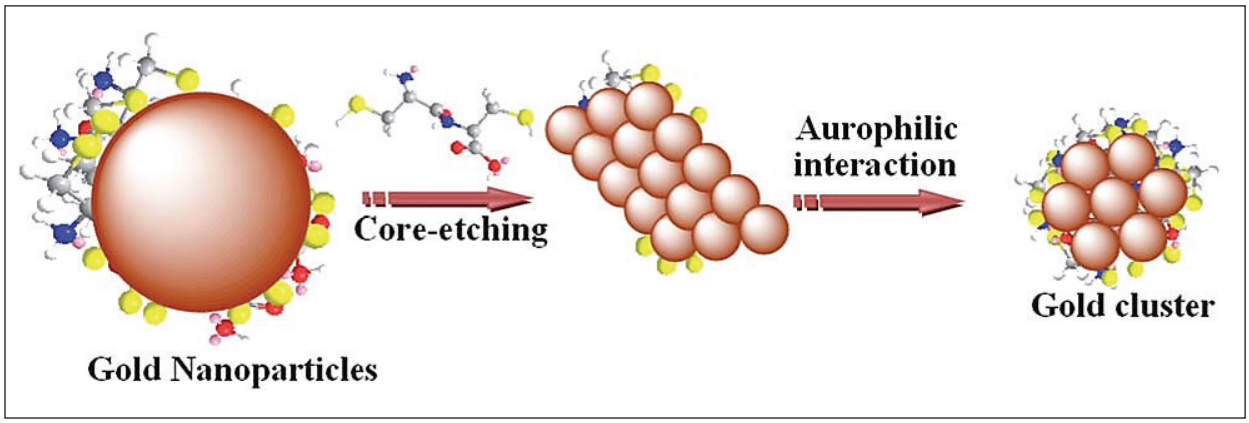

Fig. 14. Illustration of synthetic route for the formation of gold clusters through a top-down mechanism [79]

quenching of the nanoparticles due to changes in the electronic structure and accelerating the non-radioactive nature of excitons. This sensor has many advantages, including ease of use, selectivity, sensitivity, and cost-effectiveness [80]. A cysteine-functionalized tetraphenyl ethane (TPE)-based "Turn-on" fluorescent probe was developed for the highly selective detection of arsenite with a low detection limit. Free-SH groups in cysteine can bind with arsenite through As-S bonds, forming As $(CystPE)_3$ structure. TPE present in this complex promotes the formation of π-π aggregation, which results in turn-on fluorescence depending on the nature of the induced emission feature (AIE), thus creating a tendency to increase the fluorescence of the TPE complex. Figure 15 depicts the formation of As $(CystPE)_3$ complex and fluorescence activation [81].

There are many biological and chemical sensors developed for arsenite detection due to their redox properties and strong thiophilicity. The usage of thiol ligands can obtain many advantages, including high sensitivity and selectivity due to the presence of a vast number of sulphur that utilizes As-S bonds with arsenic in aqueous solutions. Also, CQDs show many advantages, including superior chemical stability, high aqueous solubility, tuneable surface functionalities, and resistance to photobleaching. However, complicated thiolated modifications lack visual analysis and limit their applications [75-81].

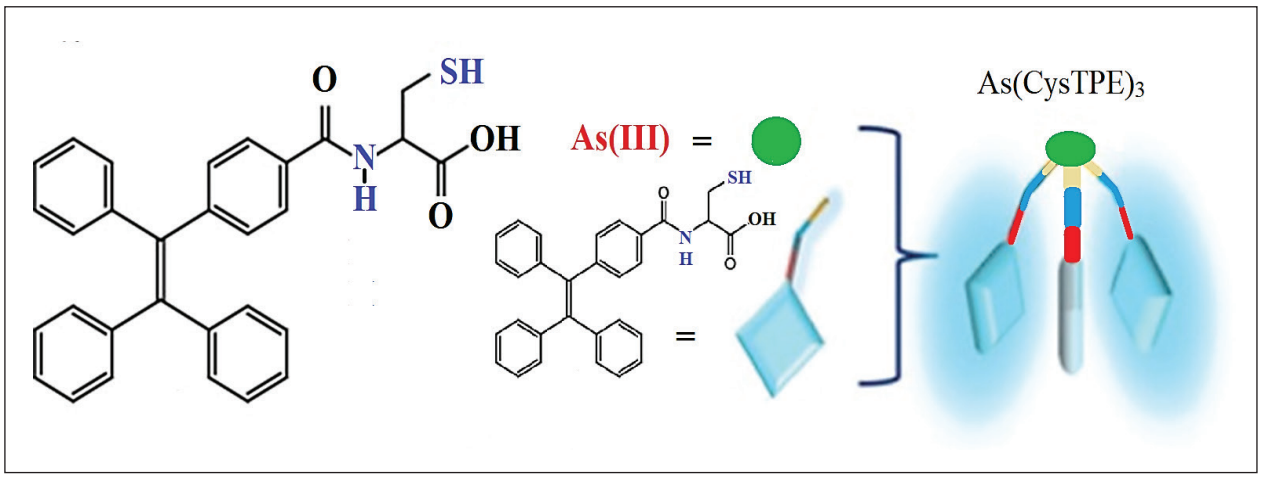

Fig. 15. Illustration of Asv$(CystPE)_3$ complex and florescent formation [81]

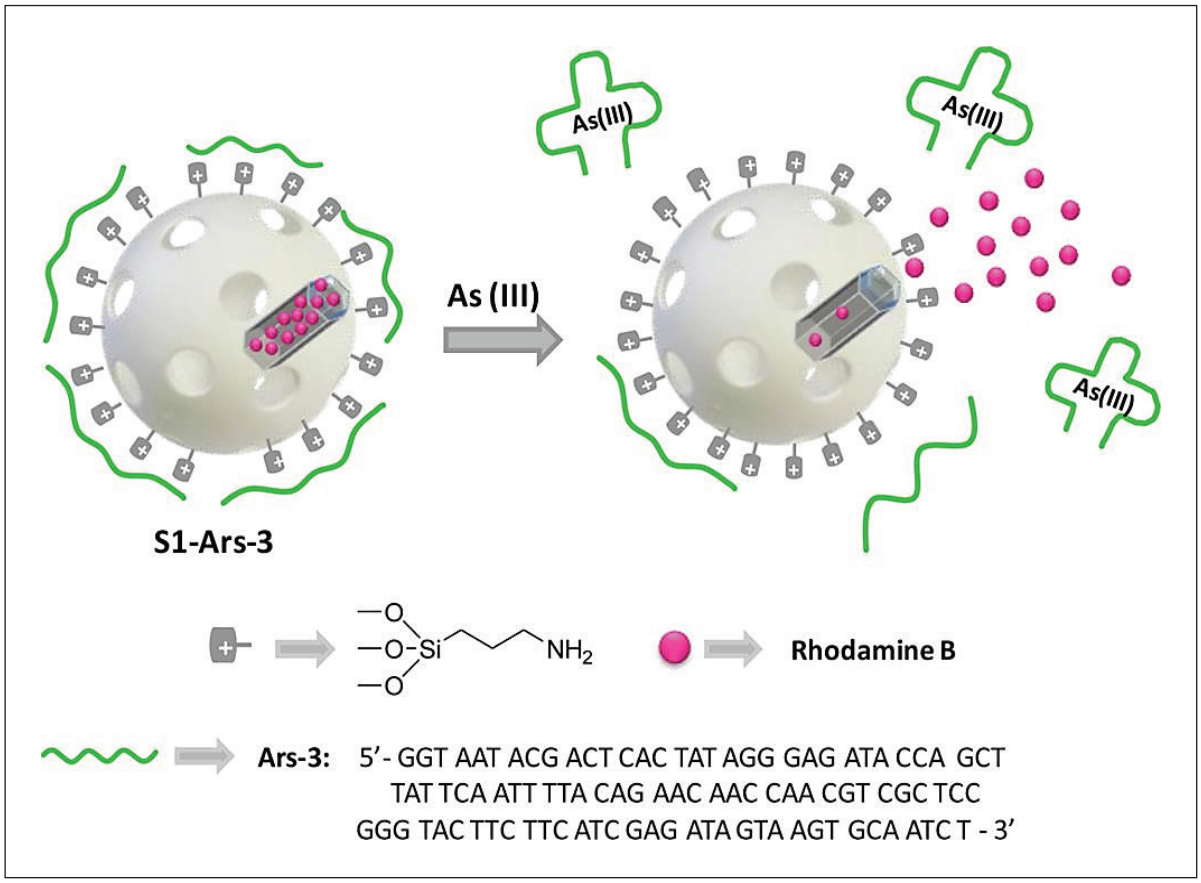

Fig. 16. Schematic representation of proposed modified Ars-3 aptamer and the sensing process of arsenite detection [86]

2.2.2.Detection based on arsenite interacts with biologically functionalized nanomaterials.

Nanostructure-based sensors provide many advantages including rapid and sensitive responses to detecting arsenic in cell living. For example, Mesoporous Silica Nanoparticles (MSNs) are considered a promising material for arsenic detection due to their high inner surface area and flexible surface modification capacity [82-86]. Therefore, MSNs can be functionalized by capping materials which enhance the detection capability. Aptamers are DNA sequences and act as capping material. Aptamer-based fluorescent sensors show high affinity, selectivity, and long-term stability for determining arsenite [83]. Oroval *et al.* [86] fabricated an arsenite sensing system using aptamer-capped MSNs. They used MCM-41 mesoporous silica nanoparticles and pores of MCM-41 inorganic support were loaded with rhodamine B. Rhodomine B was capped by Arsenite aptamer (Ars-3) in MSNs pores. As (III) has a high potential to bind with aptamer and then displace it from the MSN surface. Resulted of the fluorescence difference can be used for quantitative detection of As (III). This fluorescence system can be used for environmental analysis due to its simplicity. Figure 16 shows the functionalized Ars-3 aptamer in the sensory system for arsenite detection.

The dye-labeled G/T rich single-strand DNA-wrapped single-wall carbon nanotubes (SWCNT)-based fluorescent probe was designed to analyze arsenite quantitatively at the femtogram level. Here, 5-hexachloro-fluorescein phosphoramidite (HEX) was used to label the ssDNA, and that structure is the wrapping material of SWCNT. Arsenite can bind with the G/T bases of ssDNA in living cells, reducing the π-π interaction between ssDNA and SWCNTs. As a result, ssDNA can be dissociated

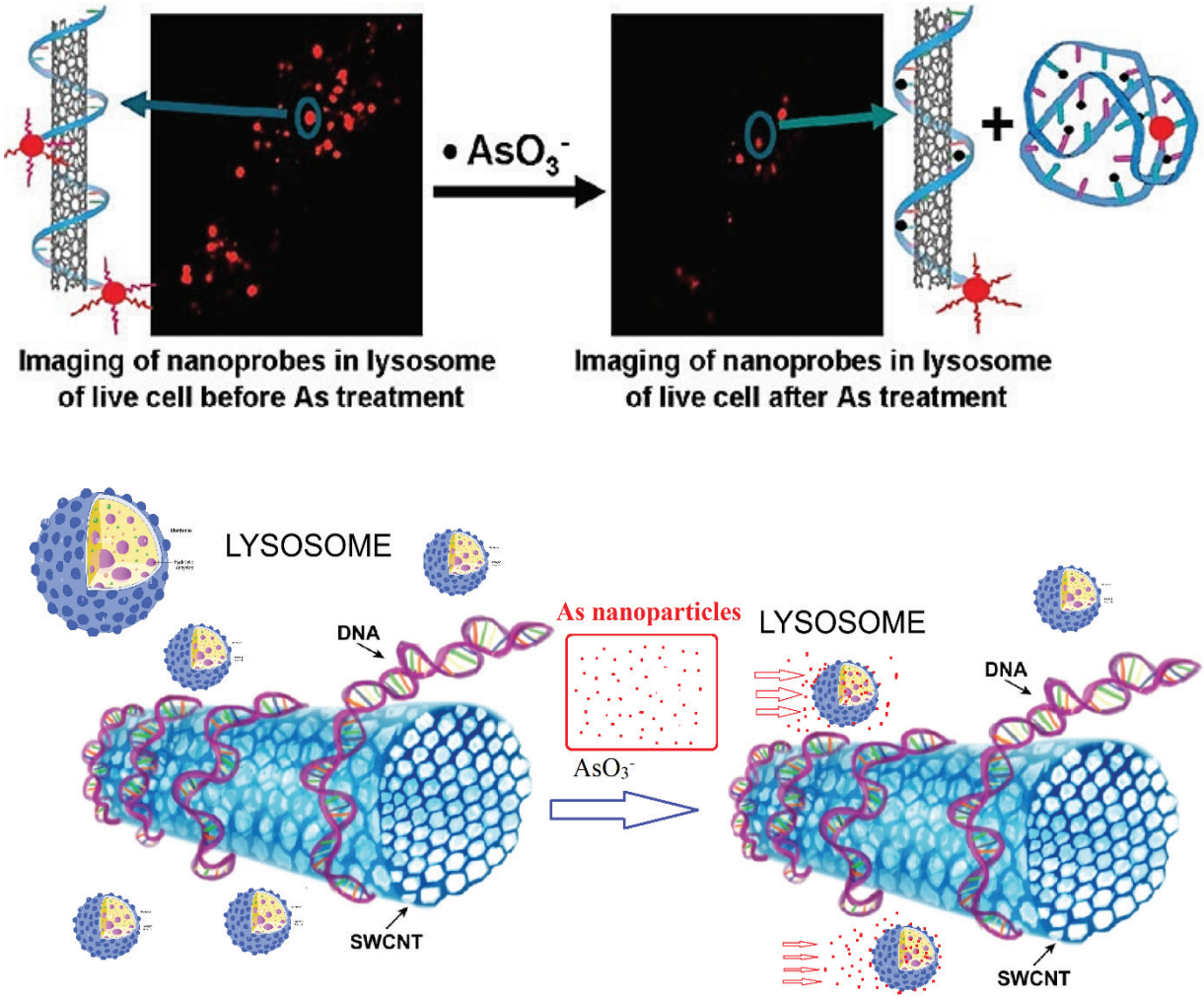

Fig. 17. Illustration of nanoprobe interaction with arsenite in living cells [87]

from the surface of SWCNTs and condensed in the live cells. The condensed structure of ssDNA facilitates the HEX to interact with G/T bases bound arsenite ions, resulting in significant fluorescent quenching of HEX dye. Figure 17 illustrates the nanoprobe interaction with arsenite in the lysosome of a living cell [87].

2.2.3.Detection based on arsenite interacts with chemosensor.

A simple Schiff base fluorescence probe (HL) was fabricated for "turn on" detection of arsenite through the intermolecular hydrogen bonding induced chelation-enhanced fluorescence (CHEF) process. Arsenite selective HL probe is synthesized by condensation of 2,6-diformyl-p-cresol with 4-aminoantipyrine. The absence of arsenite ions can obtain fluorescence with weak intensity. And the presence of arsenite, fluorescence intensity is higher than in the absence of arsenite. Fluorescence differences can be used for the quantitative detection of arsenite. When arsenite ions are present in the sample, intermolecular hydrogen bonds are formed between arsenite and the probe to form HL-As (III) complex and a resulting fluorescent signal. Its intensity increases with the increment of As (III) concentration. Arsenite ions interact with phenolic O-H to form strong hydrogen bonds, which can affect the photo-induced electron transfer (PET) process and enhance the fluorescence intensity through the chelation-enhanced fluorescence (CHEF) process. This probe can be used to imagine

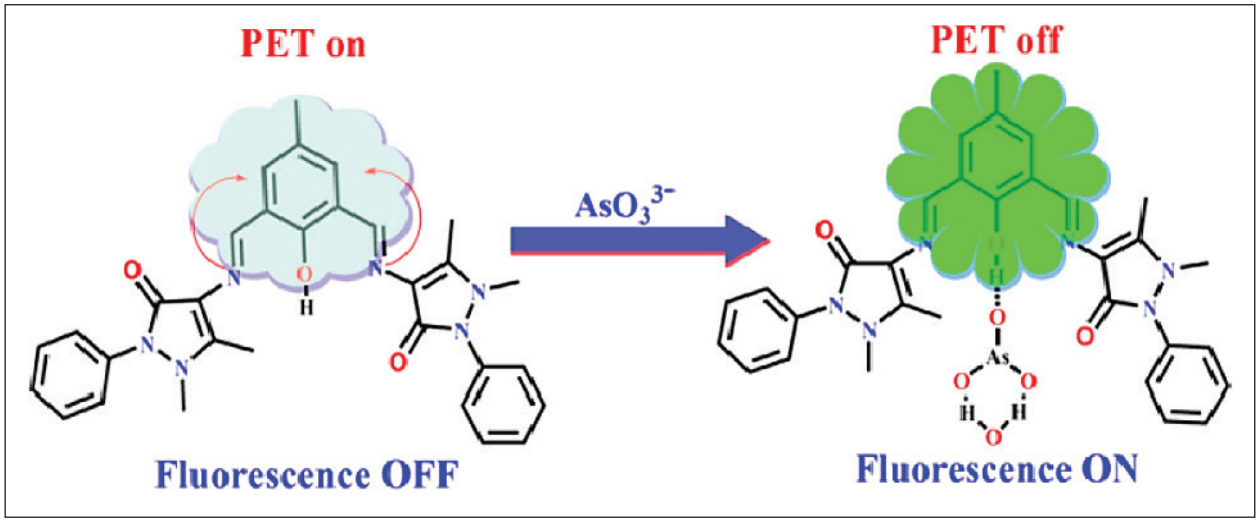

Fig. 18. Illustration of the fluorescence enhancement of HL probe in the presence of arsenite [88]

arsenite contributions in living cells, such as cancer cells, and is applicable for detecting trace-level arsenite in different water samples. Other competitive ions do not affect the fluorescence enhancement. Figure 18 depicts the fluorescence formation of HL in the presence of arsenite [88].

2.3. Fluorometric determination of arsenate

2.3.1. Detection based on the interaction of arsenate with iron-modified materials.

Nowadays, various types of solid nanomaterials are used as an adsorbent for arsenic removal processes. But most are toxic, difficult to separate after adsorption, and ineffective in impurities. Therefore, iron oxide and its modifications overcome these limitations [89-93]. Liu and co-workers [93] proposed an arsenate detection method that relies on the strong interaction between As (V) and the surface of metal oxides. They synthesized fluorescent DNA-loaded ferric oxide nanoparticles by incubating carboxyfluorescein (FAM)-labeled DNA with nanoparticles. DNA adsorbs on ferric oxide nanoparticles through phosphate in its backbone. This configuration covered the fluorescence signal of FAM-labelled DNA. The presence of arsenate competes with adsorbed DNA for binding sites and displaces adsorbed DNA by removing adsorbed DNA from the surface of nanoparticles. As a result, fluorescence is recovered. The sensitivity can be improved by increasing DNA adsorption affinity using shorter DNA due to adsorbing properties with high density and ease of desorption. In addition, they enhanced their scope for increasing adsorption capacity by using different nanoparticles. They used and compared three types of nanomaterials; CeO_2, $CePO_4$, and Fe_3O_4, which contain hard Lewis acids and a bonding preference for phosphate in DNA. Among them, CeO_2 nanoparticles perform better than the other two, achieving a ten times lower detection limit compared to Fe_3O_4 nanoparticles. The advantages of this method included the requirement for small sample volumes for the analysis and being highly sensitive to shorter DNA. They can be used to compare and identify nanomaterials' DNA adsorption affinity and sensitivity. Figure 19 shows the fluorescence recovery procedure with the induction of arsenate into the sensory system. The same group proposed a study based on fluorescent-labeled DNA-functionalized iron oxide nanoparticles for arsenate detection in environmental analysis. Polyphosphate present in DNA has a specific ability to adsorb on the surface of iron oxide. As the nature of DNA and Fe_3O_4 nanoparticles, salt induces the system for absorbance of DNA with high adsorption efficiency. Figure 20 shows the schematic of sensing arsenate by DNA-functionalized iron oxide nanoparticles. The configuration of fluorescent-labeled DNA binds to nanoparticles that quench the fluorescent

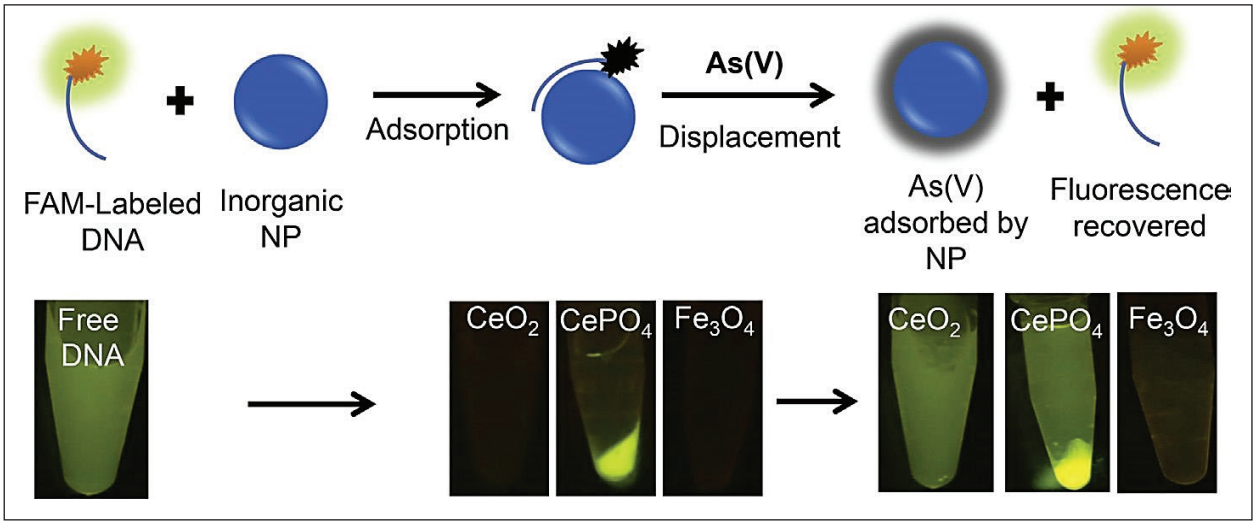

Fig. 19. Schematic representation of fluorescence recovery process with the presence of arsenate [93]

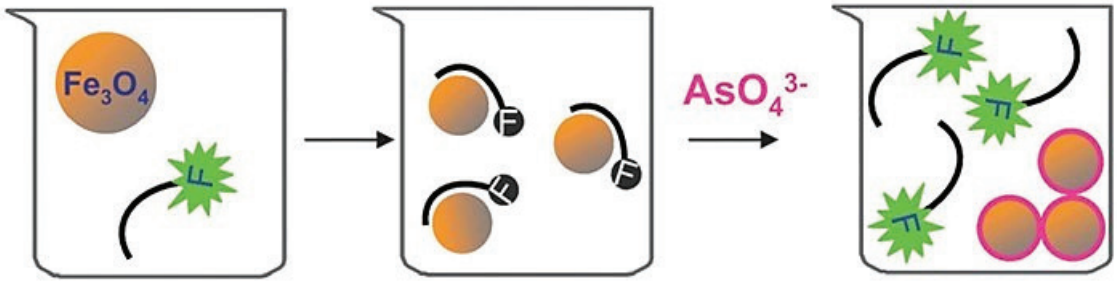

Fig. 20. Illustration of sensing arsenate by DNA functionalized iron oxide nanoparticles [94]

yield. In contrast, in the presence of arsenate, arsenate competes for the binding sites of DNA and replaces them. As a result, the fluorescent signal recovered in the system. The intensity of the fluorescent recovery is arsenate concentration-dependent [94].

In addition, Metal-Organic Framework (MOF) materials are considered sensing materials, facilitating many advantages in analyzing target materials. Because it has attractive characteristics such as the presence of organic binding ligands or metal centers and a large surface area that allow effective trapping and removal of target ions [95,96]. For example, amino-functionalized iron-containing MOFs were synthesized with powerful fluorescence emission capacity for detecting and removing arsenate. They fabricated NH_2-MIL-88(Fe) nano octahedra by solvothermal treatment of $FeCl_3$.6H2O and NH_2-BDC in DMF. Unsaturated iron sites in the sensory system have a highly selective affinity for arsenate by formatting As-O-Fe bonds. Synthesized MOFs show weak fluorescence due to the electron transfer from the NH_2-BDC organic linker to Fe_3-$_3$-oxo clusters. However, fluorescence recovery increases with the introduction of arsenate into the system. Moreover, fluorescence intensity increases with the increment of arsenate concentration. In the presence of arsenate, it can rapidly diffuse into the MOFs, and then aggregation occurs between iron-oxo-clusters and arsenate. This method has great potential for sensing arsenate compared to other methods, such as fluorescent DNA-loaded iron oxide NPs, CeO_2, and FAM-labelled DNA. Other MOFs' advantages include cost-effectiveness, readily available ease of fabrication, rapid response, high sensitivity

nature, and anti-interference ability. And applicable for polluted environmental analysis due to the fluorescence stability of MOFs [96].

2.3.2. Detection based on the interaction of arsenate with enzyme activity

Enzymes are biological molecules that speed up the reaction rate by binding to the reactant/substrate. Some arsenic detection processes involve enzymes' catalytic or inhibitory activity [97-99]. For example, a highly selective enzymatic catalysis system was synthesized using inexpensive glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to detect a low arsenate concentration with a low detection limit. GAPDH catalytic system contains an enzyme (GAPDH), a coenzyme (NAD^+), and a substrate (G3P). In the reaction, cysteine in the holoenzyme interacts with the substrate to form an acyl-enzyme intermediate through hydride transfer from intermediate hemithioacetal to NAD^+. As a result, NADH is released, and another NAD^+ has an affinity to the enzyme-bound substrate to produce NAD^+-acyl-enzyme. Arsenate can act as an acyl acceptor, and present arsenate interacts with NAD^+-acyl-enzyme to form stable 1-arseno-3-phosphoglycerate (APG). The better performance of arsenate as a nucleophile leads to hydrolysis of arsenoglycerate, and the resulting holoenzyme re-enters the catalytic cycle. The result is that a fluorescent yield of NADH is amplified in the system. In addition, APG hydrolyzed rapidly to regenerate arsenate, which leads the catalytic cycle by continuously generating NADH with excess NAD^+. Fluorescence yield increases with the amount of arsenate are increased in the sample. Furthermore, the catalytic system is inexpensive, rapid response, highly sensitive, and useful for arsenate in environmental samples and safety applications. Figure 21 illustrates the enzyme catalytic system for sensing arsenate and APG hydrolysis reaction [97].

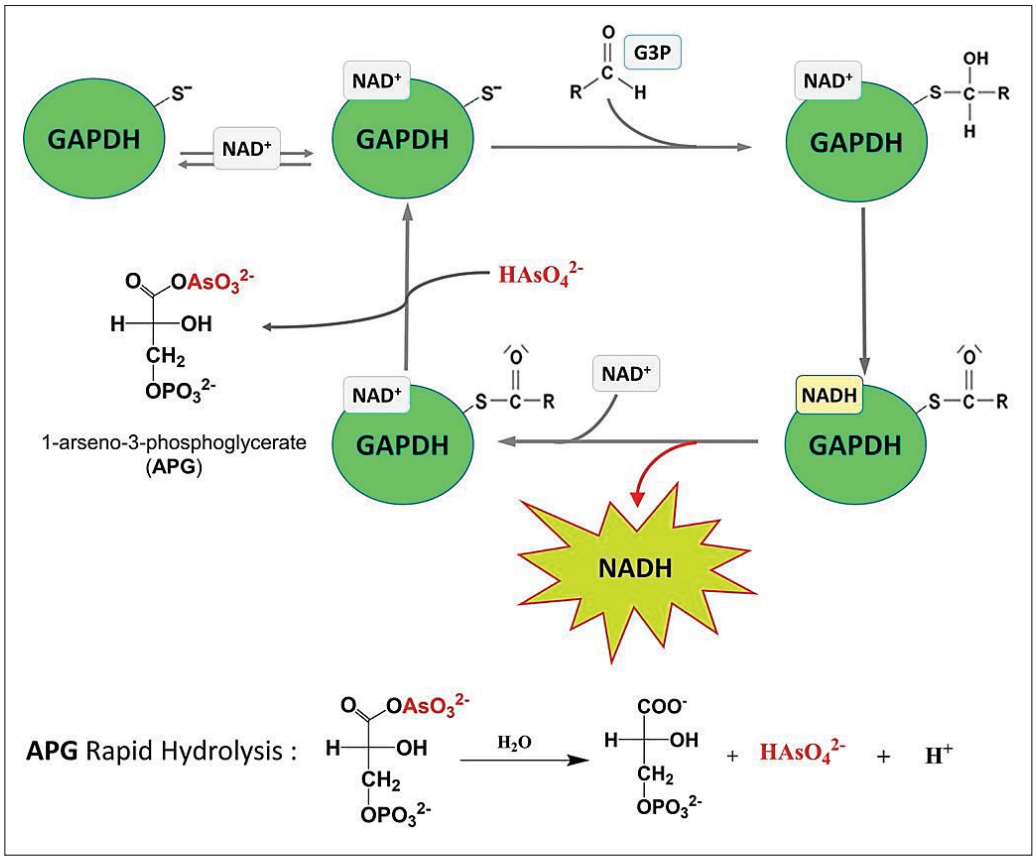

Fig. 21. Schematic illustration of catalytic enzyme mechanism for sensing of arsenate and APG hydrolysis reaction [97]

The involvement of arsenate inhibitory activity on phosphatase enzymes shows remarkable pathways to arsenate detection. As an example, Jian-Ding Qie and co-authors [98] synthesized a fluorescent nanoprobe containing CdSe/ZnS quantum dots (QDs) coated with the terbium (III) complex of guanosine monophosphate (Tb-GMP) using one-pot adaptive self-assembly process. Fabricated QD/TB-GMP composite which exhibited dual fluorescence emission and single wavelength excitation properties are used for ratiometric determination of arsenate. The presence of acid phosphatase enzyme (ACP) can catalyze the hydrolysis of GMP and as resulting phosphate ions and guanosine particles. The fluorescent intensity of Tb-GMP shows a signal off with ACP. But in the presence of arsenate, As (V) inhibits the catalytic activity of ACP. Therefore, hydrolysis of GMP does not take place effectively. So Tb-GMP complex recovered its fluorescence intensity and its intensity increased with the increment of arsenate concentration. This visual analysis method can be used to quantitatively determine arsenate with a 0.39 μg L^{-1} detection limit and high selectivity toward As (V). High solubility, facile preparation process, excellent single excitation, and dual emission fluorescence properties, visual analysis are advantages of nanoprobe. And it is a feasible method for environmental analysis. Figure 22 shows the synthesis of QD/Tb-GMP and fluorescent signal with As (V) and the absence of As (V).

2.3.3. Detection of arsenate that present in the living cell using chemosensors

Arsenate detection is essential in living cells due to its high toxicity. That might have a poisonous effect on cells. As an example, destroy the conversion of ATP into ADP permanently. Arsenate selective fluorescence sensors (APSAL) were designed by condensing salicylaldehyde with 4-amino-antipyrine. In the presence of arsenate, strong hydrogen bonds are created between APSAL and As (V) to form APSAL-As (V) complex. The molecular level interaction between As (V) and APSAL can be described using density functional theory (DFT). The presence of arsenate fluorescence intensity increases significantly without significant interference from other common ions. APSAL is highly selective for arsenate and obtains micromolar range detection limits. Optimal pH maintenance is essential for the efficiency of the sensor. This system applies to detect intracellular arsenate in living cells [99].

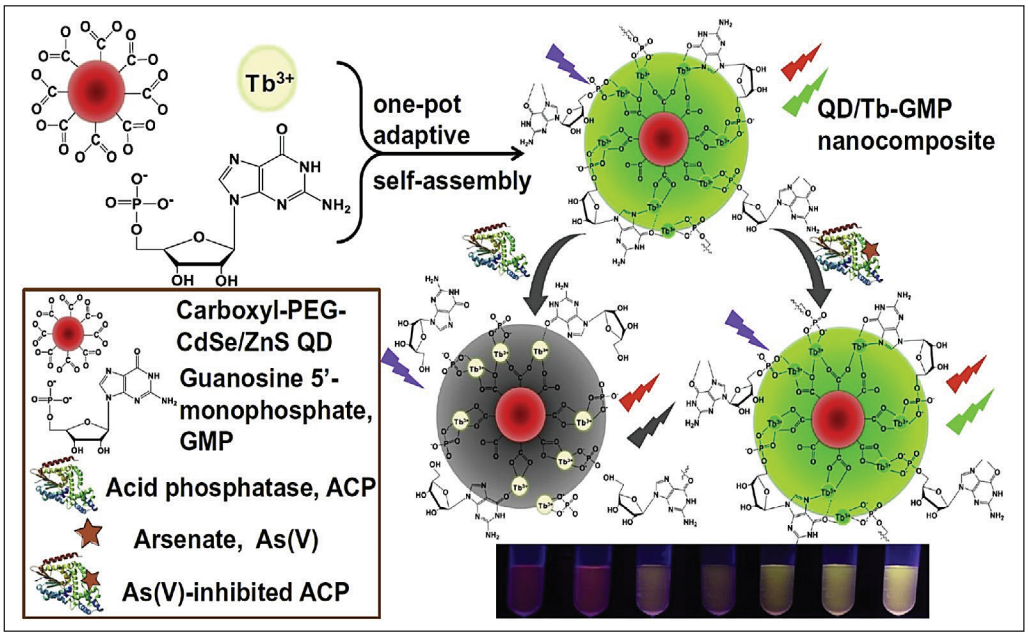

Fig. 22. Schematic representation of the formation of QD/Tb-GMP and fluorescent signal with the presence of As (V) and without As (V) [98].

Abu *et al.* proposed an oxime-based fluorescent probe to detect arsenate and arsenite in living cell-imaging applications [100]. Detection relies on formatting nano/microstructures by H-bonding interactions in the presence or absence of arsenate and arsenite. Arsenic (arsenate and arsenite) interacts with 2,6 diformyl-*p*-cresol-dioxime (DFC-DO) ligand, which has a remarkable sensing capacity for arsenite and arsenate detection and forms DFC-DO-$H_2AsO_4^-$ and DFC-DO-AsO_2^- respectively through intermolecular hydrogen bonding. As a result, the quantum yield of the free ligands increases. Above proposed methods exhibit low detection limits below the WHO permissible limit of 10 µg L^{-1} with high selectivity towards either arsenate or arsenite. Table 2 shows an overview of fluorometric methods mentioned in this review for determining arsenate and arsenite. Among them, MOF materials showed the lowest detection limit.

3. Conclusion

This review has covered colorimetric and fluorometric methods for detecting arsenate and arsenite. Discussed colorimetric methods highlighted the evolution of colorimetric methods, detections based on structurally modified molecules, and usage of nanoparticles with/without modifications. Among them, nanomaterials showed better performance compared to traditional methods. Discussed all methods are reliable, easy in usage, can be used for field analysis, relatively cost-effective compared to instrumental methods, modifications are enhanced sensitivity and selectivity and further indicated lower detection limits (below the WHO recommendations). Thiol-functionalized AgNPs showed the lowest detection limit (0.36 µg L^{-1}). However, modifications are complicated, and unmodified nanomaterials showed much cost-effectiveness. Therefore, the usage of unmodified nanomaterials is economically important. Proposed fluorometric methods were mostly based on nanostructural probes and facilitated high sensitivity and selectivity. Those are reliable, simple, non-destructive, and cost-effective. Also, they can use for field analysis with low detection limits. Among them, MOF materials showed the lowest detection limit (0.056 µg L^{-1}). Modifications in nanomaterials are enhanced sensitivity and accuracy of the fluorometric sensor. Especially thiolated modifications are very sensitive to arsenic.

4. Acknowledgement

We thank Dr. Arjuna Wijekoon for his kind guidance throughout the process. We acknowledge the Wallumattagal clan of the Dharug nation as the traditional custodians of the Macquarie University land. We strongly support equity, diversity, and inclusion in science [101]. The authors come from different countries (Sri Lanka and Australia) and

Table 2. Comparing Fluorometric methods for the detection of As (III) and As (V)

Materials	Arsenic	DR (µg L^{-1})	LOD (µg L^{-1})	References
DTT/CQDs	As (III)	5-100	0.086	[77]
GSH/DTT-QDs/CDs	As (III)	5-100	1.7	[78]
Dipeptide-AuCs	As (III)	-	~4.04	[79]
Aptamer/ MSNs	As (III)	-	0.9	[86]
HL probe	As (III)	-	4.1	[88]
DNA/CeO2 NPs	As (V)	0-150	2.2	[96]
NH2-Fe-MOF	As (V)	0.1-50	0.056	[95]
Enzyme catalytic activity	As (V)	0-200	10	[97]
QD/Tb-GMP	As (V)	0.5-200	0.39	[98]
APSAL probe	As (V)	-	5	[99]

LOD: Limit of Detection
DR: Detection Range

represent different career stages (Master's student, Ph.D. student, and ECR). The authors have no conflicts of interest to declare.

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