Development of Bioanalytical Test Kits for Rapid Monitoring of Lead and Arsenic Contamination in Water (Review)

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Abstract
The health hazards associated with heavy metal exposure have become better understood over the years including those resulting from lead and arsenic contamination. Early and accurate detection of both heavy metals in water is crucial, hence, the use of bioanalytical test kits or biosensors for real-time detection of lead and arsenic in water has been recommended as a viable alternative to the use of conventional analytical techniques for onsite water monitoring. Current advances in the design of bioreceptors and their signal pathways also suggest that various bioanalytical kits can be designed to produce significant and quantifiable signals indicating the presence of lead and arsenic in water samples. This review describes the general structure, principle of operation and classification of biosensors. It evaluates available reports on the design of bioanalytical kits for lead and arsenic detection including enzyme immobilization on different nanoparticle-based platforms, non-enzyme bioreceptors, whole-cell bacteria-based bioreceptors as well as the signal pathways utilized by these kits. This review also evaluates the efficiency of some bioengineering constructs and highlights their limitations in order to proffer guidance on adopting the appropriate strategy for developing viable rapid test kits for onsite water monitoring.

Keywords: Biosensor, bioreceptors, heavy metals, lead (Pb), arsenic (As),

1.0 Introduction

Bioanalytical kits or biosensors can be described as devices that utilize the activity of a biological recognition element (bioreceptor) to generate signals that can be translated into quantitative information about different analytes such as heavy metals (Thevenot *et al.,* 2001). Various reports have highlighted the effects of heavy metals on the environment and human health even at low concentrations; particularly, lead and arsenic are known to be among the most prevalent and toxic heavy metals available (Balali-Mood *et al.,* 2021; Collin *et al.,* 2022; Fatoki, and Badmus 2022). Therefore, various biosensor kits have been designed to determine the presence and concentration of lead and arsenic in water.

Generally, lead and arsenic occur in different concentrations and oxidation states when present in water. The conventional techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), anodic stripping voltammetry and X-ray fluorescence spectrometry, used for heavy metal analysis require well-trained personnel to operate the equipment involved. The process might involve sample preparation and also require specific time for the results to be available (Odobašić *et al.,* 2019). The use of biosensors therefore presents a simpler, faster, reliable and cost-effective approach for onsite water monitoring, particularly for the presence of lead and arsenic. The target of any biosensor design or construct is high sensitivity, short response time and specificity, hence detailed understanding of the behavior of both heavy metals in water, the signal pathways of different bioreceptors and the limitations of available biosensor designs becomes necessary.

This study describes the general structure of biosensors and evaluates available reports on the use of various biosensor kits for lead and arsenic detection in water.

2.0 *General structure of biosensors*

A basic biosensor design is composed of three distinct sections; a bioreceptor element for analyte recognition, a signal transduction system and an amplification /processing system. Some designs also incorporate a selective membrane which functions in the transportation of the analyte to the bioreceptor element (Velasco-Garcia and Mottram, 2003; Velusamy *et al.,* 2022). Generally, the heavy metal analyte is recognized by the bioreceptor after which the signal transducing system converts the response of this metal-bioreceptor interaction into well-defined signals. These signals are then processed, amplified, quantified and displayed in different forms preferably readable values. The displayed values or features can be matched to the heavy metal concentration based on pre-developed metal calibration curves (Ali*et al.,* 2017; Bhalla *et al.,* 2016). A typical illustration of a biosensor system can be seen in Figure 1.

Biosensors can be classified based on their mode of detection, mainly the transduction system and bioreceptor component. Based on the transduction system, biosensors could be electrochemical, optical and thermometric. Electrochemical biosensors utilize the electrical signals generated after the bioreceptor/ metal interaction while optical and thermal biosensors are based on optical and temperature changes respectively (Gautam *et al.,* 2012). The choice of a suitable transduction system should be based on the physicochemical properties of the heavy metal and the

nature of the signals produced after its interaction with the bioreceptor (Mohanty and Kougianos, 2006).

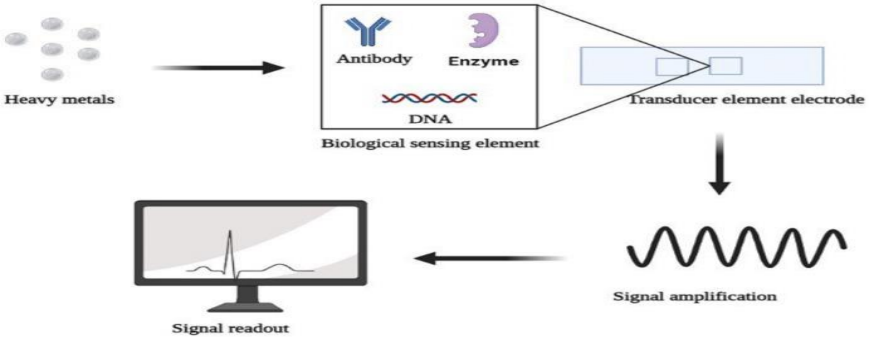

Figure 1: Schematic illustration of a biosensor general working principle (Velusamy, *et al.,* 2022*)*

Based on the bioreceptor component, biosensors could be immunosensors, enzyme-based or whole cell-based; the use of other biomolecules have also been reported. Immunosensors are based on the recognition ability of antibodies; particularly, IgG has been applied in heavy metal detection due to its higher affinity and specificity compared to other antibodies. Several reports suggest that monoclonal and polyclonal antibodies could have either narrow or broad specificity while recombinant antibodies could be designed to provide both. Difficulty in reproducing the immune-reactivity over time remains one of the main limitations of immunosensors (Ferrigno, 2016; Sharma *et al.,* 2016).

Enzyme-based biosensors are generally based on enzymatic interactions, particularly enzyme activation or inhibition by heavy metals. Enzymes that have been reported for the identification of lead include acid phosphatase, alkaline phosphatase, β-galactosidase, glucose oxidase, laccase and urease, while arsenic (+3) methyltransferase (AS3MT), alkaline phosphatase, urease, glutathione s-transferase (GST) and peroxidases have been reported for arsenic detection (Turdean, 2011). Several reports also indicate that the use immobilized enzymes could ensure faster analysis, reduce interference from other metal analytes and lower the consumption of the enzymatic material (Berezhetskyy *et al.,* 2008; Nguyen *et al.,* 2019; Bounegru and Apetrei, 2023). The design of enzyme-based biosensors therefore requires clear understanding of the specific enzyme kinetics based on heavy metal interaction, hence, environmental factors such as temperature, pH and contact time must be considered (Bachan Upadhyay and Verma, 2013; Lieswito *et al.,* 2019).

Whole cell-based biosensors (WCB) involve the integration of whole cells, including microorganisms, plant cells, algae, fungi, and protozoa into the biosensor design. Signals are usually generated from changes in cellular activities such as the modifications of the fatty acid composition in the cell membrane and cell energy fluctuations over time (Turdean, 2011; Gu *et al.,* 2004). Whole cell-based biosensors are suggested to be relatively less expensive compared to enzyme-based biosensors as they do not require distinct enzyme isolation and purification processes. Also, whole-cell biosensors are more tolerant to temperature variations, however, the interference of other analytes has been reported to affect their detection efficiency (Odobašić *et al.,* 2019, Moraskie, *et al.,* 2021).

3.0 Detection of lead and arsenic using biosensors

Most commercially available bioanalytical kits for lead and arsenic detection take the form of test strips, assay kits, or portable analyzers which differ in the type of bioreceptor, the bioreceptor-metal interaction and signal transduction pathways.

3.1 Biosensors for lead detection

The immobilization of enzymes such as DNAzyme on various nanoparticle-based platforms has been reported for lead detection (Unnikrishnan *et al.,* 2021; Rigo *et al.,* 2020). For instance, the immobilization of DNAzyme on magnetic beads was applied in the design of biosensors for lead detection (Zhuang *et al.,* 2013; Rong *et al.,* 2018). Also, magnetite-based nanoparticles have been applied for the immobilization of DNAzyme for the rapid detection of lead in water (Xu *et al.,* 2020; Li *et al.,* 2021). There are also a number of reports on the use of gold-based nanoparticles for DNAzyme immobilization. Wu *et al.* (2020) developed a surface plasmon resonance (SPR) biosensor by immobilizing DNAzyme on gold nanoparticles for the detection of lead (II) ions while the use of reduced graphene oxide-tetraethylenepentamine-gold nanoparticles (rGO-TEPA-Au) (Yu *et al.,* 2018) and streptavidin (SA) modified tin dioxide-functionalized reduced graphene oxide/gold nanoparticles (Ji *et al.,* 2019) were reported to effectively detect lead ions in water samples.

Other enzymes such as urease have been utilized in the design of enzyme-based biosensors for lead detection. Gumpu *et al.* (2017) reported the design of a biosensor based on urease-inhibition. The bioreceptor electrode was developed using the immobilization of selenium nanoparticles and urease on a Nafion layer and the detection limit of this device was observed to be 0.019 µM with a sensitivity of 89.2×10^{-3} µA μM^{-1}. Urease and alkaline phosphatase were used in the design of cantilever biosensors for the detection of lead in river water samples. The signal was initiated by changes in the deflection of the cantilever nano-biosensors after contact with water (Rigo *et al.,* 2020). The inhibition of urease and acetylcholine esterase (AchE) was used to design biosensor kits for the detection of lead ions in water with a detection limit of 1 ppb (Haron and Ray, 2006).

Other non-enzyme bioreceptors have been utilized in the design of lead biosensors. For instance, a tripeptide glutathione linked to gold nanoparticles (GNPs) was applied for the detection of lead (Chu *et al.,* 2015). The authors reported the limit of detection to be 47.6 nM, with a distinct red-to-blue colour change. Similarly, Lai *et al.* (2022) reported a portable fluorescence resonance energy transfer (FRET) based biosensor kit for the onsite detection of lead in water. The structure of the device included a laser diode cased in a 3D printed frame, a fluorescence emission image source, a biochip (Met-lead 1.44 M1) and an

optical lens; the detection limit of this device was determined to be 24 nM. The device was also designed to ensure smart phone compatibility and the results were comparable to other analytical methods such as ICP-MS. The authors, however, suggested that the interference of other metals such as copper, mercury, and cadmium could limit the accuracy of the results obtained from this device.

3.2 Biosensors for arsenic detection

Various enzyme-based biosensors have also been reported specifically for the detection of arsenic. A highly sensitive biosensor for arsenic (III) detection using DNAzyme was developed by Zeng *et al.* (2019) and the authors reported the limit of detection of this device to be 2 ppm. Similarly, Sanllorente-Méndez *et al.* (2010) applied the inhibition of acetylcholinesterase enzyme for the inhibition of arsenic.

There are reports of the use of whole-cell bacteria (WCB) as bioreceptors for arsenic detection; some authors only describe the functionality of the bioreceptor element, while others provide details of some genetic modification applied in the bioreceptor design (Li *et al.,* 2021; Valenzuela-García *et al.,* 2023). Jia *et al.* (2019) reported two whole-cell biosensors (WCB) designs using *E. coli* DH5α; The first WCB involved the coupling of an arsenic-inducible promoter (Pars) and its regulatory gene (arsR) with the reporter gene mCherry while the second involved the introduction of a transcriptional activator (a variant of luxR) as a positive feedback circuit where it activates its expression as well as that of the reporter gene. The minimum limit of detection of this device was observed to be 0.1 µM, which is significantly lower than the acceptable standard for the arsenic. The design of bacteria-based biosensor kits involving the use of bacterial reporter protein for arsenic detection was reviewed by Diesel *et al.* (2009). The authors demonstrated that the activity of the reporter proteins produced by the bacteria in response to arsenic can be measured and further suggested that reporter proteins obtained from genetically modified strains of *E. coli*, *B. subtilis S. aureus* and *Pseudomonas putida,* were viable for this type of device.

Merulla *et al.* (2013) reviewed various reports on the biotechnological approaches to arsenic detection

Table 1: Some biosensor kits for lead and arsenic detection available

Heavy metal	Bio-receptor design	Detection Limit (as reported)	Reference number
Lead	DNAzyme-based/Magnetic-bead sensor.	37 pM	57
		32pmol L^{-1}	37
	DNAzyme/gold-nanoparticle based	80 pM	52
		2 pM,	54
		38 fg mL^{-1}	22
	DNAzyme-modified Fe_3O_4@Au@Ag nanoparticles	5 pM	53
	Fe_3O_4@TiO_2core-shell nanocomposites	10^{-10}ppm,	27
	Glutathione/gold nanoparticles (GNPs)	47.6 nM	8
	Urease/CeO_2 nanoparticles.	0.019 ± 0.001 µM	18
	Fluorescence Resonance Energy Transfer (FRET) / biochip (Met-lead 1.44 M1)	24 nM.	26
	urease and acetylcholine esterase (AchE)	1 ppb	19
Arsenic	DNAzyme as abiocatalytic amplifier.	2 ppM	56
	WCB/ genetically modified strains of *E. coli*, *B.subtilis*, *S.aureus*, and *Pseudomonas putida.*	Not stated	11
	WCB/ *Salmonella typhimurium*flagellin	Not stated	21
	WCB/ *Escherichia coli* DH5α	0.1 µM	23
	E. coli DH5α with the plasmid pJSKV51	5 to 100 ppb	46
	Detectable drop in pH and a chromogenic system using endospores	less than 10 ppb arsenate	24
	Acetylcholinesterase enzyme activity.	1.1 × 10−8 M	39
Lead and arsenic	*Clostridium guangxiense*; inhibition of fermentative gas	101.33 mg/L (Ar)and 243.45 mg/L(Pb)	13

particularly available bioreporter genes and pathways that are activated or expressed during arsenic/bioreceptor interaction. The authors described several bioengineering constructs for the optimization of bioreporter gene circuits and highlighted the importance of clear understanding of the arsenic signaling pathways when designing biosensors. Jankovics *et al.* (2021) reported the design of a highly sensitive flagellin-based biosensor for arsenic detection. This construct was done using an arsenic-binding peptide motif to replace the D3 domain of *Salmonella typhimurium* flagellin and the device displayed high sensitivity and rapid response time. Gudlavalleti *et al.* (2017) developed a biosensor design through the transformation of *E. coli* DH5α with plasmid pJSKV51 in addition to other bacterial genetic modifications. The device was capable of rapid and onsite detection of arsenic in groundwater within the range from 5 to 100 ppb and had a liquid crystal display system showing the concentration of arsenic present in parts per billions. Another biosensor was developed for the detection of arsenic using *E. coli* bioreporter cells (Truffer *et al.,* 2014). The authors demonstrated that the results from this device were reproducible and could be transmitted in real time to mobile networks for direct water monitoring. A simple chromogenic biosensor was designed using endospores that utilized changes in pH level for the detection of arsenic in drinking water; the device had less than 10 ppb arsenate as the detection limit (Joshi *et al.,* 2009). This device was further improved such that pH variations of up to 50 samples could be monitored simultaneously (de Mora *et al.,* 2011).

Designing a biosensor kit that can detect both lead and arsenic simultaneously would be ideal for onsite water monitoring. Eom *et al.* (2020) reported the use of a spore-forming fermentative bacterium, *Clostridium*

guangxiense for the screening of water contaminated with lead and arsenic alongside other heavy metals. The toxicity of the water sample was evaluated based on the inhibition of fermentative gas production by both heavy metals. The authors demonstrated that the kit was easy to use, had high reproducibility and could be applied for on-site environmental monitoring. This suggests the possibility of designing multifunctional bioreceptors that can detect both lead and arsenic in water.
A summary of the reports of biosensor designs for lead and arsenic is described in Table 1.

4.0 Challenges and limitations of Lead and Arsenic Detection in Water using bioanalytic test kits

Generally, the accuracy, sensitivity and specificity of rapid-test biosensor kits are some of the major concerns in heavy metal detection which can be attributed to the varying levels of sensitivity for dissolved and particulate metal ions in water. (Velusamy *et al.,* 2022; Odobašić *et al.,* 2019; Verma and Kaur 2016). A report by Kriss *et al.* (2021) demonstrated the limitations of some "at-home" test kits, such as overestimation of lead concentrations and variation in detection limits. The authors suggested that the high detection limits of some of these test kits indicate that they are more suitable for analyzing water bodies and may not be reliable for lead detection in drinking water. Also, despite the total lead concentrations being above15 µg/L, only two out of the seven lead water samples were detected for lead by these test kits. These findings highlight the limitation of the use of rapid test kits for the detection of particulate lead in water.

Arsenic is usually present in two oxidation states, with arsenite (Ars^{111}) reported to be more toxic than arsenate (Ars^{v}). This variation could affect detection results, hence, the importance of designing kits that can differentiate between both forms (Shankar and Shanker 2014). There are reports of analytical kits that can detect both arsenic ions (Chen and Rosen, 2014; Singh and del Valle, 2015; Nagaraj *et al.,* 2024). Also, further oxidation could occur during sampling, transportation and storage which might affect the accuracy of arsenic detection (Kumar and Riyazuddin, 2010).

Some reports have highlighted the interference of other trace elements in the detection of arsenic indicating the need for a bioreceptor that is highly selective to arsenic (Yogarajah and Tsai, 2015). Some authors have also highlighted calibration issues associated with arsenic detection devices and there are suggestions that samples with concentrations around 50 µg/L need to be re-analyzed (Safarzadeh-Amiri *et al.,* 2011; Jakariya *et al.,* 2007). More studies might be required to clearly determine the variations in detection results of different rapid test kits compared to the values obtained using conventional analytical techniques (Amiri *et al.,* 2011; Jakariya *et al.,* 2007).

Summary

Developing biosensors for the real-time detection of lead and arsenic in water is essential, however, the choice, structure and signal mechanism of the bioreceptor/recognition element remains the most critical aspects in the design of these kits. Reports of the use of various enzymes and non-enzyme bioreceptors, nanoparticle-based immobilization platforms and other biosensor designs for lead and arsenic detection have all been reviewed. This review highlights the preference of enzyme-based biosensors (particularly DNAzyme and either magnetite or gold nanoparticle platforms) for lead detection and whole cell-based biosensors for arsenic detection by the authors in most of the reports (as seen in Table 1). The detection limit of some of the devices in this review indicates that a low-cost biosensor with high sensitivity and accuracy can be designed for onsite and real-time monitoring of lead and arsenic in water, however clearer understanding and more information is required. Also, certain considerations must be made when designing these kits including the variability of water quality, environmental factors, suitability of the bioreceptor, device calibration challenges and the interference of other metals present on result accuracy. Currently, achieving high detection accuracy using biosensor kits still remains challenging, however, they can provide viable information on the levels of lead and arsenic contamination during onsite water assessment. It can be suggested that these test kits should include information about stagnation protocols, sample volumes, water flow rates, additional dissolution steps and other environmental conditions which could be sources of error or generally affect the interpretation of results.

Declaration of interest

The authors declare that no conflict of interest exists.

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