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Impact of Heavy Metal Ions on Environment and Their Detection by Using Enzyme Biosensor

Meghna Malik[1], Vinay Narwal[2], Ritu Deswal[3], Sapna Grewal[*1]

[1]Guru Jambheshwar University of Science & Technology, India
[2]Deenbandhu Chhotu Ram University of Science & Technology, India
[3]Maharshi Dayanand University, India
*E-mail: *sapnagrewal29@gmail.com*

Abstract—*Heavy metal ions are considered as one of the most toxic substances affecting the environment. Accumulation of these substances increases continuously in the environment and land water due to industrial pollutants and effluents. Heavy metals have a certain threshold value to perform their function efficiently in living organisms. If the tolerance level of this value exceeds for respective organism then it turns into toxic actions. The presence of heavy metals in excess affects plants, water and soil. The plant grown in such areas accumulate heavy metals like cadmium, zinc, copper and lead. Due to high toxicity of heavy metal ions there is an obvious need of biosensor system to detect the heavy metal ions. In today scenario, biosensors are considered as an important tool for detection and quantitation of heavy metal ions. They prove very promising as the system is rapid, selective, sensible, low cost and easy to use.*

Keywords: *Heavy metal ions, analysis and monitoring of heavy metal ions, enzyme and DNA biosensor for heavy metal ion detection.*

1. INTRODUCTION

The accumulation of toxic substances in the environment continuously increases due to diverse pollutants from the industries. Contamination of land and water due to disposal of industrial effluents is the most significant problem. Heavy metal ions are regarded as one of the most toxic substances affecting the environment [1]. Heavy-metal ions are ubiquitous in nature, thus resulting in a serious environmental problem. Due to their high toxicity, there is an obvious need to determine them rapidly on site at trace levels. Although the typical detection methods such as atomic absorption spectrometry and inductively coupled plasma mass spectrometry are widely used for the determination of heavy-metal ions [2], both methods require very sophisticated equipment and cannot be used for field monitoring. Therefore, there is a need for simple and potable detection method. In general, electrochemical methods are able to selectively detect heavy-metal ions with less complex instrumentation. The techniques developed so far include ion-selective electrodes, polarography, and other voltammetric methods [3]. Conventional analytical techniques for heavy metals (such as cold vapour atomic absorption spectrometry, and inductively coupled plasma mass spectrometry) are precise but suffer from the disadvantages of high cost, the need for trained personnel and the fact that they are mostly laboratory bound. In recent years, electrochemical biosensors have received a great attention as promising alternatives for the determination of heavy-metal ions. Biosensors have the advantages of specificity, low cost, ease of use, portability and the ability to furnish continuous real time signals. The analysis of heavy metal ions can be carried out with biosensors by using both protein (enzyme, metal-binding protein and antibody)-based and whole-cell (natural and genetically engineered microorganism)-based approaches [4].

2. ANALYSIS OF HEAVY METAL IONS

Heavy metal poisoning due to contamination of groundwater, surface water, and soil has been a serious concern in many areas of the globe. The current method for detection of heavy metal ions in water and other matrices still largely relies on sending a technician to the field, collecting samples, and bringing them to a laboratory for analysis. This approach is not only time-consuming and inconvenient but also expensive and prone to errors that may occur during sample transportation and handling. A number of portable devices have been developed, which include anodic stripping voltammetry (ASV) [5, 6], X-ray fluorescence [7], and immunoassay- based detection kits. These devices are not widely used due to various technical or other limitations. A low-cost, easy-to use, and reliable device is still much needed for environmental monitoring and analysis. Such a device could also be used in medical diagnostics for early detection of heavy metal poisoning in children, which is especially important in developing countries.

ASV is an established technology for sensitive and selective detection of metal ions and other electrochemically active substances [5]. However, ASV has several limitations. First, the sample must be dissolved in supporting electrolyte.

Second, ASV often uses mercury as the working electrode, which is not environmentally friendly. Third, the presence of background current in ASV measurement makes it difficult to detect the small stripping current associated with the oxidation of the analytes. Finally, ASV measures only redox-active species and processes, and the formation of inter metallic compounds between two different metals can disturb the individual stripping peaks.

Surface plasmon resonance spectroscopy (SPR) is a sensitive method for detecting analytes adsorbed on a metal surface [8]. It has been widely used as a biomedical and pharmaceutical research tool to screen biological and chemical analytes [9]. Surface plasmons are collective oscillations of free electrons in a metallic film. Under an appropriate condition, the plasmons can resonate with an incident light beam and absorb the energy of the beam [10, 11]. Because the resonance condition is extremely sensitive to the refractive index of the medium adjacent to the metallic film, the presence of molecules on the surface of the metallic film can be accurately detected. Efforts to combine SPR with ASV techniques have been reported for metal ion detection [12, 13, 14, and 15].

3. BIOSENSORS FOR HEAVY METALS

3.1 Enzyme based biosensors

A variety of enzymes have been used in the analysis of heavy metals ions based on the activation or inhibition of their activities. Heavy metal causes activation when they form an integral part of the structure and function of the enzyme as cofactors in metalloproteins. For example, A biosensor based on the use of urease enzyme immobilized by glutaraldehyde crosslinking with bovine serum albumin on electrode surface [16]. The determination of heavy-metal ions using the urease-immobilized biosensor is based on the measurement of the urease enzymatic activity which is inhibited by heavy-metal ions [17]. It is well known that the inhibition of the urease by these ions results from the reaction with sulfhydryl groups of the active site of the enzyme [18]. The urease converts urea into ammonium and bicarbonate ions. the response time and signal magnitude, the urea concentration used in the inhibition test of the biosensor to heavy-metal ions was chosen to be 1.0 mM [19].

a) Protein-functionalized microcantilever sensors

Microcantilevers functionalized with metal-binding protein, AgNt84-6, are demonstrated to be sensors for the detection of heavy metal ions like Hg^{2+} and Zn^{2+}. AgNt84-6, a protein that has the ability to bind multiple atoms of Ni^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+} was attached to the gold-coated side of silicon nitride cantilevers via linker groups. Upon exposure to 0.1 mM $HgCl_2$ and 0.1 mM $ZnCl_2$ solutions, the microcantilevers underwent bending corresponding to an expanding gold side. Exposure to a 0.1 mM solution of $MnCl_2$ solution did not result in a similar bending indicating a weak or no interaction of Mn^{2+} ions with the AgNt84-6 protein. The microcantilever bending data were consistent with data from electrophoresis carried out on SDS-PAGE gels containing metal ions that showed protein interaction with Zn^{2+} ions but not with Mn^{2+} ions. Thus, we demonstrate that microcantilever bending can be used to discriminate between metal ions that bind and do not bind to AgNt84-6 protein in real time [20].

b) Fibre optic based biosensors

Trace analysis of heavy metals is important in the chemical, environmental and biomedical fields. Chemical and biochemical methods using optical or electrochemical techniques of signal transduction to detect metals have been studied [21-25]. Biochemical means of detection of metal ions often involve metalloenzymes, which involve metals as cofactors for their enzyme activity [26]. Inhibition of the native metalloenzyme, alkaline phosphatase, in the existence of some metal ions, and the reactivation of its apoenzyme by Zn(II) ions is utilized to determine metal ion concentrations. Alkaline phosphatase-catalysed hydrolysis of a chemiluminescent substrate, chloro 3-(4-methoxy spiro [1,2-dioxetane-3-2'-tricyclo-[3.3.1.1]-decan]-4-yl) phenyl phosphate, produces light. By evaluating the chemiluminescence signal strength in the presence or absence of metal ions, this reaction can be used to detect and determine metal ion concentrations. The immobilization of alkaline phosphatase on different glass surfaces by covalent coupling using a bifunctional reagent, glutaraldehyde, was demonstrated. Using chemiluminescence measurements, Zn(II), Be(lI) and Bi(III) were detected in trace levels. This technique forms the basis in the development of a metal ion-based fibre optic sensor [27].

c) Novel conductometric biosensor based on three-enzyme system

A differential pair of planar thin-film interdigitated electrodes, deposited on a ceramic pad, were used as conductometric transducer. The three-enzyme system (invertase, mutarotase, glucose oxidase), immobilized on the transducer surface, was employed as a bioselective element. The ratio involving enzymes in the membrane was found experimentally considering the highest biosensor sensitivity to substrate (sucrose) and heavy metal ions. Sucrose concentration optimum for inhibitory analysis was 1.25 mM and incubation time in the studied solution amounted to 10–20 min. The developed biosensor exhibited the best sensitivity toward ions Hg^{2+} and Ag^{+}. A principal possibility of the biosensor reactivation either by EDTA solution after inhibition with silver ions or by cysteine solution after inhibition with mercury ions was shown [28].

Whole cell biosensor

Heavy metals are the most serious pollutants among us and thus there is need to develop sensitive and rapid biomonitoring methods for heavy metals in the environment. Critical parameters such as bioavailability, toxicity and genotoxicity can't be tested using chemical analysis, but only can be

assayed using living cells. A whole cell biosensor uses the whole cell as a single reporter incorporating both bioreceptor and transducer elements. Example: T.thermophila transformed strains were created as heavy metal whole cell biosensor and turned on bioassays were deigned to detect, in about 2h , the bioavailable heavy metals in polluted soil or aquatic samples. Validation of these whole cell biosensors was carried out using both artificial and natural samples, including methods for detecting false positives and negatives. Comparision with other published cell biosensors indicates that the tetrahymena metallothionein promoter based biosensors appear to be the most sensitive eukaryotic metal biosensors and compare favourably with some prokaryotic biosensors as well [29].

DNA based Biosensor

A structure-switching DNA optical biosensor for rapid on-site/*in situ* detection of heavy metal ions is reported. Mercury ions (Hg^{2+}), highly toxic and ubiquitous pollutants, were selected as model target. In this system, fluorescence-labeled DNA containing T-T mismatch structure was introduced to bind with DNA probes immobilized onto the sensor surface. In the presence of Hg^{2+}, some of the fluorescence-labeled DNAs bind with $Hg^{2+}$to form T-Hg^{2+}-T complexes through the folding of themselves into a hairpin structure and dehybridization from the sensor surface, which leads to decrease in fluorescence signal. The total analysis time for a single sample was less than 10 min with detection limit of 1.2 nM. The rapid on-site/*in situ* determination of Hg^{2+} was readily performed in natural water. This sensing strategy can be extended in principle to other metal ions by substituting the T-Hg^{2+}-T complexes with other specificity structures that selectively bind to other analytes [30].

4. CONCLUSION

The studies conducted in this review shows that the enzyme and DNA based biosensor can be used as a reliable sensor for heavy metal ion determination. It has several advantages like easy production of the sensor, low cost, sensibility, ease of operation, good sensor-to-sensor reproducibility, no chemical modification of the substrate or enzyme for the enzyme immobilization process, and further possibility to control of the biosensor performance by changing the alkoxide/water ratio in the stock sol-gel solution in the construction of the biosensor. It is clear that due to the nonspecific nature of the inhibition effect, this type of biosensor based on the enzyme inhibition assay cannot be used for the specific determination of a particular heavy-metal ion. Therefore, further study is under way to achieve selective detection of heavy metal ions.

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