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Review Article

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Biosensors for Monitoring of the Environmental Heavy Metals

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Abstract Heavy metals used in different fields of industry and/or agriculture act as inhibitors of enzymes, which, are unable to bind the substrate. The total amount of heavy metals detected by such means. Thus need arises for the fast and inexpensive methods for the detection of bioavailable heavy metals. Biosensors are useful analytical devices in this respect. A biosensor is an analytical device, which converts a biological response into an electrical signal. The two main components of a biosensor are bioreceptor and transducer. Bioreceptor: It is a biomolecule or biocomponent like enzymes, DNA, metalloproteins or microbes that recognizes the target molecule. Enzymes represent the largest class of bioreceptors, which are mainly proteins that catalyze different chemical reactions in cells. The mechanism of enzyme-based biosensor and the kinetic of detection process are described. In this context, is explainable why bioelectronics, nanotechnology, miniaturization, and bioengineering will compete for developing sensitive and selective biosensors able to determine multiple analytes simultaneously and/or integrated in wireless communications systems. The overall objective of the present review is to present information concerning sources of heavy metals, harmful effects of heavy metals and the fast, useful analytical devices and inexpensive methods (Biosensors) for the detection of bioavailable heavy metals.

Keywords Biosensors, Heavy Metals, Bioreceptor

Introduction

Many definitions of heavy metals have been proposed—some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity [1]. From chemical point of view, the term heavy metal is strictly ascribed to transition metals with atomic mass over 20 and specific gravity above 5 [2]. It mainly includes the transition metals, some metalloids, lanthanides, and actinides. Unfortunately, a more in-depth consideration reveals a huge amount of problems with this simple definition. This definition is meant to suggest that the density of a heavy metal is high, but this physical property is quite meaningless in the context of plants and other living organisms. Plants do not deal with metals in their elemental (valence state of 0) forms; they are not accessible to plants. Metals are only available to them in solution, and it is necessary for metals to react with other elements and form compounds before they can be solubilised. Once such a chemical compound is formed (e.g. a salt), the density of the metal does not play any role. The correlation between the density of a metal and its physiological or toxicological effects and even the chemical properties of its compounds are not known till date [3].

Some of these heavy metals, such as Co, Cu, Fe, Mn, Mo, Ni and Zn, are essential 5 elements required for normal growth and metabolism of plants. These elements can easily lead to poisoning when their concentration rises to supra-optimal values. Others, such as As, Cd, Hg, Pb or Se, are not essential, since they do not perform any known physiological functions. For some heavy metals, toxic levels can be just above the background concentrations naturally found in nature. Therefore, it is important for us to inform ourselves about the heavy metals and to take protective measures against excessive exposure. If unrecognized or inappropriately treated, toxicity can result in significant illness and reduced quality of life [4].

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential

settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children [5].

Heavy metals are introduced into the environment either by natural means or by human activities:

Natural Sources

Natural sources: In nature excessive levels of trace metals may occur by geographical phenomena like volcanic eruptions, weathering of rocks (Acid rock drainage) and leaching into rivers, lakes and oceans due to action of winds.

Anthropogenic Sources

In modern times, anthropogenic sources of heavy metals, i.e. pollution, have been introduced to the ecosystem. People have always been exposed to heavy metals in the environment. Metals leaching from eating utensils and cookware lead to metallic contamination of food and water. Metallic constituents of pesticides and therapeutic agents are additional sources of hazardous exposure. The burning of fossil fuels containing heavy metals, the addition of tetra-ethyl lead to gasoline, and the increase in industrial applications of metals, such as metal plating factories, mining industries, tanning, dye and chemical manufacturing industries, etc., have made heavy metal poisoning a major source of environmental pollution [6].

Lead, chromium, cadmium, copper, zinc and mercury are among the most frequently observed metal contaminants [7-8].

Determination of Heavy metal

Monitoring of the heavy metals is vital due to the potential health and ecological hazard they present. Laboratory techniques routinely used for metal ion analysis, such as atomic absorption spectrometry**,** inductively coupled plasma mass spectrometry, anodic stripping voltammetry, X-ray fluorescence spectrometry **a**nd microprobes [9-11] require sophisticated equipment, sample pretreatment, or skilled operators. Most techniques can detect the total amount of metal ions. However, several studies have established that only certain oxidation states of watersoluble or bioavailable metal ions pose the most risk to human health and the environment. For example, Cr(III) is an essential nutrient required in insulin action and sugar and fat metabolism, while Cr(VI) is believed to be highly toxic and carcinogenic [12].

Therefore, simple, rapid, inexpensive, selective, and sensitive methods that permit real-time detection of bioavailable metal ions in their different oxidation states are very important in the assessment of concentration, speciation, and stability of these metal ions [13], In addition, due to the dangers that certain toxic metal ions may pose to operators, remote sensing devices are desirable [14].

Consequently, with the comparable sensitivity and selectivity, the electrochemical methods such as ion-selective electrodes, biosensors, polarography, and other voltammetric techniques are also extensively used as attractive choice to the classical methods, due to their less complex instrumentation and shorter measuring period [15]. Also, simple, inexpensive, and portable instruments are attractive and desirable for real-time sampling/measuring and online and continuous analysis/monitoring/control of natural samples [16].

The two main components of a biosensor are bioreceptor and transducer. Bioreceptor: It is a biomolecule or biocomponent like enzymes, DNA, metalloproteins or microbes etc. that recognizes the target molecule. Enzymes represent the largest class of bioreceptors, which are mainly proteins that catalyze different chemical reactions in cells. Enzyme reacts to a substrate molecule and produces a reaction that can be measured and is repeatable (i.e., the enzyme is stable). Enzymes/whole cells can be immobilized on to the transducer and their enzymatic activities can be studied electrochemically. These whole cell enzymatic biosensors have the advantage of being more stable as the enzymes are in their natural environment. Transducer: It is a device for converting the recognition event or the interaction/reaction between analyte and bioreceptor into a measurable signal. Transducers can be subdivided into the following four main types. x • Electrochemical Transducers (Potentiometric, Conductometric, Voltammetric, FET-based sensors) • Optical Transducers • Piezo-Electric Devices • Thermal Sensors Several biosensor configurations have been described in the past for heavy metal detection. Wide spectrum of biological recognition elements and transducer systems has been used for the fabrication of biosensors [17-18].

Enzymes are the most widely used biological sensing element in the fabrication of biosensors [19-21]. Although purified enzymes have very high specificity for their substrates or inhibitors, their application in biosensors construction may be limited by the tedious, time-consuming and costly enzyme purification, requirement of multiple enzymes to generate the measurable product or need of cofactor/coenzyme. Microorganisms provide an ideal alternative to these bottle-necks [22].

The many enzymes and co-factors that co-exist in the cells give the cells the ability to consume and hence detect large number of chemicals; however, this can compromise the selectivity. They can be easily manipulated and adapted to consume and degrade new substrate under certain cultivating condition. Additionally, the progress in molecular biology/recombinant DNA technologies has opened endless possibilities of tailoring the

microorganisms to improve the activity of an existing enzyme or express foreign enzyme/protein in host cell. All of these make microbes excellent biosensing elements. Of the different matrices used for the fabrication of biosensors, conducting polypyrrole (PPy) has attracted attention of various researchers due to its operational compatibility at physiological pH and the ease of conductivity modulation (with the counter ions). The electrical conductivity of polypyrrole can be modulated in the range of 10−3 to 103 Ω/cm [23-24]. Various forms of polypyrroles can be easily prepared by electrochemical techniques and oxidation of pyrrole in presence of desired dopant ions results in a doped film deposited at the surface of the electrode [25-26].

Conducting polymer matrices have been reported to have improved environmental stability, biocompatibility, increased polymerization growth with higher compactness and conductivity when used with large polymeric anions such as para-toluene sulfonate (pTS), xi polystyrene sulfonate (PSS), polyvinyl sulfonate (PVS) that helps in maintaining the charge neutrality during reduction process [27-28].

It has been suggested that size of dopant ions induces changes in molecular confirmation resulting in increased electrical conductivity [29]. Polypyrrole-polyvinyl sulfonate composite membrane has been shown to play important role as a 'charge controllable membrane' in which the fixed charges can be controlled electrochemically by an internal electrode. The second category of the biorecepotors used for the fabrication of the heavy metal sensors are metalloenzymes/metalloproteins and are potentially most promising because of their specificity for metal binding [30].

Different metalloproteins/peptides have been used for developing heavy metal sensors [31-32]. The high selectivity of these metal binding molecules even in complex natural solutions like sea water or blood when combined with a suitable transducer has a great promise as an indicator system that may in the future replace the current techniques of measuring very low concentrations of metal ions [33].

Toxicity Mechanism of Heavy Metals

Heavy metal toxicity may result from alterations of numerous physiological processes caused at cellular/molecular level by inactivating enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for essential elements and disrupting membrane integrity. A rather common consequence of heavy metal poisoning is 7 the enhanced production of reactive oxygen species (ROS) due to interference with electron transport activities, especially that of chloroplast membranes [34]. This increase in ROS exposes cells to oxidative stress leading to lipid peroxidation, biological macromolecule deterioration, membrane dismantling, ion leakage, and DNA-strand cleavage [35].

Formation of Reactive Oxygen Species

The bleaching effects of many heavy metals in light have been known for a long time and are connected with the formation of reactive oxygen species (ROS) [36] and methylglyoxal (MG)**.** Heavy metals are known to disturb redox homeostasis by stimulating the formation of free radicals and reactive oxygen species (ROS) such as singlet oxygen (1O2), superoxide radicals (O2 •−), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH)**.** Recently, methylglyoxal (MG), a cytotoxic compound, was also found to increase in response to various stresses including HMs [37-38]**.**

The increase in ROS and MG cause the following physiological damages in the cells [39]: • They directly disturb electron transport, causing electrons to be transferred to oxygen instead of the natural electron acceptors in chloroplasts and mitochondria • Disturbances to metabolic reactions feedback to electron transport • Redox-active metals in different oxidation states under physiological conditions can participate in the Fenton and Haber–Weiss reaction, producing hydroxyl radicals • Inactivation and down regulation of enzymes of the antioxidant defence system • Depletion of antioxidant substrates.

Biosensors

The promising tools Improvement of "life quality" is one of the most important objectives of global research efforts. Naturally, the quality of life is closely linked to the control of diseases, food quality 13 and safety, and quality of our environment. In all these fields, a continuous, fast, and sensitive monitoring is required, to control key parameters. Biosensors, combining a biological recognition element and a suitable transducer, represent very promising tools in this context [17]. Because of their exceptional performance capabilities, which include high specificity and sensitivity, rapid response, low cost, relatively compact size and user-friendly operation, biosensors have become an important tool for detection of chemical and biological components for clinical, food and environmental monitoring [18].

In a biosensor, a biorecognition phase (e.g., enzyme, antibody, receptor, and single-stranded DNA) interacts with the analyte to produce a signal, which may be due to (i) a change in proton concentration, (ii) a release or uptake of gases such as ammonia or oxygen, (iii) a release or uptake of electrons, (iv) a light emission, absorption, or reflectance, (v) a heat emission, or (vi) a mass change, and so forth. A biosensor is an analytical device that consists of an immobilized biological material in intimate contact with a compatible transducer, which will convert the biochemical signal into a quantifiable electrical signal. Biosensors are the offspring of the

first successful marriage between biotechnology and modern electronics. The biomolecules are responsible for the specific recognition of the analyte whereas the physicochemical transducer supplies an electrical output signal which is amplified by the electronic component [40].

Bioreceptors

The specificity of enzymes is the main reason for their use in biosensors. Since most of the enzymes employed for use in sensors have been isolated from microorganisms, it is logical that the organisms themselves should be regarded as potential biocatalysts. In microorganisms, the enzymes remain in their natural environment, increasing stability and activity. Cell membranes and organelles can also be used for biosensor construction [41]. Specific binding between antibody and antigen can be exploited in immunobiosensors. During last years, there has been a huge increase in the use of nucleic acids, as a way in the recognition and monitoring of many toxic compounds of analytical interest, because many of these molecules, and especially heavy metals, show a high affinity for DNA and thus can be used as bioreceptors for heavy metal detection [42].

Immobilization

The immobilization of the bioreceptor is one of the most important steps involved in the biosensor design. The choice of the technique used for connecting the biological component to the transducer is crucial, since the stability, the longevity and the sensitivity of biosensor largely depend on bioreceptor layer configuration.Various immobilization procedures have been used in biosensor construction. In general, the choice of procedure depends on the nature of the biological element, the type of transducer used, the physicochemical properties of the analyte and the operating conditions in which the biosensor is to function. Perhaps over-riding all the considerations is the necessity for the biological component to exhibit high activity with appropriate specificity in its immobilized microenvironment. The four main approaches to enzyme and microbial immobilization are entrapment and encapsulation, covalent binding, cross linking and adsorption [40]. The immobilization of the bioreceptor has various advantages [43-44]:

i. Thousands times lower consumption of immobilized enzyme; ii. Reduction of interferences by the differential mode of operation; iii. Unnecessary pre incubation; iv. Rapid analysis procedure, less than 5 min; v. In the case of reversible inhibition, sometimes the reactivation of enzyme activity is not necessary. The immobilization methods can be broadly divided into two categories:

• Physical methods • Chemical methods.

Physical methods

Adsorption and entrapment are the two widely used physical methods for immobilization of microbes and enzymes. Because these methods do not involve covalent bond formation and provide relatively small perturbation of the native structure and function of enzymes and microbes. These methods are preferred when viable cells are required. Physical adsorption is the simplest method for microbe immobilization. Typically, a 16 microbial suspension is incubated with the electrode or an immobilization matrix, such as alumina and glass bead [21, 45-46]**,** followed by rinsing with buffer to remove unadsorbed cells. The microbes are immobilized due to adsorptive interactions such as ionic, polar or hydrogen bonding and hydrophobic interaction. However, immobilization using adsorption alone generally leads to poor longterm stability because of desorption of microbes. The immobilization of microorganisms by entrapment can be achieved by either retention of the cells in close proximity of the transducer surface using dialysis or filter membrane or in chemical/biological polymers/gels such as (alginate, carrageenan, agarose, chitosan, collagen, polyacrylamide, polyvinylachohol, poly(ethylene glycol), polyurethane [20, 22].

A major disadvantage of entrapment immobilization is the additional diffusion resistance offered by the entrapment material, which will result in lower sensitivity and detection limit. Acetylcholinesterase (AChE) was encapsulated in sol gel film on a glass cap that could be fixed on an optical fiber [47].

Chemical Methods

Chemical methods of immobilization include covalent binding and cross-linking. Covalent binding methods rely on the formation of a stable covalent bond between functional groups of the enzyme/microorganisms' cell wall components such as amine, carboxylic or sulfhydryl and the transducer such as amine, carboxylic or epoxy. To achieve this goal, whole cells are exposed to harmful chemicals and harsh reaction condition, which may damage the cell membrane and decrease the biological activity. How to overcome this drawback is still a challenge for immobilization through covalent binding. To our knowledge, this method has therefore not been successful for immobilization of viable microbial cells [20, 48-49].

Transducers

The function of the transducer is to convert the signal into an appropriate measurable response (e.g., current, potential or temperature change). Through signal processing, this interaction is converted into digital values that

relate to the build-up of concentration/activity of the analyte in the environs of the device, which in turn relates to the ambient levels in the bulk investigated sample. A biosensor is not necessarily an individual entity, but is considered as part of a general designed instrumentation [50]**.** Some of the major attributes of a good biosensing system are its specificity, reliability, portability, (in most cases) ability to function in optically opaque solutions, realtime analysis and simplicity of operation [45].

Materials and Methods

Biosensors for Heavy metals

The bio-recognition element is the main sensing component of any biosensor.

Protein Biosensor

Protein Biosensors are of two types:

1. Enzymatic
2. Non enzymatic

Enzymatic Biosensors for heavy metal detection Enzyme based

Heavy metal biosensors are based on the principle of enzyme inhibition. The problem with biosensors based on enzymatic inhibition is that only a few enzymes are sensitive to heavy metals [51].

Enzyme Inhibitor System.

The long-term function of enzyme-based biosensors may be severely limited by the powerful inhibitors which are measured. Because the enzyme-inhibitor reaction is habitually complicated, the inhibition of the enzyme can be either reversible or an irreversible inactivation. Sometimes the effect of an inhibitor can be reversed by decreasing the concentration of inhibitor (e.g., by dilution or dialysis). It is the case of the reversible inhibition. Once inhibition has occurred and there is no reversal of inhibition with decreasing the inhibitor concentration, the inhibition is called 22 irreversible. The difference between reversible and irreversible inhibition is not absolute and is difficult to do, if the inhibitor binds very strongly to the enzyme and if it is released very slowly. Reversible inhibitors that work in a way that is difficult to distinguish from irreversible inhibition are called tight-binding inhibitors [52].

Reversible Inhibition

Reversible inhibitors are molecules that bind reversibly to enzymes with rapid association by noncovalent interactions and rapid dissociation rates. This chemical equilibrium between the enzyme and the inhibitor can be displaced in favour of the enzyme and so the activity of the enzyme can be regained, by the removal of the inhibitor by dialysis, gel filtration, and so forth [42].

Degree of Inhibition

Irreversible inhibition is usually quantified in terms of the rate of inhibition. In order to investigate the heavy metals inhibition an experimental method is used consisting in recording the bio-electrode amperometric response to successive additions of substrate, before and after its incubation in an inhibitor solution, for a given period of time [53-54].

Regeneration of Biosensor

Understanding the mechanisms of inhibition and regeneration of enzymes is a general problem of great importance for many biochemists and biotechnologists, especially when using immobilized enzymes. The mode of analyte inhibition of enzymes such as peroxidase, tyrosinase and catalase can occur through blocking of the active sites of these enzymes due to complex formation with copper cofactors and blocking of the electron transfer chain. Organophosphates inhibit acetylcholinesterase (AChE) by blocking the serine in the active site through nucleophilic attack to produce a serine phosphoester (via phosphorylation) [55].

Parameters generally affecting the performance of enzymatic biosensors

Effect of pH

The pH of the solutions containing substrates can affect the overall enzymatic activity since, like all natural proteins, enzymes have a native tertiary structure that is sensitive to pH; denaturation of enzymes can occur at extreme pHs. It is well known that the enzyme activity is highly pH dependent and the optimum pH for an enzymatic assay must be determined empirically. It is best to choose a plateau region so that the pH should not have any effect on enzyme activity and will not interfere with the results obtained relative to the inhibition of the enzyme by the inhibitor. The activity of the immobilized acetylcholinesterase as a function of pH has been studied between pH 2 and 9 [56].

Effect of enzyme concentration

The highest sensitivity to inhibitors was found for a membrane containing low enzyme loading [57]**.**

Examples of Enzyme-Based Biosensors for Heavy Metal Detection

For heavy metals detection, different enzymes such as acetylcholinesterase, alkaline phosphatase, urease, invertase, peroxidase, L-lactate dehydrogenase, tyrosinase, and nitrate reductase, have been used [42].

Antibody-based biosensors Immunoassays

Have emerged as an alternate approach for metal ion detection since they offer significant advantages over traditional detection methods such as high sensitivity, selectivity and species-specificity and are theoretically applicable to any pollutant for which a suitable antibody can be generated [58].

Whole Cell Biosensor Enzymes

Are the most widely used biological sensing elements in the fabrication of biosensors [21].

DNA based Metal Biosensor

During last years, there has been a huge increase in the use of nucleic acids, as a way in the recognition and monitoring of many toxic compounds of analytical interest, because many of this molecules, and especially HMs, show a high affinity for DNA and they can interact with nucleic 40 acids. The interaction between metal ions and DNA is important in living organisms, because it could have, either favourable, or adverse effects in life science reported to the damage, replication and transcription of DNA in vivo, mutation of gene, action mechanism of some synthetic chemical nucleases, and molecular analysis [59].

Conclusion

The intention of this article is to reflect the advances and describe the trends on biosensors for environmental applications. Biosensors are useful analytical tools for environmental monitoring, capable of providing results in real time, simple to use, portable and cost-effective. Some examples of biosensors in advanced stage of development, which have been applied to real samples, as well as of commercial devices, are given. Biosensors designed for measurement of specific chemicals are discussed. Heavy metal ions constitute a serious environmental problem due to their persistent and non biodegradable nature. They are toxic to biological systems even at low concentration and there is an obvious need to determine them at trace level. Bioaccumulation of the heavy metals has been reported to be higher in the upper trophic levels at concentrations surpassing those found in water supplies. The conventional methods used for the determination of the heavy metals based on spectrophotometry, chromatography, mass spectrometry and various hyphenated techniques; require sophisticated and expensive equipments, highly trained staff and is usually time-consuming**.** The total amount of heavy metals detected by such means may not always be related to toxicity of such samples because the original biological availability of the metal ions is not taken into account. Thus need arises for the fast and inexpensive methods for the detection of bioavailable heavy metals. Biosensors are useful analytical devices in this respect. A biosensor is an analytical device, which converts a biological response into an electrical signal.

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