Ravindra Pratap Singh ·
Kingsley Eghonghon Ukhurebor ·
Jay Singh · Charles Oluwaseun Adetunji ·
Kshitij RB Singh *Editors*

Nanobiosensors for Environmental Monitoring

Fundamentals and Application

Springer

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Dedicated
to those who
care, conserve, protect
but do
not spoil or destroy the beauty
and
unique characteristics
of
Kshit (Earth)
Jal (Water)
Pawak (Fire)
Gagan (Sky)
and
Sameera (Air)

Preface

Globally, the world population's continuous growth is projected to reach 9 billion by the year 2050. Hence, there is a need to provide a safe environment that could guarantee humankind and the ecosystem's safety. The higher level of anthropogenic action has been identified as a threat to humankind's existence due to the higher level of xenobiotic and toxic substances that could interrupt the normal ecosystem. This has prompted numerous local and international agencies that could play a significant role in environmental pollution mitigation. The application of nanobiosensor has been identified as a sustainable technique that could be applied to ensure proper detection and identification of several environmental contaminants. Hence, this book entails detailed information on the utilization of nanobiosensor as an effective technology for the effective detection, monitoring, and management of environmental contaminations to ensure its sustainability and humanity's well-being.

Nanomaterial's possible applications created an innovative domain called nanomaterial-based biosensor machinery as one of nanotechnology's ultimate subdivisions. The application of nanomaterials-based biosensors machinery and their advancements could be applied globally to resolve numerous environmental sectors' challenges to guarantee the environment's quality and safety. This book, *Nanobiosensors for Environmental Monitoring: Fundamentals and Application*, will be an excellent collection of reviews based on contemporary research and developments on nanomaterials utilization and applications in environmental monitoring along with their prospects. The book has attempted to give a comprehensive idea of nanomaterial concepts for nanobiosensors applications in an environmental context to help students, researchers, and professionals/practitioners recognize nanomaterials' significance in the environmental domain. This book will also help understand and address the environmental sectors' complications via nanomaterials' utilization and applications. Hence, this book can also serve as a textbook to help students, professionals/practitioners, scientists, researchers, and academicians in various research domains.

Aims and Scope

This book, *Nanobiosensors for Environmental Monitoring: Fundamentals and Application*, will be useful to several readers from various domains. The book has introduced the basic concept of nanobiosensors in environmental monitoring. Further, it has also highlighted some of the nanomaterials' foremost applications in environmental monitoring and some challenges in utilizing nanomaterials in environmental monitoring and nanobiosensors legal implications concerning environmental monitoring. The major areas which this book has covered are listed in the facets below.

- The basic concept of nanobiosensors in environmental monitoring.
- Nanomaterial capabilities for environmental monitoring.
- Applications and developments of nanomaterials in environmental monitoring.
- Utilization of nanobiosensors for environmental monitoring, such as monitoring and analyzing the environment, detrimental environmental constituent detection, and several other emerging domains related to environmental sustainability and safety.
- Legal implications of nanobiosensors concerning environmental monitoring and analysis.

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Dr. Jay Singh is currently working as an Assistant Professor at the Department of Chemistry, Institute of Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, since 2017. He received his Ph.D. degree in Polymer Science from Motilal Nehru National Institute of Technology in 2010 and did M.Sc. and B.Sc. from Allahabad University, Uttar Pradesh, India. He had a postdoctoral fellowship at National Physical Laboratory, New Delhi, Chonbuk National University, South Korea and Delhi Technological University, Delhi. Dr. Jay has received many prestigious fellowships like CSIR (RA), DST-Young scientist fellowship, DST-INSPIRE faculty award, etc. He is actively engaged in the development of nanomaterials (CeO2, NiO, rare-earth metal oxide, Ni, Nife2O4, Cu2O, graphene, RGO, etc.)-based nanobiocomposite, conducting polymer and self-assembled monolayer-based clinically important biosensors for estimation of bioanalytes such as cholesterol, xanthine, glucose, pathogens, and pesticides/toxins using DNA and antibodies. Dr. Jay has published more than 80 international research papers with total citations more than 3400 and an h-index being 34. He has completed/running various research projects in different funding agencies. He has many edited/authored books (under pipeline) and has authored more than 15 book chapters of internationally reputed press for publications, namely Elsevier, Springer Nature, IOP, Wiley, and CRC. He is actively engaged in fabricating metal oxide-based biosensors for clinical diagnosis, food packaging applications, drug delivery, and tissue engineering applications. His research has contributed significantly toward the fundamental understanding of interfacial charge transfer processes and sensing aspects of metal nanoparticles.

Dr. Charles Oluwaseun Adetunji (Ph.D.) is presently a faculty member at the Microbiology Department, Faculty of Sciences, Edo State University Uzairue (EDSU), Edo State, Nigeria, where he utilized the application of biological techniques and microbial bioprocesses for the actualization of sustainable development goals and agrarian revolution, through quality teaching, research, and community development. He was formally the Acting Director of Intellectual Property and Technology Transfer, the Head of department of Microbiology, Sub Dean for Faculty of Science and currently the Chairman Grant Committee, and the Ag Dean for Faculty of Science, at EDSU. He is a Visiting Professor and the Executive Director for the Center of Biotechnology, Precious Cornerstone University, Ibadan. He is presently an external examiner to many academic institutions around the globe including Department of Microbiology, University of Namibia. He has won several scientific awards and grants from renowned academic bodies like Council of Scientific and Industrial Research (CSIR) India, Department of Biotechnology (DBT) India, The World Academy of Science (TWAS) Italy, Netherlands Fellowship Programme (NPF) Netherlands, The Agency for International Development Cooperation, Israel, and Royal Academy of Engineering, UK among many others. He has published many scientific journal articles and conference proceedings in refereed national and international journals with over 390 manuscripts. He was ranked among the top 500 prolific authors in Nigeria between 2019 till date by SciVal/SCOPUS. His research interests include microbiology, biotechnology, postharvest management, food Science, bioinformatics, and nanotechnology. He was recently appointed as the President and Chairman Governing Council of the Nigerian Bioinformatics and Genomics Network Society. He was recently appointed as the Director for International Affiliation and Training Centre for Environmental and Public Health, Research and Development, Zaria. He is presently a series editor with Taylor and Francis, USA, editing several textbooks on agricultural biotechnology, nanotechnology, pharmafoods, and environmental sciences. He is an editorial board member of many international journals and serves as a reviewer to many double-blind peer-review journals like Elsevier, Springer, Francis and Taylor, Wiley, PLOS One, Nature, American Chemistry Society, Bentham Science Publishers, etc. He is a member of many scientific and professional including bodies like American Society for Microbiology, Biotechnology Society of Nigeria, and Nigerian Society for Microbiology, and he is presently the General/Executive Secretary of Nigerian Young Academy. He has won a lot of international recognition and also acted as a keynote speaker delivering invited talk/position paper at various universities, research institutes, and several centers of excellence which span across several continent of the globe. He has over the last 15 years built strong working collaborations with reputable research groups in numerous and leading universities across the globe. He is the convener for Recent Advances in Biotechnology, which is an annual international conference where renown microbiologists and biotechnologists come together to share their latest discoveries. He is the president and founder of the Nigerian Post-Harvest and Food Biotechnology Society.

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Chapter 1
Introduction to Nanobiosensors

Kshitij RB Singh, Vanya Nayak, Charles Oluwaseun Adetunji, Kingsley Eghonghon Ukhurebor, Jay Singh, and Ravindra Pratap Singh

Abstract The versatile utilities of nanobiosensors in the different domains have made them a next-generation device, which will bring a revolutionary impact in the science and technology domain. The use of nanomaterials in biosensors to develop nanobiosensors and enhance their properties is commendable and is remarked by growing literature publications, patents, and devices. The unique fusion of nanotechnology with biotechnology has created a tremendous impact on the lifestyle of humans. The chapter highlights a brief overview of the unique properties, types, fabrication techniques of nanobiosensors. Further, the chapter also combines the updated literature on various applications of nanobiosensors in the environment domain, their limitations, and future potentialities.

Keywords Nanobiosensors · Nanomaterials · Environmental monitoring · Biomedical and agricultural applications

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1.1 Introduction

Technological advancements have led to the development of various devices which have made human lives smooth and convenient, but at the same time, it has also caused harms to the environment. For these purposes, various new devices and techniques have been developed to detect and combat various environmental pollutions efficiently. Currently, nanobiotechnology has been employed to develop sensors that can efficiently detect the various pollutants, and techniques are also being developed that could efficiently eliminate the contaminants from the environment. Besides the applications in the environment domain, nanobiotechnology finds its profound uses in other domains like biomedical, agriculture, robotics, sensors, electronics, etc. Nanobiotechnology is a unique blend of biotechnology with nanotechnology, which utilizes nanoparticles with biotechnological approaches to develop novel methods and techniques useful for human lives. The nanoparticles' small size helps them exhibit a high surface-to-volume ratio and unique chemical, physical, optical, biological, catalytic, mechanical properties that distinguish them from the bulk matter (Adetunji et al. 2021b; Singh et al. 2021b; Nayak et al. 2021). With time, the nanobiosensors have found their wide utilities as they offer a big platform for detecting various analytes by utilizing biomolecules and nanoparticles. Moreover, it has been observed that the biosensors fabricated by nanoparticles tend to show enhanced functions and utilities.

Further, many works of literature are available that describe the various roles of nanobiosensors in different domains (Singh 2010, 2019; Singh et al. 2012; Singh and Singh 2021a, b). Additionally, the demand for nanobiosensors has increased with the sudden outbreak of severe acute respiratory syndrome coronavirus-19 (SARS-CoV-2), which has increased the development of nanobiosensors to detect SARS-CoV-2 in the biological medium as well as in the environment. Not only SARS-CoV-2 has affected human beings, but also it has affected the environment, which makes the need of the hour to develop rapid and accurate nanobiosensors which can specifically detect the particles of SARS-CoV-2, and further Fig. 1.1 represents a schematic illustration of the key topics highlighted in the chapter.

1.2 Types of Nanobiosensors

The nanobiosensors can be broadly classified on the basis of transducers and bioreceptors. Based on the transducer's working principles, nanobiosensors are classified as optical, electrochemical, gravimetric, and electronic nanobiosensors. Electrochemical nanobiosensors are the most widely researched because they exhibit high sensitivity and low response time. In electrochemical nanobiosensors, the electrochemical signals generated from the interaction between biorecognition element and analyte are detected on the surface of the transducers, which are analyzed in the form of capacitance, voltage, and current (Shanker et al. 2014; Malhotra and Ali

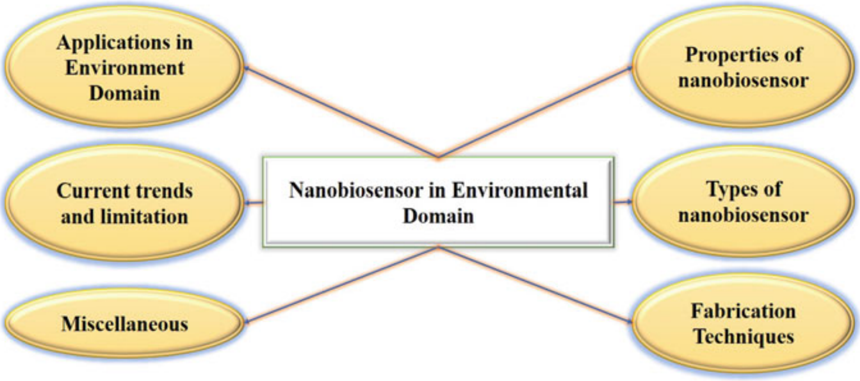

Fig. 1.1 A schematic illustration of the major topics covered in the chapter

2018a). Further, electrochemical nanobiosensors can be classified as impedimetric, conductometric, voltammetric, amperometric, and potentiometric.

Similarly, when a biorecognition element is conjugated with the optical transducer, it is known as nanobiosensors. The optical nanobiosensors utilize antibodies, tissues, whole cells, aptamers, etc., to generate label-free and real-time detection nanobiosensors. Moreover, it has been observed that the concentration of an analyte affects the working of the optical nanobiosensor. There are two types of optical nanobiosensors: label-free and labeled nanobiosensors, which are also classified based on the working principle of the optical nanobiosensors. Another nanobiosensors, which is highly utilized in the environmental domain, are the gravimetric nanobiosensors that can detect a small signal generated by the mass of the binding materials. The mass-based transducers consist of piezoelectric-based, quartz-crystal microbalance (QCM), and magnetoelastic (MES)-based nanobiosensors.

Further, different biorecognition elements like enzymes, antibodies, nucleotides, aptamers, whole cells, etc., also help classify the nanobiosensors. One of the highly used nanobiosensors is enzyme-based nanobiosensors. The enzyme acts as the biorecognition element that is immobilized on the surface of the transducer and is highly used in biomedical, agriculture, and environment domains. Similarly, when the antibodies act as the recognition elements, the nanobiosensor is known as immunonanobiosensor that detects the signals generated by antigen–antibody interactions. Moreover, the use of aptamers, nucleotides, and whole-cell sensors as biorecognition elements has also gained much attention.

1.3 Properties and Fabrication of Nanobiosensors

Unique properties of nanobiosensor and their optimization help in the development of high-performance nanobiosensor. Various properties like selectivity, sensitivity, detection limit, response time, linearity, etc., are required to develop a high-performance nanobiosensor. A nanobiosensor should exhibit high selectivity, which is defined as the bioreceptor's capability to specifically distinguish and detect the desired analyte from the sample causing the least interference (Wu et al. 2019; Qian et al. 2020; Zhang et al. 2020; Hu et al. 2020). Similarly, atmospheric disturbances also hinder the activity of the nanobiosensor as they alter the output signals during measurement. The stability of a nanobiosensor is defined as the degree of susceptibility to atmospheric disturbances present in and around the biosensing system; therefore, monitoring stability is considered an important criterion to develop a precise nanobiosensor. Further, sensitivity or limit of detection (LOD) is considered another important property to develop a nanobiosensor, as the minimum amount of analyte is detected by the nanobiosensor. A precise nanobiosensor exhibits high sensitivity as it can efficiently detect the minute change by observing the change in the output signal (Wang et al. 2019; Domènech et al. 2019; Liu et al. 2020; Song et al. 2020; Wei et al. 2021). Moreover, reproducibility also plays an important role in developing accurate nanobiosensor as it is determined by the precision and accuracy of the transducer of the nanobiosensor. These signals show high robustness and reliability to the interference created on the response of a nanobiosensor. Additionally, linearity is determined to study various responses of the nanobiosensor, forming a straight line of different responses recorded (Bhalla et al. 2016; Malhotra and Ali 2018b).

Various nanomaterials like metal and its oxide nanoparticles, quantum dots, carbon nanotubes, magnetic nanoparticles, etc. are highly used to fabricate nanobiosensors to enhance their properties. The biomolecules are linked with the nanomaterials by establishing a biointerference, which can easily detect the minimum concentration of the target molecules that help rapidly detect the various analytes. Fabrication techniques can be broadly divided into three types: physical, chemical, and surface fabrication, each playing their unique roles in developing a rapid and accurate nanobiosensor. The physical fabrication technique comprises physical adsorption, physical entrapment, etc., in which the biorecognition elements are immobilized on the surface of the outer surface through various weak attractive forces like hydrogen bonding, van der Waals force, etc. (Sassolas et al. 2012; Martinkova 2017; Arya et al. 2019). Moreover, physical fabrication techniques are simple and cost-effective and also enhance the sensitivity and selectivity of a nanobiosensor.

Further, the chemical fabrication technique uses covalent bonds to join the biorecognition elements with the surface of the transducer. Various chemical fabrication techniques, such as cross-linking, sol–gel, covalent linkage, spray pyrolysis, and electropolymerization, are utilized to fabricate diverse environment-based nanobiosensors to detect environmental pollutants toxic chemicals (Singh 2011; Sassolas et al. 2012; Martinkova 2017). Another fabrication technique that is widely used is the surface functionalization technique, in which the binding affinity of nanomaterials is increased so that they can specifically bind to the target analyte (Sang et al. 2015). Nowadays, the development of label-free nanobiosensors is growing as they have efficiently enhanced the properties of the nanobiosensors (Fang 2010; Citartan et al. 2013; Musayev et al. 2014; Liu et al. 2014).

1.4 Potentialities of Nanobiosensors in the Environment Domain

Currently, microbial biosensors have gained much attention in the environment domain as they utilize microorganisms like viruses, fungi, bacteria, etc., to detect various analytes. Initially, the microbial biosensors were used to study the biological oxygen demand (BOD) and monitor general toxicity. The standard BOD approaches are time-consuming and costly and sometimes lead to wastewater discharge in the river bodies before the test results are released. Therefore, various microbial biosensors were developed to ease the long process and make it cost-effective to overcome this monitoring process. A two-mediator biosensor system was proposed by Kharkova et al., in which they showed that interaction of graphite paste-immobilized ferrocene with phenazine mediators was successfully established using yeast *Blastobotrys adeninivorans*, as they help in the oxidation of large substrates by increasing the rate of electron transfer. The sensor exhibited high sensitivity, and it was observed that the data obtained from the two-mediator ferrocene-neutral red system and the yeast *B. adeninivorans* highly matched with the data of the standard BOD analysis method ($R = 0.9693$). Therefore, this unique combination of microorganisms with the mediator can be highly utilized to develop a BOD detecting biosensor exhibiting high sensitivity (Kharkova et al. 2021).

The sudden outbreak of SARS-CoV-2 has effectively boosted the advancements in science and technology as it became the need of the hour to efficiently detect and remove the SARS-CoV-2 as soon as possible from all the expected areas (Singh et al. 2021a). Moreover, the emergency to develop the nanobiosensors increased when several countries started to report the presence of genetic material of SARS-CoV-2 in the sewage treatment plants, which quickly shifted the researchers' attention in developing nanobiosensors for the detection of SARS-CoV-2 from the environment as well as sewage waters, wastewater, and dumping grounds (Mallapaty 2020; Medema et al. 2020; Chen et al. 2020; Randazzo et al. 2020; La Rosa et al. 2020). Additionally, it has been suggested to analyze wastewater to detect SARS-CoV-2 rather than testing each individual. However, the current state-of-the-art detection platform methods lack the SARS-CoV-2 in the sewage as various limitations like well-trained human resources, and well-equipped laboratories are still lacking. However, these nanobiosensors will play a vital role in the early detection of the SARS-CoV-2 and ensure the eradication of SARS-CoV-2 from the environment. Moreover, with the development of the Internet of Things (IoT), lab-on-a-chip, point-of-care analysis, etc., the fabrication

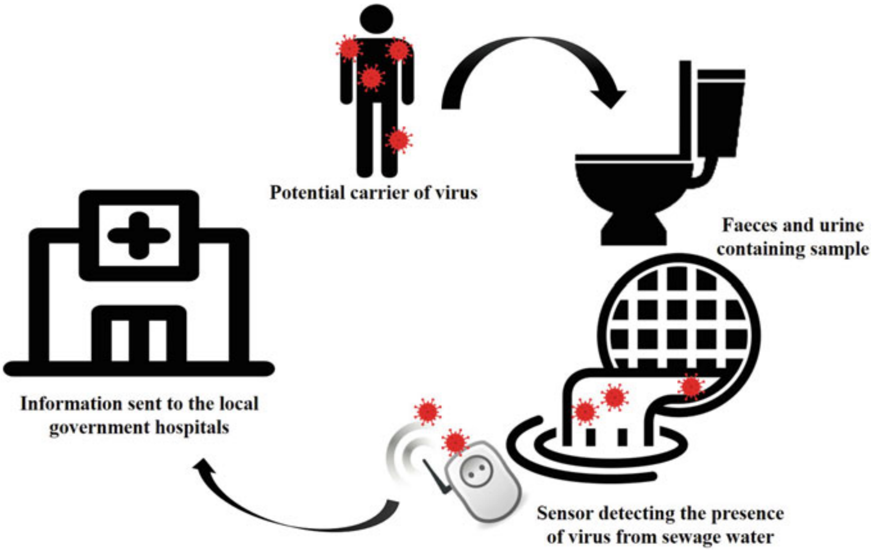

Fig. 1.2 An illustration of detection of SARS-CoV-2 from the sewage water and sending the detected case to hospital

of advanced nanobiosensors to detect SARS-CoV-2 will also grab much attention (Mao et al. 2020). A schematic illustration of the working of the nanobiosensors to detect SARS-CoV-2 is illustrated in Fig. 1.2.

Enzymatic biosensors exhibit high specificity and sensitivity because of adsorption and inhibition activity that leads to the generation of a stress tension on the cantilever surface, which is further measured by the change in the deflection. Nowadays, cantilever nanobiosensors are capturing the highest attention as they show high versatility, compactness, and further can also be utilized for in real-time monitoring, which holds a promising future for on-site analysis (Lang and Hegner 2004; Chen et al. 2012; Steffens et al. 2012, 2014). Moreover, according to Hansen and Thundat (2005), no other sensing technology has been observed that can provide this much versatility (Hansen and Thundat 2005). Moreover, carrying this idea, Rigo et al. (2020) developed a cantilever nanobiosensor functionalized with alkaline phosphatase and urease that efficiently detected lead (Pb) concentration in different rivers waters. Lead is considered as one of the hazardous heavy metals which can result in the generation of harmful effects on the marine environment, as well as when it enters the food chain; it can generate various life-threatening diseases like Hashimoto's disease, memory loss, genetic drift, cancer, etc. (Benson et al. 2018). According to the World Health Organization (WHO), only ten parts per billion (ppb or µg/L) of Pb is considered acceptable in drinking water. In their experiment, the functionalized cantilever nanobiosensor efficiently detected the concentration of Pb in river water, and therefore, it can be used for real samples of water. The prepared cantilever nanobiosensor exhibited high-sensitivity, real-time monitoring at low concentrations and facilitated enhanced monitoring of the contaminated areas (Rigo et al. 2020).

The wastewater treatment plants treating industrial wastewater are always at the risk of getting overloaded by toxic influents like phenolics and cyanides that can partially or completely destroy the biological treatment system. However, bioluminescent bacteria were a considerable organism for monitoring toxic chemicals in the wastewater treatment plant. However, various factors like temperature sensitivity, salt dependency, variation in pH, etc., make it difficult to utilize bioluminescent bacteria to treat industrial wastewater. However, a whole-cell genetically modified bioluminescent biosensor was developed by Philp et al. (2003), and their immobilization was used to regularly monitor the toxicity of the industrial wastewater loaded with phenolics. The immobilization technique provides various advantages as it can enhance the biological and plasmid stability of the sensor (Cassidy et al. 1996). The poly(vinyl alcohol) (PVA) was used as a synthetic hydrogel, which was used to develop a thin film of immobilized biocatalysts.

Further, it was used to detect the pure toxicants phenol and 3-chlorophenol and coke oven effluent at various processing stages before, during, and after biological treatment. Further, two organisms were compared: *Photobacterium* (*Vibrio*) *fischeri* and *Pseudomonad strain* that consisted of chromosomally integrated *luxCDABE* operon obtained from the terrestrial bacterium *Photorhabdus luminescens*. The experiment results reported that the immobilization technique was useful in detecting pure toxicants and distinguishing the toxicity of various zones in the wastewater treatment plants. Additionally, it was also observed that the genetically modified Pseudomonad species could detect the 3-chlorophenol and phenol in the wastewater when immobilized with PVA. The results were obtained within 5 min, suggesting the real-time use of the developed luminescent biosensor in industrial wastewater treatment (Philp et al. 2003).

The molecularly imprinted technique (MIT) is a novel technique for enhancing the sensitivity and selectivity of a sensor through modifying the chip's surface and helps in recognition of specific target molecules. Copolymerizing cross-linkers can prepare molecularly imprinted polymer-based biosensors with template molecules via hydrogen, covalent, and non-covalent bonding (Xie et al. 2008). Since MIP-based biosensors show high stability and sensitivity, they find their broad utilities in various domains like environment, biomedical, agriculture, etc. (Xie et al. 2006). However, these MIP-based biosensors face limitations like poor availability of recognition sites, low potential binding sites, and heterogeneous binding (Yáñez-Sedeño et al. 2017). But, it was suggested that integrating nanomaterials into these MIP biosensors increases the surface area, which leads to the increase in the number of imprinted sites on the surface of the chip, increasing efficiency and sensitivity, and decrease in the response time (Wackerlig and Lieberzeit 2015). These properties of MIP-based nanobiosensor have started gaining much attention from researchers and have increased their utilities in various domains. Further, it was observed that the combination of MIP-based nanobiosensors with carbon nanotubes (CNTs) increased the catalytic oxidation, surface area, and rate of electron transfer to the surface of the electrode. A highly sensitive electrochemical nanobiosensor utilizing gold nanoparticles (AuNPs) on CNT-modified glassy carbon electrode (GC) surface for imprinting of TAP template through electropolymerization of o-hydroxyphenol on the GNPs/CNT/GC electrode was developed by Li et al. (2012). The proposed MIP-based nanobiosensor was used to detect triazophos (TAP) pesticides from the soil. This coupling approach showed an increase in the electrochemical response of TAP, and additionally, enhanced sensitivity and selectivity of the MIP-based nanobiosensor were also observed (Li et al. 2012).

Nowadays, mercury (Hg^{2+}) accumulation in water bodies has become a major concern, causing water pollution. Moreover, it has been observed that the entrance of mercury into the food chain has led to the development of neurodegenerative diseases, genetic drift, mutations, oncogenesis, etc., which becomes a major issue to be resolved quickly. Various detection methods have been developed in order to rapidly detect the concentration of mercury in the water bodies accurately as well as during the treatment of industrial wastewater, but somehow, some limitations like low sustainability, low sensitivity, high response time, etc. always hamper the process. One of the efficient methods found to overcome these limitations is colorimetry, a low-cost sensor highly utilized for detecting trace metals and is extremely famous as results can be seen through naked eyes in the form of color change. The colorimetric assay detects the difference in the optical properties which are induced by various surface chemical reactions. The colorimetric recognition units consist of various biomolecules like antibodies, enzymes, antigens, aptamers, nucleotides, etc., but it has been suggested that the use of nanomaterials for the detection of mercury can be a futuristic approach (Xing et al. 2014; Qiu et al. 2016; Zangeneh Kamali et al. 2016; Ren et al. 2018a, b). One of the potential plasmonic nanobiosensor was proposed by Faghiri and Ghorbani (2019), which used nanocomposite of sodium alginate-silver nanoparticles (SA-AgNPs) synthesized using solvent casting method as shown in Fig. 1.3. The nanobiosensor was used for the naked-eye detection of the trace Hg^{2+} in different samples of water. The proposed nanobiosensor showed a limit of detection (LOD) of 5.29 nM, with a recovery range of 81.58–114.73%. Additionally, the nanobiosensor also exhibited excellent selectivity and was found to be a potential nanobiosensor with high prospects and good market value (Faghiri and Ghorbani 2019).

Moreover, it has been observed that the sensor chip modified with metal nanoparticles can efficiently enhance surface plasmon resonance (SPR) signals, catalytic activity and increases biocompatibility (Mahmoudpour et al. 2019). Additionally, the conjugation of metal nanoparticles with biomolecules can also enhance the properties of the nanobiosensor, which can be used in the food industry to detect various mycotoxins. Moreover, single domain antibodies, also known as nanobodies (Nbs), have gained much attention because of their small size and hydrophobicity, which help them exhibit excellent stability, elasticity, and ease in manufacturing. These properties of nanobodies have made them gain their wide applications in biomedical, food packaging, environmental, agricultural, drug delivery, sensors, and pharmaceutical domains (Könning et al. 2017). Recently, a group of researchers proposed an indirect competitive nanobody-based enzyme-linked immunosorbent assay (Nb-ELISA) for the detection of ochratoxin A in cereals. The ochratoxin A is one of

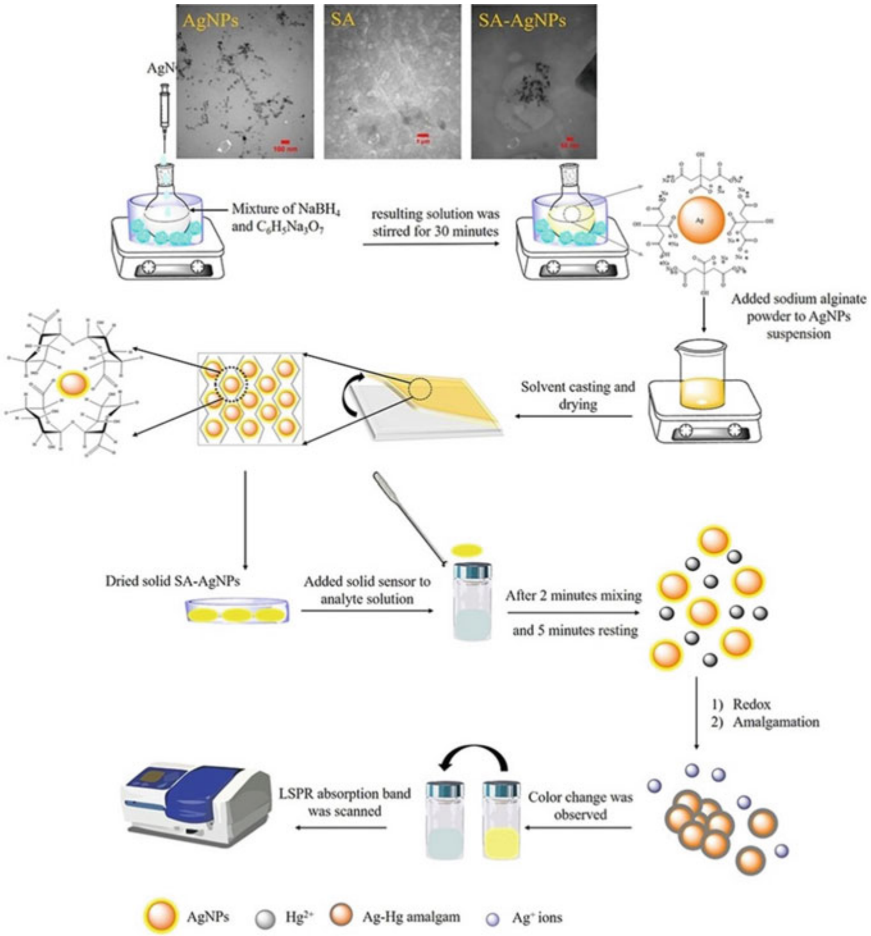

Fig. 1.3 Preparation of nanocomposite of sodium alginate-silver nanoparticles (SA-AgNPs). Reproduced with permission from Faghiri and Ghorbani (2019)

the neurotoxic metabolites secreted by *Peniciliium* and *Aspergillus* fungi that can cause neurodegenerative diseases and carcinogenic effects. The proposed Nb-ELISA showed high sensitivity and a linear range of 0.27–1.47 ng mL^{-1} (Könning et al. 2017; Mahmoudpour et al. 2019).

1.5 Miscellaneous Applications of Nanobiosensors

Nanotechnology in relation to nanobiosensors is an emerging research precedence, owing to the extensiveness of its potential benefits to humanity (Ghaffar et al. 2020;

Ukhurebor et al. 2021a; Onyancha et al. 2021). Such benefits/applications, especially in the environmental domain, are the core of this book. Apart from the environmental applications of nanobiosensors, there are presently other areas or domains where nanobiosensors are being utilized. However, other miscellaneous applications of nanobiosensors in other domains apart from the environmental domain (such as the agricultural and biomedical domains and the food safety and monitoring applications of nanobiosensors) are briefly discussed in the preceding part of this section.

1.5.1 Agricultural

Nanotechnology in relation to nanobiosensors can transform, evolve, and revolutionize the agricultural sector, which is one of the utmost sectors crucial for human existence. Nanotechnology entails considering the smallest constituent part (particles) that plays a significant role in encountering the complications that are unsolved by conventional means, raising the desire anticipation for the improvement of the agricultural sector (Ghaffar et al. 2020; Ukhurebor 2021). The progression in the synthesis of new-fangled nanomaterials vis-à-vis nanodevices represents innovative agricultural sector applications (Ghaffar et al. 2020). One of such applications of nanotechnology is the development of progressive biosensors that has led to the development of minuscule structures called nanobiosensors. Reportedly, these nanobiosensors are more effective and efficient than other conventional biosensors (Ukhurebor 2021). Nanobiosensors have been reported to find it useful for efficiently sensing the soil's pH and moisture context (Ukhurebor and Adetunji 2021), as well as a widespread diversity of pathogens and pesticide insecticide and herbicide (Adetunji et al. 2021a). According to Ukhurebor and Adetunji, nanobiosensors are also employed in utilizing cost-effective and eco-friendly fertilizers (Ukhurebor and Adetunji 2021). Therefore, the appropriate and meticulous use of nanobiosensors would support the sustainability of the agricultural sector for improving agricultural productivity and mitigating climatic and other environmental consequences (Ukhurebor et al. 2021b, c). Generally, the application of nanobiosensors in the agricultural sector will possibly complement the management of affluence and controlled use of most natural resources (Ghaffar et al. 2020).

1.5.2 Biomedical

According to Mishra and Rajakumari (2019), evolving scientific inventions aided by next-generation devices will outline the background for the imminent commercialization of biomedical devices such as nanobiosensors (Mishra and Rajakumari 2019). In this regard, countless studies are now being focused on the advancement of nanobiosensors for biomedical applications. As reported in some recent review studies, the advancements of nanobiosensor are fast attaining remarkable attention in the biomedical domain due to their wide-ranging applications (George Kerry et al. 2021; Mohankumar et al. 2021). Nanobiosensors have presently been efficaciously employed for detecting, diagnosing, and treating diseases and monitoring and managing other aspects of human health (Qiao et al. 2010; George Kerry et al. 2021). They have also been utilized in the assessment and diagnosis of biomedical-related in vivo features. At present, newly advanced nanobiosensors assist in transduction that is utilized for sensing biomolecules bearing huge sensitivity. Also, various nanobiosensors are now prominently employed in plummeting poison in products from human cells, diagnosis of diseases, and several other biomedical applications owing to their homogeneous or heterogeneous nature and correspondingly function in sensing devices (Banigo et al. 2020). Qiao et al. (2010) and Ahangar et al. (2019) reported that nanobiosensors are employed for diagnosing, clinical testing, preventing, monitoring, and treatment of countless human diseases (Qiao et al. 2010; Ahangar et al. 2019). Similarly, Rai et al. (2012) reported the applications of nanobiosensor for the detection of diabetes, immunoassay, cancer, and pathogenic bacteria (Rai et al. 2012).

The applications of nanobiosensors for the detection, monitoring, and management of most deadly communicable and non-communicable diseases such as diabetes, SARS, cancer, Ebola, Hendra, Nipah, Avian influenza, tuberculosis, malaria, AIDS, etc. as well as the recent trending SARS-CoV-2 have brought great succor to humanity. According to Rai et al. (2012), the prompt assessment of telomerase action in biological samples is beneficial, and nanobiosensors are accomplishing this. Presently, owing to the application of nanobiosensors, the detection of microbial pathogens and their contaminants in affected persons is now conceivable (Rai et al. 2012).

Nanobiosensors have opened innovative possibilities for improving reduced (miniaturized) arrays of nanoelectrodes for the determinations of multiproteins. Owing to their significant enhancements in specificity, sensitivity, and parallel nature, they are now used for in vitro biomedical diagnostics, discovery/detection of pharmaceuticals and pathogens. The utilization of nanobiosensors for biomedical purposes has opened up new views in biomedical studies by making medical diagnosis fast, simple, precise, economical, and painless.

1.5.3 Food Safety and Monitoring Applications

Nanobiosensors also have wide-ranging applications in the domain of food safety and monitoring (Raghu et al. 2020). Presently, nanobiosensors have been utilized for the mycotoxin detection in food substances (Gonçalves et al. 2019; Agriopoulou et al. 2020). In recent times, nanobiosensor has emerged as a simple, facile, dependable, and innovative strategy for detection and analytic platforms for different mycotoxins such as aflatoxins and ochratoxins citrinin, patulin, and fusarium in foodstuff. Also, several categories of nanobiosensors such as DNA-based, electrochemical, quartz-crystal microbalance, carbon nanotubes, etc. are used to detect various fungal toxins in foodstuff. Certainly, nanobiosensor has enabled instant, cost-effective, high-throughput, convenient, and ultrasensitive determination of mycotoxins with a further feature of multiplexing, allowing accessibility to food safety and monitoring.

A recent report by Raghu et al. (2020) showed that nanobiosensors have wide-ranging applications in the area of microbial quality as well as the safety monitoring in dairy production packaging ingredients integration as a major indicator for the quality and safety of the food products. In all these, it is affirmed that nanobiosensors play an utmost role in food safety and monitoring (Raghu et al. 2020). Nevertheless, to completely accomplish the potentialities of nanobiosensors, further and more research and incessant advances in the development of nanomaterials for the fabrication of nanobiosensors should be invigorated and implemented.

1.6 Recent Trends and Limitations

The versatility of biosensors is not an unknown topic these days as they find their vast utilities in almost every domain, from biomedical to pharmaceutical and from agriculture to the environmental domain. Additionally, their low cost, simple handling, and small size make them a potential candidate in science and technology. Further, with the introduction of nanomaterials, the prospects and utilities of biosensors have increased and are now capturing the molecular levels, which was not possible previously with the bulk matter. In the environment domain, the nanobiosensors find their wide utilities for detecting pollutants, heavy metals, pathogens, etc., from soil, air, and water, but their applications become limited as to achieve real-time monitoring in the field is yet the toughest challenge to overcome. Moreover, the nanobiosensors have enabled rapid detection, enhanced sensitivity, stability, robustness, and portability, along with their high selectivity and cost-effectiveness. However, with the development of lab on a chip, microarrays, and the Internet of Things, it has been suggested that the nanobiosensors will soon rule over the market, as they will be able to develop smart delivery systems, maps, etc. and treat specific affected areas.

Additionally, with the outbreak of SARS-CoV-2, nanotechnology applications in developing rapid and sensitive nanobiosensors have paced up to detect the virus in both living samples and the environment. Moreover, many nanobiosensors are focused on preserving limited natural resources like water in the agriculture field. Another important trend in nanobiosensor is developing a nanobiosensor that can work under extreme conditions like highly alkaline, acidic, high temperature, etc., with low cost and rapid results. The various advances in nanobiosensors are evident from the exponentially increasing publications, patents, projects, and utilities.

1.7 Conclusion

The nanobiosensors are highly demanded sensors due to their unique properties and low cost, and therefore, they find their immense utilities in almost every domain of science. They have started to become a regular application in our day-to-day life as they have eased the lifestyle of humans. Moreover, the unique properties of nanomaterials like large surface-to-volume ratios, small size, physical, chemical, biological, mechanical, optical, catalytic properties have made them grow their utilities in the environment domain. The different nanobiosensors are used to detect pollutants, pesticides, toxins, and heavy metals from the environment. Various characteristics of nanobiosensors like sensitivity, selectivity, robustness, linearity, reproducibility, and detection time play an important role in developing an accurate nanobiosensor. Further, their fabrication with different materials using physical, chemical, or surface modification techniques enhances their properties. Nanobiosensors can be broadly classified on the basis of transducers and biorecognition elements. With the advancements in technology, nanobiosensors have also started to develop lab-on-a-chip, point-of-care devices, Internet of Things (IoT) and wearable devices, making them suitable for real-time and on-spot detection of pollutants. However, apart from all the advantages, the nanobiosensors face certain limitations like their results can be hampered in the real field due to the presence of various elements, and their high cost also reduces their utilities in various fields. To overcome these limitations, various regulatory laws should be implicated, which are updated regularly.

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Chapter 2
Classification, Properties, and Fabrication Techniques of Nanobiosensors

Vanya Nayak, Kshitij RB Singh, Ranjana Verma, Shweta Rathee, Ajaya Kumar Singh, Jay Singh, and Ravindra Pratap Singh

Abstract With the successful establishment of biosensors in the bioanalytical field, nowadays, nanobiosensors are also trending and are carving their ways in various domains, but they have gained much attention in the environmental domain as they can easily detect various pollutants from the environment and can help in remediation of dyes, contaminants, etc. Moreover, nanobiosensors are expected to overcome multiple hindrances faced by conventional biosensors without creating any major drawbacks. The various properties like sensitivity, stability, response time, the limit of detection (LOD), etc., and different fabrication techniques, including chemical, physical, and surface modification techniques, majorly define the utilities of nanobiosensors. Further, nanobiosensors can be broadly classified based on transducers and biorecognition elements. This chapter deals with the classification of nanobiosensors, various properties, and different fabrication techniques used to develop nanobiosensors.

Keywords Nanobiosensors classification · Properties · Fabrication methods · Nanomaterials

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2.1 Introduction

With the advancement in technology, the branches of science domain are also expanding; leading to the evolution and development of various domains, but it has majorly benefitted humans in one way or another. One of the well-known branches of science is nanotechnology, which has gained enormous attention for the past few decades. Nanotechnology utilizes particles whose size ranges from 1 to 100 nm, and due to this unique size, these particles help the nanotechnology to find its versatile applications in various domains like robotics, aeronautics, cosmetics, pharmacology, sensing, biomedical, environmental, agriculture, food packaging, veterinary sciences, etc. Moreover, nanotechnology also broadens its uniqueness by merging with biotechnology and forming a novel domain, nanobiotechnology. Nanobiotechnology dominates the biomedical field as it utilizes the biologically derived nanoparticle, which increases biocompatibility, decreases toxicity, and is a cost-effective technique. There are various literature data available that show the utilities of different nanoparticles in the biomedical domain (Li et al. 2012; Pratap Singh 2016; Singh et al. 2021c, d; Adetunji et al. 2021a, b; Singh and Singh 2021).

Furthermore, nanobiotechnology also deals in the development of nanobiosensors that utilizes various nanomaterials in developing the biosensors as these engineered nanomaterials give high-electrical conductivity, increase the sensitivity, stability, and decrease the LOD and response time. Moreover, as the properties of nanomaterials are growing, the development of various new bionanodevices likes smart sensors, point-of-care, lab-on-a-chip, wearable sensors, Internet of things, etc., is also increasing (Nayak et al. 2021; Singh et al. 2021a, b). A nanobiosensor generally consists of four parts, namely a bioreceptor, transducers, signal processor, and an interface, as shown in the Fig. 2.1. Various nanobiosensors have been developed to detect multiple biological analytes like DNA, RNA, aptamers, glucose, uric acid, etc. Further, the development and commercialization of nanobiosensor still face many challenges and need various regulatory rules for proper commercialization, and therefore, researchers now need to focus on their regulatory activities.

This chapter is dedicated for exploring different types of nanobiosensors classified based on transducers and biorecognition elements, properties of nanobiosensors, their fabrication technique, and future opportunities and limitations. The chapter presents the updated and detailed literature on nanobiosensors.

2.2 Classification of Nanobiosensors

The nanobiosensors can be broadly classified based on transducers and the type of bioreceptors. A brief overview of the classification of nanobisoensor is illustrated in Fig. 2.2.

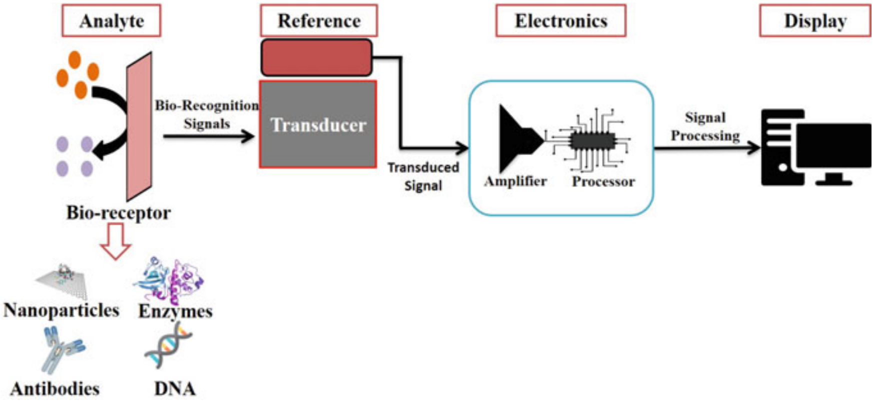

Fig. 2.1 It illustrates different constituents of a biosensors

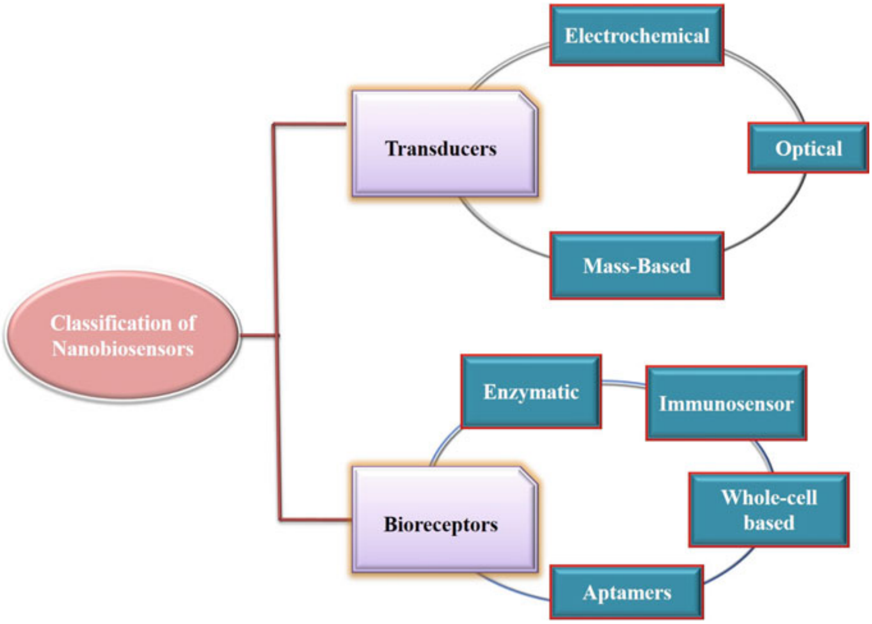

Fig. 2.2 It demonstrates the classification pattern of nanobiosesnors, which are divided on the basis of transducers and bioreceptors

2.2.1 Classification Based on Transducer

On the basis of the operating principle of transducers, nanobiosensors can be further classified on as electrochemical, optical, electronic, and gravimetric nanobiosensors

(Naresh and Lee 2021). The most widely studied nanobiosensors are electrochemical nanobiosensors, as they show high sensitivity, selectivity, and low-response time. The operating principles of these sensors depend on the electrochemical properties of the transducer and analyte. The nanobiosensors detect the electrochemical signals generated when an analyte interacts with the biorecognition element on the surface of the transducers. These electrochemical signals are studied in the form of the current, voltage, capacitance, and impedance (Shanker et al. 2014; Malhotra and Azahar Ali 2018). Moreover, electrochemical nanobiosensors are classified as amperometric, potentiometric, impedimetric, conductometric, and voltammetric nanobiosensors. The amperometric nanobiosensors are highly sensitive, rapid, and accurate and show linear responses that make them suitable for mass production. These sensors consist of two or three electrodes that measure the current produced when a constant potential is applied on the working electrode with respect to the reference electrode that causes the electroactive species to undergo electrochemical reduction or oxidation. Moreover, the concentration of the analyte plays an important role in sensing as the current generated on the working electrode's surface depends on the concentration of the analyte present in the solution (Grieshaber et al. 2008). Domínguez-Renedo et al. designed a urease amperometric biosensor-modified screen printed carbon (SPC) electrodes with gold nanoparticle, which detected the mercury (Hg^{2+}) ions in the soil. The decrease in current intensity was observed and was suggested that Hg^{2+} ions affected the amperometric response of urea (Domínguez-Renedo et al. 2009). The potentiometric nanobiosensor measures the charge, which is accumulated due to the interaction of bioreceptor with analyte at the reference and working electrode under zero potential. Ion-sensitive field-effective transistor and ion selective electrodes are used for the conversion of the biochemical reaction into potential signal (Malik et al. 2013; Shanker et al. 2014; Pisoschi 2016). Conductometric nanobiosensors measure the change in the conductance between the pair of electrodes caused due to the generation of an electrochemical reaction. Both conductometric and impedimetric nanobiosensors are generally utilized in the biomedical domain to analyze different metabolic processes in the living beings (Grieshaber et al. 2008; Dzyadevych and Jaffrezic-Renault 2014). Further, by applying small sinusoidal excitation signal, an electrical impedance is generated at the electrode/electrolyte interface, and these electrical impedance is analyzed with the help of an impedimetric nanobiosensors. In this, a low-amplitude alternate current (AC) voltage is applied at the electrode of the sensor, which generates an in/out-of-phase current response that is analyzed as a function of frequency (Ronkainen et al. 2010; Radhakrishnan et al. 2014). Moreover, the analytes can be detected using voltammetric nanobiosensors, which work by quantifying the current by applying variation in the potential. Additionally, they can potentially detect several analytes simultaneously with high sensitivity; therefore, they have found their profound utilization in many domains (Grieshaber et al. 2008).

When a biorecognition element is combined with an optical-based transducer system, the device is known as an optical nanobiosensor. The optical nanobiosensor utilizes various enzymes, antibodies, tissues, whole-cells, aptamers, nanomaterials, etc., to impart label-free and real-time detection, and their working principle depends on generating signals that are proportional to the analyte's concentration. The biorecognition elements produce chemical and physical changes, which are then transduced by the transducer by initiating change in various reactions like absorption, amplitude, transmission, phase, reflection, or frequency. Moreover, the optical nanobiosenors are divided into label-based and label-free optical nanobiosensors. In label-based sensing, the fluorescence, calorimetric, or luminescent-based methods are responsible for generating the optical signals, whereas, in the label-free sensors, the optical signal is generated by the direct interaction between the transducer and analyte (Lazcka et al. 2007; Martinkova 2017; Chen and Wang 2020). Further, based on their working principle, various optical nanobiosensors are produced like fluorescence-based optical nanobiosensors, which are highly used in the biomedical, environment, and food packaging domain as they exhibit low-response time selectivity and sensitivity. In the fluorescence-based optical nanobiosensors, the analytes which are to be detected are fluorescently labeled. Moreover, fluorescence-based nanobiosensors majorly involve three techniques: fluorescent quenching (turn-off), fluorescent enhancement (turn-on), and fluorescence resonance energy transfer (FRET), but FRET has gained a lot of attention in the therapy of cancer and analysis of aptamers, as they exhibit high sensitivity and can also detect the changes from nanometers to angstrom (Demchenko 2015). A carbon dots (CDs)/silver nanorod assembly-based FRET sensor was developed to detect lead ions. The prepared nanobiosensor exhibited a linearity of 0–155 μM and LOD of 0.05 μM (Jaffrezic-Renault and Dzyadevych 2008). Chemiluminescence-based optical nanobiosensors are based on the phenomenon of chemiluminescence in which chemical reactions cause the release of light energy. These nanobiosensors are simple and cost-effective and also show a low-detection limit. Further, Niu et al. (2018) designed an optical florescent biosensors utilizing DNAzyme based on graphene quantum dots and gold nanoparticle for detection of lead (Pb^{2+}) ion, which exhibited an extremely broad detection range of Pb^{2+} from 50 nM to 4 μM, and LOD of 16.7 nM (Niu et al. 2018). Moreover, currently, nanomaterials are used to enhance detection applications by increasing intrinsic sensitivity (Dippel et al. 2018). Similarly, a ternary electrochemical chemiluminescence (ECL) biosensor for the detection of miRNA-141 which was based on DNA walkers and utilized three-dimensional reduced graphene oxide (3D-rGO@silver nanoparticle (AuNPS) as conducting material was developed. It was observed that the numerous luminophores were able to react on extremely weak ECL signals due to the presence of DNA markers and exhibited high sensitivity, low LOD of 31.9 aM in the concentration range from 100 aM to 1 nM. Therefore, these properties of the ECL nanobiosensor make them an alternative approach that can be used to detect nucleic acids and biomarkers in various clinical analyzes (Chen et al. 2017). SPR-based optical nanobiosensors are label-free nanobiosensors and can detect the surface plasmon waves generated by the changes in the refractive index caused by molecular interactions. The SPR method highly depends on the variations of the refractive index that are connected to the transducer where an analyte binds with the biorecognition element. The SPR-based nanobiosensors are used for various clinical purposes and monitor pollutants and food quality (Damborský et al. 2016; Solaimuthu et al. 2020). An SPR-based nanobiosensor was developed that used silver nanoparticles to detect endotoxin *Escherichia coli* and exhibited a LOD of 340 pg mL^{-1} (Zandieh et al. 2018). Optical fiber-based optical nanobiosensors are used to measure the biological species like aptamers, proteins, whole-cells, etc., as it utilizes optical field and can be used as a potential technique for biological assessment. A fiber-optic sensor was developed which was based on poly(N-isopropyl acrylamide)-co-acrylamide)(P(NIPAAm-co-AAm))-magnetic immobilized glucose oxidase(GOD) complex (PMIGC) and glucose oxidase (GOD). It was used for the detection of cholesterol and glucose, and it efficiently detected glucose concentration (at 25 °C) and cholesterol (at 38 °C), by exhibiting the detection ranges of 50–700 mg dL^{-1} and 25–250 mg dL^{-1}, respectively (Huang et al. 2017).

Mass-based transducers are also known as gravimetric nanobiosensors that can detect a small signal produced in the mass of binding material, like antibodies, proteins, etc., generating a measurable signal. These nanobiosensors consist of thin piezoelectric quartz crystals, and when the current is applied, the piezoelectric quartz crystal vibrates at a particular frequency, therefore, current applied and the weight of the detected material highly defines the performance of the gravimetric nanobiosensors (Walton et al. 1993; Cali et al. 2020). The mass-based transducers can be further divided into piezoelectric-based nanobiosensors, magnetoelastic-based nanobiosensors (MES), and quartz crystal microbalance (QCM) nanobiosensors. Moreover, these transducers were used to detect the antigens and pathogens by using binding interactions. A piezoelectric biosensor consists of a crystal that undergoes elastic deformations whenever potential or current is applied, and it is achieved when a wave is produced inside the crystal by an alternating electric field at a specific frequency, which detects the change in the resonant frequency produced when a biorecognition element covered analyte is absorbed or desorbed on the surface of the crystal that suggests the occurrence of binding. Similarly, the QCM nanobiosensors also work based on the piezoelectric principle. In a QCM, two conducting electrodes cover a thin disk of quartz crystal, and as the mass of the detected material is changed, the resonance frequency of the crystal will also change. Further, MES is wireless and passive sensors that can detect strain, force, pressure, and stress. Since, MES consists of amorphous ferromagnetic ribbons; therefore, they show thick-film-like structure and exhibit very high-mechanical tensile strength of 1000–1700 MPa. The MES working is based on the principle of magnetostriction, in which, when a magnetic field is applied, mechanical deformation is generated. Further, a time-varying magnetic field generates magnetoelastic vibrations, resulting in the change of the field-generated strain with time, and hence produces longitudinal elastic waves. The detectable magnetic flux is produced by the elastic waves within the magnetoelastic material, and therefore, MES is widely considered in environmental applications. Moreover, they are cost-effective, durable, small in size and show wireless characteristics (Grimes et al. 2011).

2.2.2 Based on Bioreceptors

The nanobisoensors are also classified according to the biorecognition elements as catalytic and non-catalytic (affinity) nanobiosensors. In a catalytic nanobiosensor, the interaction between the analyte–bioreceptor results in developing a novel biochemical reaction product. This nanobiosensor includes enzymes, tissue, microorganisms, and whole-cells. Whereas, in the non-catalytic (non-affinity) nanobiosensor, the receptor and analyte are enclosed irreversibly, which produces no products during new biochemical reaction, and this type of sensor consists of nucleic acids, antibodies, and cell receptor as the target for detection (Naresh and Lee 2021).

An enzyme-based nanobiosensor is made up of an enzyme that acts as the recognition element immobilized on the transducer's surface. Since enzymes act as biocatalysts that can efficiently increase the biological reaction rate; therefore, they show excellent selectivity and affinity for the target molecules. These enzyme-based nanobiosensors find their immense potentialities in the biomedical field for diagnosing various disease, but also it has started to gain attention in other domains like agriculture, food industry, pharmaceutical industry, cosmetics, environmental, etc. Different magnetic nanoparticles, quantum dots, and carbon nanotubes have been used to develop nanobiosensors. For instance, a single-molecule enzyme nanocapsule as a highly stable biosensing platform was designed to cover the gap of enzyme loss at the working state in different environments. The proposed nanobiosensor showed excellent stability that could be utilized in various domains like environmental, biomedical, wearable devices, biofuel cells, etc. (Dhanjai et al. 2020).

Antibodies are highly known as affinity biorecognition elements and are most commonly used in nanobiosensors for more than two decades because of their strong antigen–antibody interactions. Antibodies show a structure similar to the immunoglobulins (Ig) in the form of a 'Y' shape composed of two light and heavy polypeptidic chains that are joined by disulfide bonds. Based on the heavy chains, antibodies are classified into IgM, IgG, IgD, IgA, and IgE (Schroeder and Cavacini 2010). Similarly, on the basis of their elective properties and the synthesis technique, they are distinguished as polyclonal, monoclonal, and recombinant (Cordeiro et al. 2018). These antibodies are either placed on a ligand, or the interaction between antigen and antibody helps develop immunobiosensors. Moreover, these immunobiosensors are classified as labeled and non-labeled. Non-labeled immunobiosensors specifically detect the physical changes caused during the formation of antigen–antibody complex that helps detect the antigen–antibody complex. Whereas, on the other hand, labeled immunobiosensor, an antigen–antibody complex, is detected with the help of a sensitively detectable label (Lim and Ahmed 2016, 2019). Bhardwaj et al. utilized gold nanobipyramid (NBPs) to develop optical and electrical label-free nanobiosensors to detect Aflatoxin B1 (AFB1). The sensor showed a linearity of 0.1–25 nM and LOD of 0.1 nM as shown in the Fig. 2.3 (Bhardwaj et al. 2021; Naresh and Lee 2021). Currently, the world has been suffering from the SARS-CoV-2 pandemic and to combat this viral pandemic, proper detection techniques are required. The onset of the pandemic has made us realize the importance of nanotechnology and

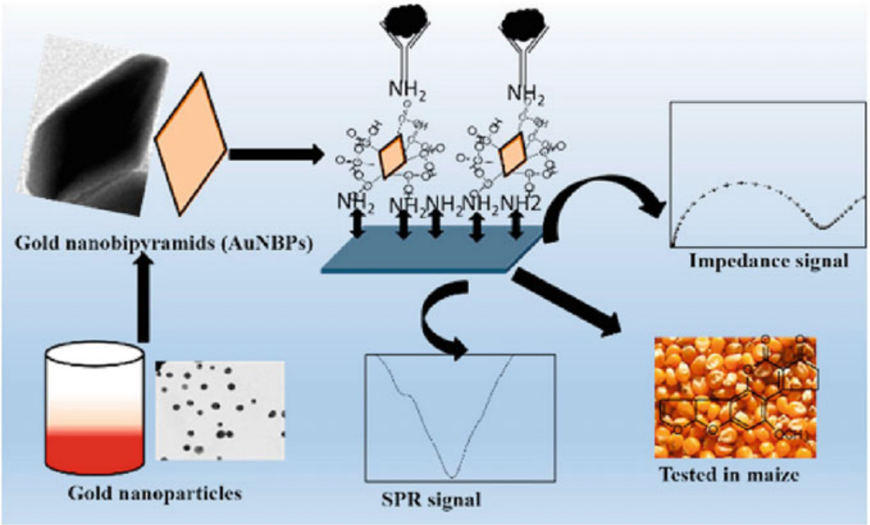

Fig. 2.3 A schematic illustration of functionalization of gold nanobipyramids for the detection of Aflatoxin B1 (reproduced with the permission from Bhardwaj et al. 2021)

biotechnology, and therefore, the work on combating this pandemic has started at a rapid pace (Ukhurebor et al. 2021). A graphene-based nanoresonator biosensor was proposed that used the finite element method (FEM).

A nanomechanical biosensor works by detecting the change in the mechanical response during the addition of a foreign object. Payandehpeman et al. (2021) proposed a sensor, which consisted of a single-layer graphene sheet (SLGS) with a specific antibody against the severe acute respiratory syndrome coranvirus-2 (SARS-CoV-2) spike S1 antigen was coated, and then, the SARS-CoV-2 viruses were spread on the layer. Moreover, the SLGS size, antibody condition, aspect ratio, and the number of viruses were detected by the frequency shift and relative frequency shift. The results exhibited that the proposed biosensor could efficiently detect SARS-CoV-2 ranging from 10 to 1000 viruses per test with a frequency shift. According to the simulation results, an analytical relationship was also proposed. By determining the geometry of the SLGS, the LOD of the sensor could be predicted with the required sensitivity index. Therefore, a highly sensitive nanoresonator sensor for SARS-CoV-2 detection for ten viruses per test was demonstrated and can be used for the point of care diagnostics of SARS-CoV-2 (Payandehpeyman et al. 2021).

Other important nanobiosensors are developed using aptamers, synthetic single-stranded RNA, or DNA that can selectively bind with the target molecule in both two-dimensional (2D) and three-dimensional (3D) structures. Since these 2D and 3D structures have less spatial blocking and large surface density, which help in high-binding performance (Tombelli et al. 2005; Hong et al. 2012; Dhiman et al. 2017), these aptamers are highly functionally and structurally stable over a wide range of temperatures. As the aptamers can be chemically synthesized, they show certain thermal refoldings and can be easily chemically modified to detect target molecules; therefore, more preferred for the development of label-free and labeled nanobiosensors (Kumar et al. 2019). Various techniques like piezoelectric, optical, surface plasmon resonance (SPR), fluorescence, electrochemical, etc., have been used to develop aptamer-based nanobiosensors. Aptamer capped near-infrared lead sulfide quantum dots (NIR-PbS-QDs) were designed for the detection of thrombin protein based on the charge transfer and show a LOD of ~1 nM (Choi et al. 2006).

Similarly, whole-cell nanobiosensors have also gained a lot of attention as they utilize microorganisms like bacteria, viruses, fungi, algae, protozoa, viruses, etc., that act as the potential biorecognition elements, like antibodies. Since these microorganisms are self-replicating; therefore, they do not require any extraction and purification methods (Choi et al. 2006; Kylilis et al. 2019). Moreover, these whole-cell-based nanobiosensors show high sensitivity and selectivity to detect the analytes helps them to find their immense potentialities in food analysis, environmental monitoring, detection of heavy metals, pesticides, drug screening, etc., the principle of the whole-cell-based nanobiosensor is based on the detection of the electrochemical responses of the microorganisms with analytes that are detected by the transducers (Ron and Rishpon 2009). Riangrungroj et al. (2019) designed label-free optical whole-cell *E. coli* nanobiosensors to detect pyrethroid, which is an insecticide that showed the LOD of 3 ng mL^{-1} 3-PBA in the linear range of 0.01–2 ng mL^{-1} (Riangrungroj et al. 2019).

Several nanobiosensors are developed that utilize nucleic acid as the recognition element and show broad-spectrum activities in various domains. For instance, peptide nucleic acid (PNA) is a synthetic analog of DNA composed of an uncharged backbone which makes it a stable complex as compared to the DNA or RNA individual complex. But, with the onset of nucleic acid analogs, nucleic acid sensing has increased rapidly, as these synthetic analogs efficiently mimic nucleic acids, helping them show excellent sensing performances. Moreover, PNA offers excellent thermal, pH, chemical, and structural stability, allowing them to exhibit a higher level of selectivity than the DNA or RNA. These biophysical properties of PNA and locked nucleic acid (LNA) make them more considerable to be utilized in the sensor field (D'Agata et al. 2017). A rapid and precise PNA-based nanobiosensor has been reported, which can be used for biomedical applications (Singh et al. 2010, 2020).

2.3 Properties of Nanobiosensors

Every nanobiosensors possess various static and dynamic properties, and their optimization can greatly affect the performance of the nanobiosensor as illustrated in Fig. 2.4. The bioreceptor's ability to detect specific analytes from a sample is known as selectivity and is considered one of the most important properties of a nanobiosensor. It can efficiently distinguish and measure the analyte with the least interference in the sample from other materials. Therefore, if the selectivity is high,

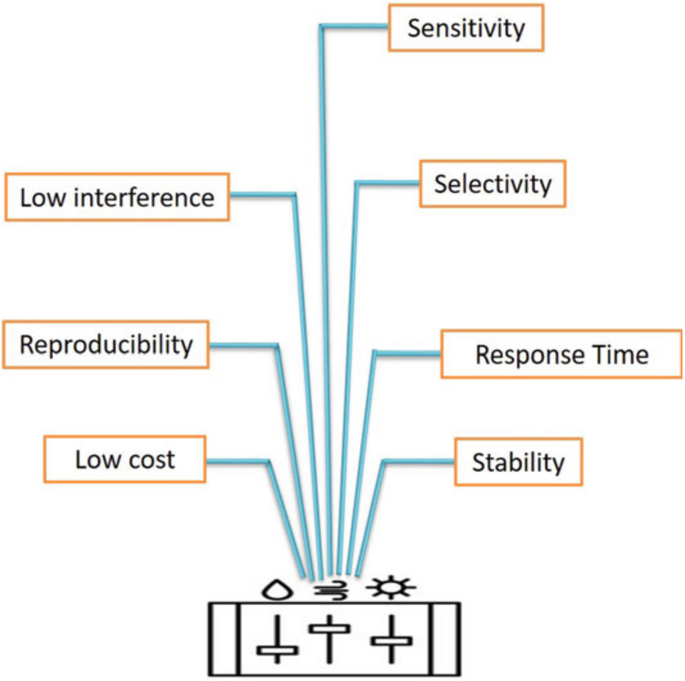

Fig. 2.4 It shows different properties of a nanobiosensor

the sensor is considered more effective and precise. To construct a biosensor, selectivity is considered as the main property while choosing bioreceptors (Wu et al. 2019; Qian et al. 2020; Zhang et al. 2020; Hu et al. 2020).

Similarly, the sensitivity of a nanobiosensor is also considered an important property, as it determines the minimum amount of analyte which is detected by the nanobiosensor and is sometimes also referred to as the LOD (Li et al. 2020). If a nanobiosensor shows high sensitivity, that means it can detect the slightest change of the analyte concentration, which is observed in the change output signal. Therefore, the bit resolution of these sensors is observed to be high. Both selectivity and sensitivity help nanobiosensors be utilized in various fields like biomedical, environmental, food industry, etc. (Wang et al. 2019; Domènech et al. 2019; Liu et al. 2020; Song et al. 2020; Wei et al. 2021).

Another important property required to develop an efficient nanobiosensor is stability, which is defined as the degree of susceptibility to atmospheric disturbances present in and around the biosensing system. These atmospheric disturbances tend to shift the output signals of a biosensor during measurement, leading to the generation of an error in the measured concentration. It also hampers the accuracy and precision of the nanobiosensor. Therefore, stability becomes an important feature in the development of nanobiosensors which requires long incubation steps and continuous monitoring. Many factors can hamper the strength of the nanobiosensors, such as temperature, sensitive nature of the transducers, degree of binding of bioreceptor with the analyte, and degradation of bioreceptor.

Moreover, bioreceptors that show high affinity tend to provide strong covalent linkage or electrostatic bonding with the analyte that helps in securing the stability of the nanobiosensors. Moreover, nanobisosensors take a certain time to convert the output state into the input state, and this period of time is known as the response time. The reaction time is defined as the time required for a sensor output to change from its previous state to a final settled value within a correct new value tolerance band. The fast reaction time helps in the development of successful, accurate, and precise nanobiosensors. Various nanomaterials like polyaniline (PANI) and carbon nanotubes have been used to develop nanobiosensors, showing fast response time and high selectivity (Bhalla et al. 2016).

Similarly, reproducibility also plays a vital role in developing a nanobiosensor. The generation of the identical response for a duplicate experiment setup is known as the reproducibility of the nanobiosensor. The reproducibility is determined by the accuracy and precision of the transducer and electronics in a biosensor. Precision in a sensor is defined as the capability of the sensor to give similar results every time a sample is measured, whereas the accuracy of a sensor is indicated by the sensor's ability to give a mean value close to the true value when a sample is measured more than once. Reproducible signals provide high reliability and robustness to the inference made on the response of a nanobiosensor.

When different concentrations of analyte are measured, different responses are recorded, and their accuracy is measured through linearity by forming a straight line. The linearity is measured as

$$Y = m.c$$

Y represents the output signal, m is the sensor's sensitivity, and c is the concentration of the analyte. Moreover, the range of analyte's concentration and the resolution of the sensor can greatly affect the linearity of the nanaobiosensors. The slightest change in the concentration of an analyte that is required to bring a change in the response of the biosensor is known as the resolution of a nanobiosensor. Further, to precisely detect and measure the concentration of the analyte over a wide working range, the nanobiosensors should exhibit good resolution. Similarly, linear range is another term that helps in measuring linearity. It is defined as the linear response of the biosensor to the change in the analyte concentration (Bhalla et al. 2016).

2.4 Fabrication of Nanobiosensors

To develop an efficient nanobiosensor, it is very important to select a substrate that can be used for dispersing the sensing material. For this purpose, various nanomaterials like nanoparticles (gold), carbon nanotubes (CNTs), quantum dots (QDs), and

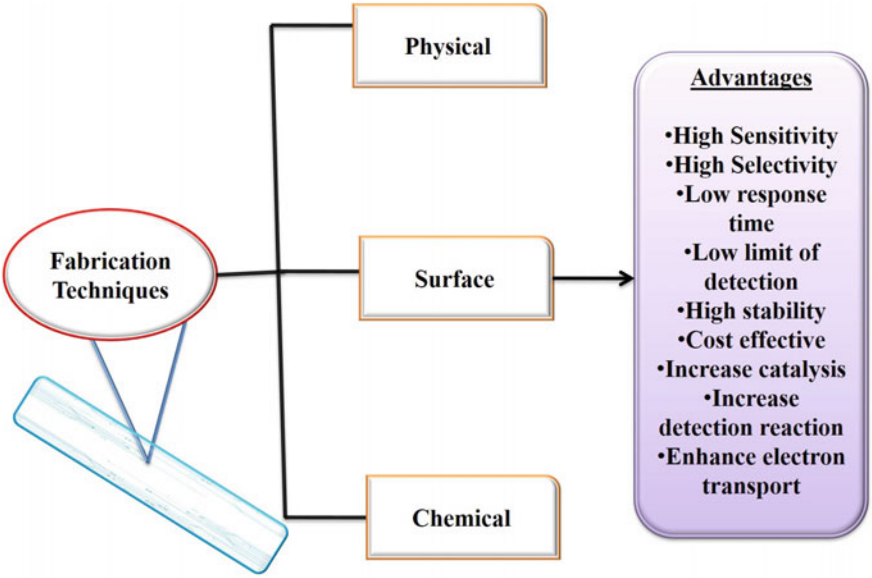

Fig. 2.5 A schematic illustration of different fabrication methods used to develop nanobiosensors and their advantages

magnetic nanoparticles have been utilized as they exhibit unique physical, chemical, optical properties, resulting in the enhancement of sensitivity and specificity of detection (Zhang et al. 2009). Moreover, these nanostructures can be used to fabricate nanobiosensors to increase catalysis, detection reaction, and electron transport as shown in Fig. 2.5.

2.4.1 Physical Fabrication

In the physical adsorption technique, the outer surface consists of immobilized biorecognition elements by some weak attractive forces, like electrostatic force, van der Waals forces, hydrogen, or ionic bonding. Mostly, this method is observed in enzyme-based nanobiosensors. Physical adsorption is an easy and cost-effective, non-destructive process that does not require modifying the biological components or building a matrix. However, with the change in ionic strength, pH, temperature, poor storage and operational stability can occur (Sassolas et al. 2012; Martinkova 2017; Arya et al. 2019). Similarly, in the physical entrapment process, the covalent and non-covalent bonds physically entrap the biorecognition elements in the 3D matrices. In this method, the enzyme and a monomer solution are mixed, then polymerized by a chemical reaction. Here, the material used for the 3D network can be either organic like photopolymer, alginate, cellulose, gelatin, etc., or inorganic like porous ceramic material or the activated carbon. Since metal and metal oxide nanoparticles are utilized for immobilizing the biomolecules in the disease diagnosis applications, therefore, waxberry-like nanoscale ZnO balls were found to be excellent materials that can be used for immobilizing the enzymes, and their wide bandgap helps them to act as the rapid electron transfer agent, which can be used to fabricate the nanobiosensors (Xia et al. 2008; Huang et al. 2008). Further, the porous structure of the ZnO balls increases the active surface area for protein binding, which creates a microenvironment for the enzymes to exhibit their enzymatic activity and stability (Lu et al. 2008). In this technique, a film consisting of metallic coating is grown on a base material by the electrochemical reduction of metal ions. For this, a nanobiosensor was developed to detect anti-HIV nevirapine from the human blood sample, which consisted of copper oxide nanoparticles electrodeposited on the carbon nanoparticle film. Therefore, the developed nanobiosensor exhibited good response time and high sensitivity, suggesting its use in the biomedical domain to detect anti-viral drugs from the blood serum (Shahrokhian et al. 2015).

2.4.2 Chemical Fabrication

Chemical fabrication forms strong chemical bonds, like covalent bonds between the functional group of the biorecognition element and the surface of the transducer. Cross-linking is one of the chemical fabrication techniques that occur by forming intermolecular covalent cross-linkers either between biorecognition elements and functionally inert protein like bovine serum albumin (BSA) or between the biorecognition elements. Multi-functional reagents are the linkers that connect the enzyme in 3D cross-linkers with the transducer sensor. Various conditions like temperature, pH, ionic strength, etc., can affect cross-linking. Cross-linking exhibits less response time, strong bonds and increases the catalytic activity of enzymes. Moreover, with the help of stabilizing reagents, the environment of the biorecognition elements can be modified. Since the cross-linkages are formed between protein molecules and not between the protein and the matrix, it can also lead to the denaturation of the protein structure, resulting in limiting the application of the cross-linking functionalization. Similarly, covalent binding is one of the widely used techniques to fabricate the biosensor is the direct covalent binding. The surface of the transducer or matrix binds tightly with the biorecognition element. The interaction between the functional protein groups and reactive groups of the transducer matrix with the biorecognition elements highly defines the covalent binding mechanism (Singh 2011; Sassolas et al. 2012; Martinkova 2017). It has been observed that the functional groups present on the carbon nanotubes (CNTs) react with the functional group of biorecognition molecules to form a covalent bond. Using this property, an electrochemical immunosensor was designed consisting of multi-walled CNTs deposited on the indium-tin oxide (ITO) electrode to detect the aflatoxin B1. In the designed sensor, the monoclonal aflatoxin B1 antibody was deposited on the electrode. It was observed that the immunosensor exhibited high sensitivity of $95.2\,\mu\text{A}\,\text{ng}^{-1}\,\text{mL}\,\text{cm}^{-2}$, an enhanced LOD of 0.08 ng mL^{-1}, and the decreased value of association constant at 0.0915 ng mL^{-1} (Martinkova 2017). Moreover, electropolymerization is considered one of the easiest processes as it involves forming a polymer film of a certain thickness by controlling the current applied to the electrode. Generally, for enzyme immobilization, aniline, pyrrole, and thiophene-based electropolymerized films have been used. The extraordinary electrical conductivity exhibited by polymer and the reversible doping, flexibility, and recognition of biomolecules makes them a considerable agent for developing various nanobiosensors. Moreover, electropolymerization is a cost-effective and simple process that can help in the development of nanobiosensor (Cosnier 2003). Mousa et al. (2019) fabricated a nanobiosensor by using conductive polyaniline nanofibers (PAnNFs) and electropolymerized poly(aniline-*co*–N-phenyl-*o*-phenylenediamine) nanoflakes [P(An-*co*-PoPD)], as they provided large surface area that resulted in rapid ion exchange and improved the sensitivity of the sensor. The PAnNFs and P(An-*co*-PD) films were then established on the gold electrode by redox polymer-modified surface, which was then further used to detect glucose level. It was reported that this sensor showed a high-amperometric response to glucose compared to the PAnNFs films, concluding that the addition of PoPD monomer to aniline results in the generation of flake-like morphology that responds to the enzyme-based electrode (Mousa et al. 2019). Spray pyrolysis is a thermal degradation of organic substances completed in the absence of oxygen. Jaymand et al. (2013) developed and performed a comparative analysis of the copper oxide nanoparticle and copper oxide nanolayer-based biosensor, which detected the *Aspergillus niger* obtained from the decayed food. The CuO nanoparticles were synthesized using the sol–gel method, whereas the nanolayers of CuO were deposited on the glass substrate through the spray pyrolysis method. Since the mold needed the oxygen to react with the surface; therefore, the sensitivity of a sensor was measured with the increase in temperature, as the temperature increased, the sensitivity of the sensor decreased. It was observed that CuO nanoparticles decreased with increasing time, and the resistance remained unchanged, whereas, in the case of nanolayers, the resistance increased dramatically initially and further became constant and stable**.** Therefore, it was concluded that the resistance of both CuO nanoparticles and nanolayers varied rapidly in the presence of the mold, and the resistance of CuO nanoparticles quickly decreased with increasing the temperature (Jaymand 2013). Sol gel technique is the most commonly used fabrication method that requires low temperature to bind an enzyme. In this method, the hydrolysis method is used to synthesize the nanoparticle, and similarly, metal alkoxides are synthesized using the condensation method, which forms the 3D matrix that is further utilized to encapsulate the biomolecule. Wang et al. (2008) fabricated the ZrO_2/Au nanocomposite films by combining electroplating with the sol–gel process to adsorb the organophosphate pesticides (Ops) on the ZrO_2/Au film electrode surface. This method was found to be effective in the quantitative detection of the Ops (Wang and Li 2008).

2.4.3 Surface Modifications

Surface functionalization of nanomaterials increases their binding affinity to specifically bind with the target analyte, which can be useful in developing nanobiosensors that could detect the pollutants present in the environment. Moreover, capping the surface of nanomaterials with certain stabilizing agents like surfactants, biomolecules, etc., can increase the stabilization of nanobiosensors. Fluorescent nanomaterials for the development of nanobiosensors have been found to be very useful as they possess large surface area, porous structure, and high-loading capacity and can also specifically bind to the analyte. Further, these fluorescence molecules can lose their luminescence; therefore, surface functionalization of these nanomaterials can help protect their luminescence characteristic and enhance their target-sensing property. Many surface-functionalized nanomaterials like carbon nanotubes, metal and metal oxide nanoparticles, quantum dots, etc., have been utilized to develop nanobiosensors that can detect pesticides from the soil. For instance, Kondekar et al. (2015) utilized the surface-modified cetylpyridinium bromide capped gold nanoparticles (CPB-AuNPs) to develop an ultrasensitive, easy, and highly specific colorimetric nanobiosensor to detect the sulfide ions (S^{2-}) from the aqueous medium. The color change was observed when the S^{2-} was added to the solution of CPB-AuNPs, which demonstrated that the results could be visualized through naked eyes, making it more suitable for use for onsite and real-time screening. Additionally, it was also shown that the S^{2-} can also be used without masking agent or any pre-treatment (Kondekar et al. 2015).

Moreover, the detection techniques used in the development of nanobiosensors can also be classified into the label-free and labeling phenomenon. The labeled technique utilizes certain ‘tags’ or ‘labels’ which help them detect a particular analyte. The fluorescence, chemiluminescence, and radioactive nanobiosensors are widely based on the labeling phenomenon. Although, the labeling of nanomaterials with tags or labels can greatly affect the decrease of sample purity, quantity, and functionality. Therefore, the development of label-free nanobiosensors has started gaining a lot of attention as it does not require labeling of the ligand with receptor and also allows the screening of the complex in low quantity (Fang 2010; Citartan et al. 2013; Musayev et al. 2014; Liu et al. 2014). As use of nanotechnology is increasing, the development of label-free nanobiosensors is also increasing as these nanobiosensors have enhanced the throughput, sensitivity, selectivity and have decreased the sample consumption and damage to the analyte (Sang et al. 2015). Lin et al. (2014) developed a fiber-optic particle plasmon resonance immunosensor that was based on gold nanorods and was utilized for label-free detection of orchid viruses. Since, the gold nanorods were functionalized by antibodies on the surface; therefore, they specifically recognized the corresponding *Cymbidium mosaic virus* (48 pg/mL) or *Odontoglossum ringspot virus* (42 pg/mL).The developed nanobiosensor was found to be rapid and can be used for the rapid viral detection in plants (Lin et al. 2014).

2.5 Challenges and Future Perspectives

Although biosensors show versatile applications in various domains of science like biomedicine, engineering, biomedical engineering, etc., the onset of nanotechnology and the use of nanomaterials to produce nanobiosensors have enhanced their effectiveness, decreased their cost, increased their sensitivity, selectivity, rapidness, etc. Moreover, these nanobiosensors have gained a lot of attention as the size of the nanomaterials helps them exhibit unique properties which are not possible to accomplish using bulk matter. The nanobiosensors have found their profound use in the environmental domain to provide accurate in situ and real-time monitoring of pollutants and detect pathogens and ecological toxicity. Mostly, metal oxide nanoparticles are preferred for the development of nanobiosensors as they have inherent biocompatibility due to their unique shape and size. Nowadays, the development of various smart nanobiosensors to detect environmental analytes has started. The wearable wireless nanobiosensors based on the Internet of things (IoT) and paper-based nanobiosensors are becoming the front runners. These nanobiosensors promise a potential future as they are mobile, accurate, simple, easy to carry, environment friendly, and dedicated to fieldwork. Moreover, a continuous and enhanced approach could definitely make the nanomaterials a breakthrough entities for the development of efficient nanobiosensors.

One of the main limitations faced in the development of environmental nanobiosensors is their lack of utilities in real environmental samples as many nanobiosensors, termed environmental nanobiosensors, have been tested under lab conditions, which makes them unsuitable to be used in the external field. Therefore, it is now a need of the hour to develop reliable, easy, rapid, and sensitive nanobiosensors which can be used in the area. Moreover, other limitations like high cost, low-storage capacity, and low stability also need to be addressed.

2.6 Conclusion

It is well known that biosensors rule almost all well-established bioanalytical techniques, but currently, nanobiosensors that constitute the inclusions of nanotechnology as well biotechnology are now revolutionizing the analytical field with generating future promising and permanent solutions by decreasing the excess conventional laboratory methods and protocols, rapid response time, increasing sensitivity, selectivity, robustness, and point-of-use portability. These nanobiosensors find their immense potentialities in various domains like biomedical, agriculture, robotics, electronics, the Internet of things, the environment, etc. The chapter here covers the classification, properties, and fabrication techniques of nanobiosensors in the aspect of environment domain. The nanobiosensors can be broadly classified based on the transducers and type of bioreceptors. Moreover, their unique fabrication techniques involve surface, chemical, and physical modifications, which help enhance the nanobiosensors. The nanobiosensors should exhibit high selectivity, sensitivity, accuracy, robustness, and less response time. Although the use of nanobiosensors in the environment domain is limited as they show limitations in the actual field, it hampers the detection process and results. Moreover, the high cost of developing the nanobiosensors also reduces their utilities; therefore, the researchers also need to address these limitations that can help them to enhance the utilities of nanobiosensors in various domains.

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Chapter 3
Nanobiosensors' Potentialities for Environmental Monitoring

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Abstract Pollutants have become the global concern for which there is an intense demand for a quick, reliable, and sustainable system for their determination in the environment and agricultural land. Quantitative analytical tools such as chromatography and spectroscopy, albeit precise and accurate, expensive, requires experienced technician, complicated sample preparation steps, and difficult to assess at high frequencies in real-time. To overcome the issues, nanoparticle-based biosensors are considered as a potential tool to detect both biotic and abiotic toxins. With headways in nanotechnology, numerous specialists have utilized the one-of-a-kind properties of nanomaterials (counting a high surface-area-to-volume proportion) to foster efficiency and sensitivity in detection techniques. Nanomaterials have enabled us to design devices at the microscale level, prompting fast, versatile, and sensitive microorganism symptomatic frameworks that can recognize airborne microbes in clinics, air vents, and planes and bioterrorism in open spaces. Hence, this chapter gives an overview of the usage of nanobiosensors in the detection of contaminants. Further, the present scenario and future scope are also discussed in the development of novel detection devices, and their advantages over other environmental monitoring methodologies.

Keywords Biosensors · Nanoparticles · Contaminants · Pathogens

3.1 Introduction

Biological response sensing has attained much attention in the present scenario in the field of altered homeostatic happenings in the environment including both in vivo and ex vivo processes. Biological analysis has been gaining great significance in various

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fields like pharmaceuticals, screening food quality, diagnosis, and environmental applications. For this purpose, the development of biosensors for analyzing every biological interactions' detail has become very important (Dzyadevych et al. 2008). A biosensor is generally defined as a measurement system that consists of a probe with biological recognition element, often called a bioreceptor and a transducer. The interaction of the analyte with the bioreceptor is designed to produce an effect measured by the transducer, which converts the information into a measurable effect, for example, an electrical signal. Biosensors are classified into different types due to their transduction and biological recognition units which include piezoelectric (Kazemi-Darsanaki et al. 2012) and potentiometric for transduction, and organelles, antibodies (Abs), and enzymes for biorecognition purpose (Schubert et al. 1991; Malhotra et al. 2017). The development of the premier oxygen biosensor by Lel and Clark in the 1960s leads to better opportunities for developing a different kinds of biosensors in Therapeutics, Biomedical Engineering and Technology (BMET), Environment, and Nanotechnology (NT) applications. A typical example is the nanobiosensor synthesized from the synergy of nanotechnology and biosensors. The incumbents of nanomaterials to biosensors make the biosensors function in a far better and more diverse manner.

Nanobiosensor has dimensions on the nanometer-size scale ranging from 1 to 100 nm. These kinds of sensors have various unique chemical, physical and physicochemical properties and their atomic constituents are present near or on the surface. The combination of both, i.e., electrical system and nanomaterial devices is responsible for the generation of nanoelectromechanical system (NEMS) which is highly active in the electrical transduction mechanism (Malik et al. 2013). The nanomaterials like nanorods (NRs), nanowires (NWs), nanotubes (NTs), nanoparticles (NPs), and nanocrystalline tubes (NCTs) were explored due to their unique electrical and mechanical properties and for their capability of improving the transduction mechanism and biological signaling. Furthermore, these nanobiosensors are best employed due to their great sensitivity, selectivity, linearity, and response time. Among all the nanomaterials, NPs are well analyzed and studied up to this moment.

The applications of nanobiosensors are gaining immense importance in various fields such as food safety, quality control, and environmental safety due to their characteristics like high specificity, selectivity, fast response, and reproducibility (Auffan et al. 2009). The chapter particularly focuses on the role of nanobiosensors in the field of environmental remediation. Environmental contamination has a lot of negative impacts on human health and socioeconomic development, which is a global concern. Contaminants in the environment, particularly biotic contaminants such as bacterial, viral, and parasite infections and their toxins, as well as abiotic contaminants including heavy metals, pesticides, bisphenols, and various other chemical compounds represent major public health risks. Diagnostic systems and test kits that are sensitive, cost-effective, and portable are desperately needed in the environmental industry. Biosensors based on nanoparticles are being investigated as promising tools for the identification of the analyte of interest in a sensitive manner (Koedrith et al. 2015). Distinct nanoparticles have different optical, fluorescent, and magnetic properties, and combining these properties has a lot of applications in environmental screening. Nanoparticle-based technology, in particular, allows us to monitor and enhance the quality of water, air, and soil. For example, silica nanoparticles are thought to be a good alternative because of their many functional capacities, such as the ability to administer antimicrobial compounds for treating certain pathogenic microorganisms and the ability to recognize the germs (Song et al. 2013). Similarly, a variety of nanoparticle-based sensors for sensing specific heavy metal ions have been tested and shown to be effective. A gold nanoparticle-based sensor with a sensitivity of 5 ng/mL (ppb) was used for quick screening of mercury (Hg^{2+}) ions in aqueous solutions (Darbha et al. 2008). The current chapter summarizes the different types of nanomaterials and a detailed discussion on the potential applications of developed nanobiosensors in the identification and determination of various kinds of environmental pollutants (abiotic and biotic).

3.2 Types of Nanobiosensors

Nanobiosensors are categorized into different categories by taking into consideration the type of nanomaterial used in the synthesis of biosensors, the type of material to be screened using these nano-bio sensors, and their sensing response, e.g., if the analysis of an antigen is performed by the nanobiosensor, then it is known as antigen nanobiosensor and if the enzyme is screened then it is named as enzyme nanobiosensor. Hence, the naming of biosensors is done on the nature of the analyte to be analyzed. Based on their sensing mechanism, the major types are colorimetric, optical acoustic, and electrochemical (De Corcuera et al. 2004) as shown in Fig. 3.1.

Electrochemical nanobiosensors transduce the biochemical events such as antigen–antibody interaction and enzyme–substrate interaction into the electrical signals. This type of sensor works on the principle of electrochemistry like cyclic voltammetry, amperometry, impedance, and voltammetry. These sensors typically consist of an electrode which is a key component of the sensor. The electrode is used as solid support for electron movement and immobilization of biomolecules (antigens and nucleic acid). Based on electrodes, these sensors are categorized into two groups, i.e., carbon nanomaterial-based and non-carbon nanomaterials-based sensors. The carbon nanomaterial-based biosensors are best employed as an electrode and also as a supporting matrix due to having unique properties like effective electron transfer rate and greater surface area (Cho et al. 2020).

Nanosensors that are typically utilized in diagnostic processes are known as Immunonanobiosensors. These sensors generally consist of receptors that are employed for the antigen–antibody interaction. The target analyte detection can be observed directly by the formation of the immune complex (antigen–antibody complex) or indirectly by using a label, e.g. gold NPs (AuNPs) to detect the binding event (Holford et al. 2012).

Colorimetric nanobiosensors are employed to detect the specific analyte through color change. The color change can be visible either by naked eyes or using some portable optical devices in these types of sensors, NPs are utilized as the colorimetric probe for detection and quantification purposes. Various types of NPs like

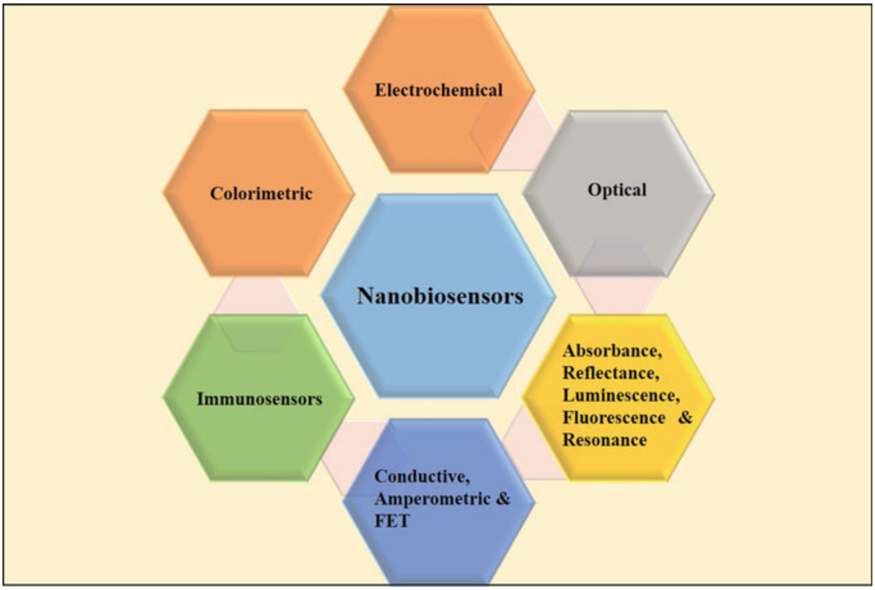

Fig. 3.1 Different types of sensing responses exhibited by nanobiosensors

AuNPs (modified and unmodified), silver NPs (AgNPs), and quantum dots (QDs) are employed for the development of colorimetric sensors (Zhao et al. 2020).

Fluorescent nanobiosensors are one of the emerging technologies that allow the biochemical parameters' measurement with high temporal and spatial resolution. These sensors typically comprise two or more fluorophore units. These units are reference and analyte-sensitive entities. The reference fluorophore unit generates a fluorescent signal that is insensitive to an analyte. An analyte-sensitive unit generates a fluorescence signal depending on the amount of target analyte. By the incorporation of more than one analyte-sensitive fluorophore unit, multiple target analytes can be detected. The best ratio metric response is observed in the case of a combination of both reference and analyte-sensitive units (Harrison and Chauhan 2018).

But, the main criteria for classification of nanobiosensors are considered as the nature of nanomaterial utilized in the synthesis of the biosensor, e.g., if carbon nanotubes (CNTs) are utilized in the synthesis of biosensors, then the biosensor is known as a nanotube-based sensor and if NPs are involved then it is a nanoparticle-based biosensor. The present enlists major types of nanobiosensors and their applications:

3.2.1 NPs-Based Biosensors (NPBS)

NPs are metal elements having a diameter of 1–100 nm. Out of all the reported NPs, metal NPs are most widely employed in the field of biosensing because of their better extinction coefficient and definite optic-electrical properties. Among all, AuNPs and AgNPs are of utmost preferred because of their distinct electrical, photothermal, and optical characteristics (Sabela et al. 2017). Metal NPs-based biosensors are used in biological studies and are of different structures and shapes like spheres, rods, stars, rings, wires, and shells that are all in the nanoscale (Anbia and Amirmahmoodi 2016). In a free state, metal NPs are unstable thermodynamically and can get precipitated or aggregated very easily which leads to high surface energy.

The synthesis of AuNPs involves Au (III) salts of different sizes and shapes. The physicochemical properties of AuNPs are found to be very different than pure Au. AuNPs-based biosensors exhibit better analytical behavior with enhanced sensitivity because of having distinct chemical, optical, electronic, and catalytic properties (Alex and Tiwari 2015; Xiong et al. 2018). The major difference between the AuNPs and pure Au is color, i.e., red for AuNPs and yellow for pure Au. But the main limitation of AuNPs-based biosensors is that the presence of heavy metal ions in various organic molecules, environment, soil, and food pigments diminishes the selectivity and sensitivity of these biosensors and leads to unreliable results.

The synthesis of AgNPs involves the reduction of silver chloride (AgCl)/silver nitrate ($AgNO_3$) in presence of reducing agents like sodium citrate/sodium borohydride. The extinction coefficient of AgNPs is higher than AuNPs. But the surface modification of AgNPs is not possible as they lead to chemical degradation upon functionalization. AuNPs are stable up to 30 days or more and AgNPs are stable only up to 15 days (Mancuso et al. 2013). That's why AuNPs-based biosensors are most widely employed in comparison to AgNPs-based biosensors.

Along with these, magnetic NPs such as ferrite-based materials have also been used in the biosensing field. The use of magnetic nanomaterials offers a wide variety of scopes in the biosensing field. Iron is combined with other transition metals, many of which have different properties, and is used in screening. Magnetic NPs have been added to traditional bio-detection devices, making them much more sensitive, stronger, and specific (Malik et al. 2013).

3.2.2 NTs-Based Sensors (NTBS)

For the synthesis of NTBSs, CNTs were utilized for enhancing the specificity of the reaction. CNTs are the pseudo-1-D allotrope of carbon. These are the hollow cylindrical NTs that behave as a sensing material in nanobiosensors. These NTs were rolled to generate the rolled graphene (Grp) sheets. The arrangement of carbon atoms in the sheet can be zigzag, armchair, or chiral consisting of the 3D CNTs and 2D Grp sheets. CNTs can be synthesized using two dynamic methods out of which one is chemical vapor deposition (CVD). The method is employed for obtaining the high yield by mass and then further purification has been done to avoid the structural damage and to eliminate the undesirable metal used as catalyst (Haniu et al. 2012).

Based on the number of Grp sheets present covered by a hemisphere called fullerenes, CNTs can be categorized into three groups: SWCNTs consist of single rolled Grp sheets having a diameter in the range of 0.4–2.5 nm and length of a few millimeters, double-walled CNTs (DWCNTs) comprised of two Grp sheets and multi-walled CNTs (MWCNTs) includes multiple sheets of single-walled CNTs (SWCNTs) organized in a coaxial manner, having a diameter of 2–100 nm and length of 1–100 μm or more (Hanein and Bareket-Keren 2013). Sometimes, SWCNTs exhibit metallic and semiconducting behavior with MWCNTs which are metallic (Haniu et al. 2012). These have specific structural properties, mechanically and electrically stable and chemically inert. These CNTs have some properties like high tensile strength, high thermal conductivity, better electrocatalytic activity, high surface-to-volume ratio, high surface area, electrochemically stability in aqueous and non-aqueous solutions, and high thermal conductivity. The specific sensitivity of CNTs to the surface makes them the preferable material for synthesizing the highly sensitive nanobiosensors with utmost output and functionality (Wang 2005; Yun et al. 2007). The key benefits of using these CNTs in nanobiosensors are enhanced enzyme loading and better electrical communication. But, the main disadvantage of these CNTs is their hydrophobic nature and strong intermolecular p-p interaction. To overcome these limitations and to enhance their stability and solubility, some modifications have been done by functionalizing them through chemical adsorption (Bianco et al. 2005). CNTs are employed as the working electrode in biosensors due to their extraordinary detection limit. But, the major concern in the modification of CNTs is their ability to inscribe several threats to biocompatibility and toxicology (Erdely et al. 2013). Though the nanobiosensors based on CNTs have been widely used due to having their most specific and sensitive performance. They also have various practical aspects in application such as Enzymatic/CNT paste electrodes, CNTs paste electrodes with immobilized enzymes, Bioaffinity/CNT DNA, and also useful in various methods of detection and recognition of biological species and environmental contaminants.

3.2.3 NWs-Based Sensors (NWBSs)

According to Touhami, NWs are 1D fibril-like nanostructures having a 1–10 nm diameter and an appreciable length (Touhami 2014). Hence, NWBSs is the biosensing devices synthesized from NWs or nanoscopic fibers covered by various macromolecules (bioNWs) which include DNA molecules and fibrin proteins (Patolsky et al. 2006a). The sensors that contain NWs are preferably used for electron carriers and transport, optical excitation, and also used to find the chemical and biological species such as viruses, proteins, and drug molecules by utilizing ultrasensitive and direct electrical methods (Patolsky et al. 2006b). NWs are assumed as the most important nanomaterials for various sensing applications because of their electrical controllability for precise measurement and chemical-friendly surface. NWs can be made of semiconducting materials, and the dopant form can be easily regulated (Cui et al. 2001). NWs' surfaces can be altered to make them more sensitive toward biological and chemical species (Seker et al. 2000). Generally, the synthesis of NWs includes two types of growth processes which include solution phase and vapor phase growth processes. The preferred synthesis process is the vapor phase growth process because of the surface contamination of nanostructure in the solution phase growth process. The thin nature of NWs is still a major factor in the development of remote nanobiosensors for environmental research and in-body sensing. Because of their unique properties and as well potential to be turned into high-density nanoscale instruments, NWs, nanobelts, and other nanomaterials are ideal for biosensing research. NWBSs possess a unique set of properties, including semi-conductivity, metallicity, biocompatibility, insulation, and a high surface-to-volume ratio, all of which help the materials perform better. In addition, the attached material aids in the identification of biomolecules using NWs. Zhang et al. reported a silicon nanowire-based biosensor for the analysis of chemical and biological studies in which silicon NWs are doped with boron (Zhang et al. 2004). A Myriad of advantages is reported for utilizing nanobiosensors which include simple preparation method leads to large-scale production, high stability, and direct real-time and label-free electrical signal transduction even at room temperature. The large S/V ratio is very important for studying the fast reaction kinetics (Patolsky et al. 2006a).

Despite having so many advantages, several challenges are faced for their use in the development of sensors. Various related studies have reported that the incorporation of NWs into the sensors does not lead to the overall improvement of electrical conductivity. To improve the sensing behavior of NWBSs, Wang (2003) signified some important features and structural aspects of NWs and nanobelts that can improve their rationale behavior in sensing properties (Wang 2003).

3.2.4 QDs-Based Sensors (QDNSs)

QDs are fluorescent inorganic semiconductors that are made up of 9–51 atoms having a diameter between 3 and 9 nm (Cai and Chen 2007; Srivastava et al. 2015). QDs are colloidal particles and synthesized from the atom of groups II–VI, III–V, or IV–VI of the periodic table. 1–5 QDs exhibit some unique electronic and optical properties which makes them the most effective and promising material in the field of biological, physical, and chemical sensing. Some groups in 1998, reported the usage of QDs in synthesizing the biosensors for the detection of different types of biological species and molecules (Bruchez et al. 1998; Chan and Nie 1998). One of the great advantages of utilizing QDs for biosensing purposes is their size-tunable optical characteristics which include emission spectrum (Han et al. 2001). In complex sample analysis, the brightness of QDs was improved by 20 folds, and stability against photo-bleaching is enhanced by 100 folds when compared with organic dyes (Chan and Nie 1998).

Furthermore, the broad absorption and narrow emission exhibited by QDs make it possible to excite multiple QDs using a single wavelength (Medintz et al. 2005b). Hence, keeping all these characteristics in mind, modification of QDs surface can be done very easily using various biosensing elements like peptides, Abs, and DNAs for the synthesis of QD-labeled probes (Biju 2014). Along with optical properties, QDs also possess photoelectrochemical characteristics. When immobilization of QDs on a conductive has been done, light excites the QDs which further leads to the electron transfer reaction between electrode and QDs to produce an electric current. Considering all these distinct properties of QDs, QDs have been used as the chemiluminescent emitters and fluorescent labels for the development of various types of bioluminescent, fluorescent, and chemiluminescent biosensors (Algar et al. 2014). The electrochemical applicability of QDs-based biosensors has been increasing very rapidly due to their great selectivity and sensitivity. Unfortunately, QDs are toxic in presence of heavy metals which are harmful to human health. Therefore, it is of great importance to perform toxicity and various other tests before human application (Algar et al. 2014).

3.3 Nanobiosensors for Detection of Environmental Pathogens

3.3.1 Viruses

Airborne contaminants are not only having a lethal effect on living organisms but are diminishing air quality in diverse ways as well. In humans, several viral contaminants including rubella, rhinovirus, varicella virus, severe acute respiratory syndrome (SARS) coronavirus, Avian influenza, and influenza virus A (H1N1), etc. are meticulously infectious viruses transmit via atmospheric contact (Morawska and Cao 2020). The potential of air to spread airborne pathogens/illness in humans, animals, and birds via dust, fine mist, aerosols, and/or liquid has led to the immense need to decontaminate air via effective techniques. Influenza is the most common and easily transmittable virus which spread through aerosols produced by coughing, talking, and sneezing. It is very difficult to control due to its short incubation time of 2–3 days, delayed symptoms (immediate after 12 h of infection), and genetic mutations (Cao et al. 2011). Among all, the H1N1 strain (influenza A) is one of the deadliest strains till now known for the death of approximately 60 million patients during the 2009 influenza outbreak (Patient 2021). Another widely spread virus is avian influenza which has imposed health issues primarily in livestock and poultry products and later transmits to humans. It mutates so quickly and causes concern among healthcare industries and affects nation economics worldwide. The avian virus has various subtypes such as H5N1, H5N2, H3N2, and H7N9 among which H5N1 is the highly pathogenic strain (Morens et al. 2012). Rubella is another lethal virus that has an incubation period of two weeks and is transmitted through droplet secretions. Rubella causes serious health sickness which mainly affects developing nations (Fronczek and Yoon 2015). In addition, SARS a member of the coronavirus is another deadly virus that has affected dozens of countries. During the 2003 outbreak this virus affected more than 8000 people and 774 deaths were reported (Anderson et al. 2004). In the year 2020, the new strain SARS-CoV-2/COVID-19 has posed a great threat and economic loss at the global level (Tian et al. 2020). Till now, more than 100 million cases and 3.2 million deaths have been reported worldwide (data extracted from WHO on 11/05/2021). The major symptoms of SARS appear after 10 days of incubation period which include coughing, high fever, chills, shivering, and difficulty in breathing, etc. the other waterborne viruses include norovirus, rotavirus, and hepatitis virus which cause deadly human diseases (Caygill et al. 2010). The potential use of various nanomaterials such as CNTs, Grp, AuNPs, and AgNPs, and QDs have been exploited to develop detection platforms such as optical, piezoelectric, and electrochemical biosensors. So far optical and electrochemical biosensors are used for the most part due to their simple operational procedure, suitability and sensitivity. Various biosensors based upon the type of signals and bioactive compound used is enlisted in the Table 3.1.

Infection in food crops and plants directly affects the food production and supplies throughout the universe. Therefore, the detection of plant infection is important to maintain the productivity level. In this concern easy and quick methods enlisted in Table 3.2 were developed to detect plant infections by using nanomaterial-based biosensors.

3.3.2 Fungus

Fungi are a common allergen and can be found in any environment on a wide range of substrates. Fungal spores play the dual role of being beneficial and hazardous. The report suggested that fungi secrete secondary metabolites in an environment that contaminates food and becomes a causative agent for the death of people around the world (Pitt and Hocking 2006). Among humans, dermatophyte infections of the skin, nails, and hairs are the most common diseases and affect nearly 20% of the worldly population. Moreover, around 100 million women are prone to vaginal fungal infections and 3 million adult populations are sensitive to fungal spores to develop lung-associated diseases. It is suggested that the burden of superficial infections and morbidity rate between 1 and 1.5 million deaths/annum is caused by *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp., and *Pneumocystis* spp. Generally, immunocompromised people are more vulnerable to infection with life-threatening fungal infections. Hence, due to personal and public health concerns, food safety monitoring of the fungal spores is a foremost task (Von Eiff et al. 1995; Morrell et al. 2005). *Aspergillus niger* is an indoor allergic fungal species that affect children and the elder especially. Causative agent leads to invasive pulmonary aspergillosis, asthma, and ear and nose infections. It is also responsible for various plant diseases

Table 3.1 Nanobiosensors for the detection of airborne and waterborne viruses

Technique/principle	Nanomaterial/activity	Target	Assay time	References
Calorimetric	Peroxidase activity of Ab/Au-CNTs	H3N2	< 10 min	Ahmed et al. (2016)
	Peroxidase activity of Ab/Grp-AuNPs	Norovirus-like particles (NoV-LPs)		Ahmed et al. (2017)
	Peroxidase activity of Ab/AuNPs-Gro oxide (GO) (Hg^{2+} as a stimulant for NPs)	RSV	20 min	Zhan et al. (2014)
	RNA aptamer (Apt)-tagged AuNPs (detection based upon the agglomeration of NPs in the presence of an analyte)	H9N2	1.5 h	Zhou et al. (2014)
Plasmon-assisted fluoro-immunoassay (PAFI) Plasmon-assisted fluoro-immunoassay	Au-CNTs	H3N2	1 h	Lee et al. (2015)
Plasmon resonance (PR)	Thiol-modified antisense oligonucleotides (ASO)-capped AuNPs (detection based upon the agglomeration of NPs in the presence of an analyte)	SARS-CoV-2	10 min	Moitra et al. (2020)
Luminescence resonance energy transfer (LRET)	Energy transfer between polyethyleneimine (PEI) modified BaGdF5: Yb/Er up-conversion NPs (UCNPs) and AuNPs	H7N2	2 h	Ye et al. (2014)

(continued)

Table 3.1 (continued)

Technique/principle	Nanomaterial/activity	Target	Assay time	References
Fluorescence	Polyclonal Abs/AgNPs (detection is done by using a sandwich immunoassay-based autocatalytic activity of NPs)	H1N1	2 h	Li et al. (2014)
Surface-enhanced Raman-spectroscopy (SERS)	Peroxidase activity of AgNPs (detection is based upon an agglomeration of NPs)	Respiratory syncytial virus (RSV)	–	Zhan et al. (2016)
Metal-enhanced fluorescence (MEF)	Apt-conjugated Ag@SiO_2NPs	Recombinant hemagglutinin (rHA) protein of H5N1	30 min	Pang et al. (2015)
Surface plasmon resonance (SPR)	AuNP-alloyed quaternary l-cysteine capped CdSeTeS QDs	H1N1, H3N2, and NoV-LPs	15–20 min	Takemura et al. (2017)
SPR-based fluorescence enhancement	CdSe-ZnS-based QDs	NoV-LPs	–	Ashiba et al. (2017)
Fluorescence	Target responsive hydrogel-based QDs	H5N1	30 min	Xu et al. (2016)
Mach–Zehnder optical waveguide system	Ab immobilized on Sol–gel glass	H1N1/HA1	15 min	Sakamoto et al. (2016)
Evanescent-field fiber optic (EFFO)	Evanescent coupling interaction between bacteria and optic fiber results in optical attenuation	Methicillin-resistant *Staphylococcus aureus* (MRSA) and Streptococcus pneumonia	6 h and 13 h respectively	Ferreira et al. (1999)
Label-free based on reflectance measurement	Abs/3D SiO_2-based inverse opal nanostructures	H1N1	–	Lee et al. (2018b)
Lateral flow immunoassay (LFIA)	Abs/lanthanide-doped polystyrene (LNPs) Abs/AuNPs	Anti-SARV-CoV-2 IgG Anti-SARV-CoV-2 IgM and Anti-SARV-CoV-2 IgG/IgM	10 min 15 min	Chen et al. (2020b), Huang et al. (2020), Li et al. (2020)

(continued)

Table 3.1 (continued)

Technique/principle	Nanomaterial/activity	Target	Assay time	References
	Selenium NPs-based LFIA strips	SARS-CoV-2	–	Wang et al. (2020)
Chemiresistive biosensing electrodes	DNA probe/CNTs	H5N1 DNA sequences	15 min	Fu et al., (2017)
Label-free conductometric sensor	DNA probe/MWCNTs	Influenza virus type A	4 min	Tam et al. (2009)
Differential-based voltammetry (DPV) based detection	Apt/MWCNTs/polypyrrole NWs/Grp nanoplatelets	H5N1	–	Liu et al. (2011)
	Paper/stencil electrodes modified with silica NPs, SWCNTs, and chitosan	H1N1	–	Devarakonda et al. (2017)
	Methylene blue (MB)-electro-adsorbed GO nanostructures Chitosan and protein A is used for sensor fabrication dual immunosensor was used	H5N1, and H1N1	< 1 min	Veerapandian et al. (2016)
	Apt/AuNPs-based carbon electrodes	H5N1	–	Diba et al. (2015)
Linear sweep voltammetry (LSV) based detection	polyclonal Abs (pAbs)-AuNPs/Grp nanocomposites sandwich assay format was used	Avian H7	–	Huang et al. (2016, p. 7)
	Au/magnetic NPs (MNPs)-CNTs conjugated with DNA probes	H1N1 and NoV	–	Lee et al. (2018a)

(continued)

Table 3.1 (continued)

Technique/principle	Nanomaterial/activity	Target	Assay time	References
	Alkaline phosphatase (ALP)-fluorescence magnetic nanosphere (FMNs)-Abs/Ag/b-AuNPs Digital enzyme-linked immunoassay-based detection	H7N9	–	Wu et al. (2018)
	Oligonucleotide-modified Au electrodes based on square wave voltammetry	cDNA of H5N1	–	Grabowska et al. (2013, p. 1)
Chronoamperometry	Reduced GO coupled with a microfluidic platform Label-free based detection	H1N1	–	Singh et al. (2017)
	DNA tetrahedral probes coupled with a microfluidic platform	H7N9	–	Dong et al. (2015)
Impedance based method	MWCNT-cobalt phthalocyanine-polyamidoamine nanocomposite	H5N1	–	Zhu et al. (2009)
	AgNPs on a polycarbonate substrate	Der p2	–	Shen et al. (2017)
	Abs-Screen-printed GO textile-based material	H1N1	–	Kinnamon et al. (2018)
	On-chip integrated rolled-up nanomembrane DNA-based electrode using electrochemical impedance spectroscopy (EIS)	H1N1	–	Medina-Sánchez et al. (2016)

(continued)

Table 3.1 (continued)

Technique/principle	Nanomaterial/activity	Target	Assay time	References
Field effective transistor (FET)	CNT-based FETs	Influenza A DNA	–	Tran et al. (2017)
	SWCNTs	H5N1	–	Van Thu et al. (2013)
	silicon NWs (Si-NWs)-FET	H1N1	30 min	Karnaushenko et al. (2015)
	Abs-Grp-based FET immunosensors	SARS-CoV-2	–	Seo et al. (2020)

Table 3.2 Nanobiosensors enlisted for the detection of the plant-based pathogen

Technique	Nanomaterial/bioreceptor/activity	Target	References
Calorimetric	DNA Probe conjugated AuNPs based on localized SPR (LSPR)	Tomato yellow leaf curl virus (TYLCV)	Obeid et al. (2003)
	Restriction enzyme *Acinetobacter calcoaceticus* II (AccII), terminal deoxynucleotidyl transferase (TdT), Mg^{2+} dependent DNAzyme, hemic/G-quadruplex DNAzyme	Cucumber green mottle mosaic virus (CGMMV)	Wang et al. (2019b)
Sandwich lateral flow immune chromatographic assay (ICA)	Abs-AuNPs	Grapevine leaf roll-associated virus 3 (GLRAV-3)	Byzova et al. (2018)
	Abs-AuNPs	Polyvalent antigen PVY	Razo et al. (2018)
SPR	Monoclonal Abs (mAbs)-QDs	Potato virus Y (PVY)	Hong and Lee (2018)
	Abs-QDs	Maize chlorotic mottle virus (MCMV)	Zeng et al. (2013)
	RNA oligonucleotides-QDs	Barley stripe mosaic virus (BSMV)	Florschütz et al. (2013)
	DNA Apt-QDs	Apple stem pitting virus (ASPV)	Lautner et al. (2010)
Fluorescence resonance energy transfer (FRET)	Surface immobilized CPMV CdSe-ZnS core	Cowpea mosaic virus (CPMV)	Medintz et al. (2005a)
	AuNPs-CTV-CP/Ads-CTV-CP Abs, AuNPs/QD	CTV	Shojaei et al. (2016)
Differential-pulse voltammetric measurement	T4DNA polymerase, Mg^{2+} dependent DNAzyme, hemic/G-quadruplex DNAzyme	Watermelon mosaic virus (WMV)	Wang et al. (2019a)
	Citrus Tristeza virus-coat protein (CTV-CP) Abs-CdTe	CTV	Hong and Lee (2018)
Fiber optic particle PR immunosensor	Abs-Au NRs	Cymbidium mosaic virus (CMV)	Lin et al. (2014)

leading to economic deprivation. Researchers are continuously updating monitoring techniques or implementing the use of nanobiosensors to progress in pathogen detection techniques. For example, the detection of *Candida albicans* was done by using pAbs conjugated on the Au electrode by using membrane-based electrochemical impedance spectroscopy. In this method, the change of charge transfer resistance of the Au electrodes was measured when the antibody binds to the target sample (Kwasny et al. 2018). Other nanobiosensors are also reported for detection and are summarized in Table 3.3.

Table 3.3 Nanobiosensors enlisted for the detection of fungal species, toxins, and spores

Technique	Nanomaterial/bioreceptor/activity	Target	References
Fluorescence microscopy	Anti-Candida Abs fabricated microfluidic device	*Candida albicans*	Asghar et al. (2019)
UV–visible spectroscopy	Oligonucleotide conjugated AuNPs	*Paracoccidioides brasiliensis*	Martins et al. (2012)
Calorimetric	Peptide ligand immobilized AuNPs	*Aspergillus niger*	Lee et al. (2021b)
Fluorescence microscopy	Fluorescein-labeled peptide nucleic acid (PNA) probes	*Candida albicans*	Rigby et al. (2002)
SERS	Probe DNAs conjugated Au-NWs	*Aspergillus fumigatus, Candida glabrata, Candida krusei, and Cryptococcus neoformans*	Yoo et al. (2011)
Ultraviolet resonance Raman-spectroscopy (UVRS)	AuNPs	*Candida* spp.	Naja et al. (2008)
Enhance-SPR (ESPR)	Molecularly imprinted polymers (MIP)-AuNPs	AFB1	Akgönüllü et al. (2020)
Magnetic resonance	DNA oligonucleotides conjugated carboxylated superparamagnetic iron oxide NPs (CSPIO-NPs)	*Candida* spp.	Neely et al. (2013)
Electrochemical impedance spectroscopy (EIS)	1,6-Hexanedithiol and Chitosan-stabilized AuNPs	*Aspergillus fumigatus*	Bhatnagar et al. (2018)
Field effect transistor (FET)	SWCNTs	*Candida albicans*	Villamizar et al. (2009)
Micro gap immunosensor	Abs conjugated interdigitated electrodes	*Aspergillus niger*	Lee et al. (2021a)
EIS	Apt fabricated GO electrode	Mycotoxin ochratoxin A (OTA)	Bulbul et al. (2015)
DPV	Screen-printed carbon paste electrodes (SPCE)-GO nanosheets-thionine-Apt	Mycotoxin OTA	Sun et al. (2017)
EIS-SERS	Au coated glass-AuNPs-Apt	Mycotoxin aflatoxin B1 (AFB1)	Li et al. (2017a)
Square wave voltammetry (SWV)	Pt microelectrode–ferrous oxide NPs-polyaniline (Fe_3O_4 NPs-PANi) Apt	Mycotoxin AFM1	Nguyen et al. (2013)
EC	*Escherichia coli* fabricated on glassy carbon electrode (GCE)	Mycotoxin AFB1 and ZEN	Chen et al. (2020a)
Paper-based colorimetric probe	Peptide-labeled MNPs fabricated Au-film	*Stachybotrys chartarum*	Suaifan and Zourob (2019)

Research is also gaining momentum in crop systems to reinforce pathogen detection approaches. Nowadays, people understand the importance of using biosensors to diagnose various plant fungal diseases majorly caused by Ascomycetes and Basidiomycetes. In such cases, the traditional use of chemical fungicides has been found effective but harms the biodiversity of native species. Such an unbalanced ecosystem undoubtedly leads to miserable health conditions, environmental risks, plant diseases, and crop loss. The use of biosensors is eco-friendly and can be easily upgraded by using nanomaterial to increase the limit of detection and overall performance. Nanobiosensors are way better to improve plant diseases and crop management. NPs are excellent markers in biosensors and can provide early detection of the pathogen. For example, AuNPs conjugated with anti-teliospore Abs-based optical immunosensor have been developed to detect *Tilletia indica* in wheat by using the SPR technique (Singh et al. 2010). A few more nanobiosensors by using different NPs have been developed for plant pathogen detection and are discussed in Table 3.4.

3.3.3 Bacteria

Detection of soil health and pollutants using biosensor is based upon the calculation of relative activity of microbes viz their different rate of oxygen utilization during respiration. NPs undoubtedly, boost up the biosensor's working efficacy leads to an accomplished biosensor with compact size and layout. Soil particles interact with bionanomaterials and are accurately interpreted for the presence of nutrients, moistures, abiotic, and biotic factors. Metal-based NPs are widely used because of their high electro-activity and conductivity. For instance, a screen-printed carbon electrode (SPCE) fabricated with AuNPs has been developed to detect plant volatiles or metabolites, specifically released during infection. The reaction mechanism includes the hydrolysis of methyl salicylate (plant volatile of soybean extract) and the oxidation of negative species. This electrochemical sensing is recorded by cyclic voltammetry and differential-pulse voltammetry (Umasankar and Ramasamy 2013). Other nanobiosensors include AuNPs conjugated with single-stranded oligonucleotides to detect potato bacterial wilt caused by *Ralstonia solanacearum* based on calorimetric detection (Khaledian et al. 2017). In the past decade, a lot of nanobiosensors have been developed for the detection of soil-borne pathogens and a few of them are further discussed in Table 3.5.

Mostly, bacteria have mutual symbiotic interaction with plants but few of them do have a pathogenic effect on them. These bacterial species belonging to the tropical or sub-tropical region and includes Agrobacterium, Pseudomonas, Erwinia, Xanthomonas, and Proteobacteria, etc. which lead to galls, wilting, blights, leaf spots, soft roots, and scabs, etc. Among various diseases, potato brown rot caused by *Ralstonia solanaceaeum* was examined by using Abs linked AuNPs fabricated lateral flow immune assay (Razo et al. 2019). In another work, a dual-function Platinum (Pt) disc microelectrode sensor was developed for the electrochemical detection of

Table 3.4 Nanobiosensors enlisted for the detection of plant-based fungal pathogens

Technique	Nanomaterial/bioreceptor/activity	Target	References
Electric resistance (ER)	Copper oxide (CuO)-NPs and nanolayers	*Aspergillus niger*	Etefagh et al. (2013)
Cyclic voltammetry (CV) and DPV	Titanium dioxide (TiO_2) or Tin dioxide (SnO_2) modified screen-printed carbon (SPC)	*Phytophthora cactorum*	Fang et al. (2014)
DPV	DNA probe conjugated AuE fabricated with Ionic liquid, ZnONPs, and chitosan nanocomposite	*Trichoderma haarzianum*	Siddiquee et al. (2014)
SPR immunosensor	Polyclonal Anti-HF1 immobilized Au coated chips	*Pseudocercospora fijiensis*	Luna-Moreno et al. (2019)
	Microarray-based detection	*Rhizocotonia solani, Spongospora subterranean, Snchytrium endobioticum, Alternaria solani, Collectorichum coccodes, Fusarium* spp.	Nikitin et al. (2018)
Lateral flow assay (LFA)	ssDNA probe conjugated AuNPs	*Phytophthora infestans*	Zhan et al. (2018)
Paper-based immunosensor	pAbs labeled fluorescent NPs	*Phakopsora pachyrhizi*	Miranda et al. (2013)
LFA	MAbs fabricated on Lateral flow device	*Peronospora destructor*	Kennedy and Wakeham (2008)
	Microfluidic microarray containing DNA probes labeled with fluorescent tags	*Botrytis cinerea, Didymella bryoniae, and Botrytis squamosa*	Wang and Li (2007)

H_2O_2 during endophytic bacterial infection caused by *Enterobacter cloacae* (Lima et al. 2018). This study has demonstrated that plant produces a variety of secondary metabolites as an endogenous signaling factor and use them as a preventive measure against pathogenic infections. The given study has opened up various gateways to target these signaling molecules to design potent biosensors for the detection of plant infections.

3.4 Nanobiosensors for the Detection of Heavy Metals

Heavy metals such as arsenic, cadmium, and lead are considered serious environmental pollutants. Being toxic and carcinogenic, heavy metal contamination is a

Table 3.5 Nanobiosensors used for the detection of soil and waterborne bacterial pathogens

Technique	Nanomaterial/bioreceptor/activity	Target	References
EC	CNTs	Salmonella	Jain et al. (2012)
Optical NC probe	MNPs and TiO_2 Nanocrystals (NCs)		HuiáShin and JoonáCha (2012)
Piezoelectric (PE)	Oligonucleotide conjugated AuNPs	*Escherichia coli* O157:H7	Purwar and Srivastava (2021)
EC immunosensor	Liposomal and poly (3,4-ethylene dioxythiophene)-fabricated CNTs	Cholera toxin	Viswanathan et al. (2006)
GCE	Fe_3O_4 NPs	*Campylobacter jejuni*	Huang et al. (2010)
Optical SPR	Au substrate with streptavidin-conjugated QDs	*Legionella pneumophila*	Foudeh et al. (2015)
Voltametric immunosensor	Abs/ZnO NRs fabricated Au electrodes	*Legionella pneumophila*	Park et al. (2014)
EC amperometric biosensor	Abs linked polydopamine (pDA) modified MNPs fabricated SPCE	*Legionella pneumophila*	Martín et al. (2015)
EC DPV	MWCNTs electrodes functionalized with oxygen plasma	*Legionella pneumophila*	Park et al. (2010)
Electrochemiluminescence (ECL) biosensor	pDA printed surface imprinted polymer (SIP) and pAbs-nitrogen-doped Grp QDs (N-GQDs)	*Escherichia coli* O157:H7	Chen et al. (2017)
EC amperometric biosensor	SWCNTs fabricated Au electrodes	*Bacillus subtilis*	Yoo et al. (2017)
EC immunosensor	Abs-molybdenum disulfide (MoS_2)/Au nanocomposite fabricated Au electrode	Microcystin-LR	Zhang et al. (2017b)
Fluorescence immunosensor	Carboxylic acid-modified magnetic beads (MBs) and MAbs-cadmium telluride (CdTe) QDs	Okadaic acid (OA)	Pan et al. (2017)
Fluorescence microscopy	Abs-pyoverdine-polydimethylsiloxane (PDMS) stamps functionalized Au plated glass chips	*Pseudomonas aeruginosa*	Doorneweerd et al. (2010)

serious global concern. For example, cadmium is a hazardous pollutant for the environment as well as humans as it can cause cancer, neurological problems, and kidney damage. (Tchounwou et al. 2012; Engwa et al. 2019). It demands the development of effective detection techniques for the determination of heavy metal levels. Owing to biocompatibility, strong adsorption, and electron transfer kinetics the nanomaterials are considered as most promising biosensing method (Table 3.6). The nanospheres, AuNPs, AgNPs, and GO (graphene oxide) are some of the commonly used nanomaterials for heavy metal detection (Kuswandi 2019; Aguilar-Pérez et al. 2020). Cantilever nanobiosensor is major proven excellent approach for biological as well as chemical detection of heavy metals. They are miniature structures produced in bulk as an economical substitute for the conventionally known expensive detection system. Rigo et al., in 2020 reported the cantilever nanobiosensor conjugated with alkaline phosphates for the detection of various heavy metals. The designed nanobiosensor is highly sensitive, stable, and has an ultralow detection limit of ppb range (Rigo et al. 2020a).. The electrochemical and modified electrode-based nanobiosensors have also gained much attention for heavy metal detection due to their selectivity and specificity. Yang et al., in 2015 reported the electrochemical nanobiosensor fabricated by polyaniline nanocomposite and three-dimensional GO specifically for the detection of mercury ions. The results showed that the sensor is highly selective and sensitive for mercury ions (Yang et al. 2015).

Table 3.6 Nanobiosensors for the detection of heavy metal

Analyte	Nanomaterial used	Biomaterial used	Detection limit	References
Copper	AgNPs	Dopamine	Microlevel	Ma et al. (2011)
Mercury	AuNPs	Grp nanocomposite	0.5–19 nM	Dong et al. (2014)
Copper, lead, palladium, and platinum	AuNPs	Peptides	Microlevel	Slocik et al. (2005)
Lead	QDs	GO	0.6 nM	Qian et al. (2015)
Chromium	AgNPs	*Xanthoceras sorbifola*	3 μM	Ha et al. (2014)
Mercury	AgNPs	Poly Glutamic Acid	1.9 nM	Guan et al. (2014)
Mercury	NFs	Single-stranded DNA	0.6 fM	Tang et al. (2017)
Lead and cadmium	Ferro ferric NPs	Polymers	0.03 nmol L^{-1}	Kong et al. (2018)
Mercury	AgNPs	*Baccaures ramiflora*	Microlevel	Alam et al. (2015)
Lead, cobalt, zinc, nickel, aluminum, and Cadmium	Nanostructured films	Urease enzyme	ppb range	Rigo et al. (2020a)
Lead	Nanostructured films	Urease and alkaline phosphatases enzyme	ppt range	Rigo et al. (2020b)
Cadmium	QDs	ds DNA	2–10 nm	Sreekanth et al. (2021)
Cadmium	Tin oxide NPs	Alkaline phosphatases enzyme	0.005–1 μg L^{-1}	Swain and Bhand (2021)

3.5 Nanobiosensor for Detection of Soil and Air Contaminants

3.5.1 Soil Contaminants

Nanobiosensors are an exciting and emerging technology that has gained significant attention in recent years for multiple purposes. These sensors have been widely used in the determination of environmental pollutants due to the consequent increase in demand for food and raw materials with the increase in population. Pollutants present in the soil, water, air, and even food can be detected using this technology and have proven to be more efficient with a selective technique like when different contaminants are detected with the help of the correct choice of biomolecules which is used as a sensing layer. Interest in these biosensors increased from their potential for detection of in-field contaminations without using any expensive lab equipment (Koedrith et al. 2015).

Contamination of soil by pathogenic fungi, bacteria, and viruses is common but additional inputs like the use of agrochemicals uncritically for enhancing the nutritional value also increase the toxicity with the remaining residues in the environment. Toxic substances like pesticides, herbicides, and organophosphates compounds are used for growing crops which leads to the contamination of soil to a great extent. The use of pesticides is very hazardous which leads to the decline in the quality of the soil directly affecting the biodiversity, nutritional cycles and also indirectly affecting the quality of air and water bodies. The organophosphorus used in the pesticides pose great concern for environmental pollution. Nanobiosensors have been developed as analytical strategies for the detection of these organophosphorus in the soil and air contamination (Justino et al. 2017).

Detection of pathogenic microorganisms can be easily identified using DNA-based nanosensors. Apt formed by the short sequences of ssDNA or RNA can also be incorporated in nanosensors (Kumar and Guleria 2020). Detection of another group of nitrogenous heterocyclic compounds named atrazine which is used extensively in herbicides is limited. This compound can be detected by an AuNPs-based biosensor that uses plasmonic nanocomposites produced in situ using Au salt in the presence of bacterial cellulose (Wei and Vikesland 2015).

Detection of chemically active toxins in the soil samples can also be detected using different chromatographic techniques. Chromatographic techniques have few limitations like require highly skilled and trained operators, lengthy and complex, and requiring high-cost materials. So, to overcome the limitations of the above technique development of simple methods with low cost, more sensitivity became important. Therefore, the use of nanosensors became more popular and an alternative for classical methods. For example, an aptasensor produced by using Au nanomaterial can effectively detect the presence of brevetoxin-2 (Eissa et al. 2015). Furthermore, different plants extract is also used to make nanobiosensors. Studies have also reported using nanostructures such as macro-microporous ZIF-8 nanostructures for carriers of nano-lipase that can efficiently detect the presence of nitrogenous diphenyl ether pesticides (nitrofen) (Cheng et al. 2020). Mirabi-Semnakolaii et al., 2011 developed an electrochemical sensor of carbon electrode modified with copper NWs for the detection of trifluralin herbicide (Mirabi-Semnakolaii et al. 2011). A liposomal nanobiosensor used for the detection of two extensively used organophosphorus pesticides: dichlorvos and paraoxon was developed by Vambakaki and Chaniotakis in 2007 (Vamvakaki and Chaniotakis 2007). The below mentioned (Table 3.7) describes a few of these important nanobiosensors that are used for the detection of soil contaminants.

The invention of sensors based on cantilevers in combination with Atomic Force Microscopy (AFM) has opened many possible ways for the detection of contaminants. It is a versatile technique with a wide range of applications in pesticide detection as it is highly sensitive because of its small size and very specific to the sensing layer (biomolecule) (Muenchen et al. 2016).

3.5.2 *Air Contaminants*

Air pollution is one of the leading risk factors worldwide for various global diseases and death rates. Therefore, it is very necessary to control the increasing pollutants coming out from various sources directly into the environment endangering the natural ecosystem. The most common sources of air pollutants are gases produced by human activity such as carbon monoxide, oxides of carbon, etc. Nanoparticle-based technology has proved to help detect and monitor the quality of air. In large cities presence of nitrogen dioxide (NO_2) in higher amounts majorly causes air pollution. Different types of biosensors have been used for the detection of NO_2 present in air samples. Further, they have effectively employed to detect contamination arising from nitrite ions and other organic waste products produced from chemical fertilizers (Sayago et al. 2019). AuNPs-based biosensors can be used for the detection of these contaminants in a sensitive colorimetric assay (Daniel et al. 2009). Another pollutant sulfur dioxide (SO_2) is a colorless gas present in the environment as a contaminant. Acidity in rain waters and fogs are produced by the sulfur dioxide present in the atmosphere which leads to corrosion in buildings and metal objects. Hart et al. developed an amperometric biosensor for the detection of SO_2 in flowing gas streams.

Different types of immune chromatographic assays are effective and are for detecting the presence of different bacterial toxins. AuNPs-based biosensors conjugated with Abs are used for aflatoxin B1 and ochratoxin A. This assay has several advantages over other processes including simpler nature, stability, and speediness (Aguilar-Pérez et al. 2020). Detection of airborne pathogens can also be detected using biosensors that use the principle of optical sensing. For example, detection of the H3N2 virus can be done by using AuNPs conjugated with Abs specific against the virus antigens. This assay yields a rapid result and has higher sensitivity compared with the conventional techniques used for the detection of contaminants (Ahmed et al. 2016).

Table 3.7 Different types of nanobiosensors used for environmental diagnosis of contamination in soil

Pollutant	Type of biosensor	Bio element	Type of electrode	References
Paraoxon	Amperometric, voltametric, colorimetric	AChE	Au, cysteamine, NRs	Arduini et al. (2013)
Methyl parathion	Impedimetric, amperometric, optical	Hydrolase/AChE/*Sphigonomonas* sp.	Graphite, Fe_3O_4, microalgae, AuNPs	Zhao et al. (2013)
Chlorpyrifos	Impedimetric, voltametric, amperometric	Tyrosinase, Apt, AChE	Carbon black, Fe_3O_4, IrO_x	Mayorga-Martinez et al. (2014)
Dichlorvos	Impedimetric, voltametric, fluorescence	AChE, ChO^4	Acetylcholine, ionic-liquids, Au	Meng et al. (2013)
Acetamiprid	Colorimetric, impedimetric	Apt	Au, platinum, multi-walled carbon NTs	Liu et al. (2014)
Atrazine	Impedimetric, voltametric, amperometric	mAbs, Apt	Au and platinum	Oliveira et al. (2013)
Pirimicarb	Voltametric, amperometric	Laccase, AChE	Carbon paste electrode, prussian blue	Jeyapragasam and Saraswathi (2014)
Carbofuran	Voltametric, amperometric	AChE	Glassy carbon electrode, chitosan nanocomposite	Li et al. (2017b)
Carbaryl	Impedimetric, amperometric	AChE	Au electrode, porous glassy carbon electrode	Santos et al. (2015)
Hg^{2+}	Fluorescence, optical, voltammetric	Nucleic acids, DNA	Single-walled carbon NTs, optical fiber platform	Long et al. (2013)
Pb^{2+}	Fluorescence	DNAzyme, Apt	Micro spin column, carboxylated magnetic beads, Grp, AuNPs	Ravikumar et al. (2017)

(continued)

Table 3.7 (continued)

Pollutant	Type of biosensor	Bio element	Type of electrode	References
Brevetoxin-2	Impedimetric, voltametric	Apt, cardiomyocyte cells	Au electrode, microelectrode array with platinum nanoparticles	Eissa et al. (2015)
Saxitoxin	Voltammetric, interferometry	Apt, cardiomyocyte cells	Microelectrode array with platinum nanoparticles	Wang et al. (2015)
Microcystin	Impedimetric, voltametric	MAbs, Protein phosphate-1	Grp, Au electrodes, AuNRs	Zhang et al. (2017a)
Okadaic acid	Optical, electrochemical, fluorescence	MAbs	Grp, Au electrode with a carboxymethylated surface	McNamee et al. (2013)
Domoic acid	Optical, electrochemical	MAbs	Glass side chip with an Au surface, SWCNTs	Marques et al. (2017)

3.6 Conclusion and Prospects

Biosensors are useful and applicable in every field of health, engineering, biomedical engineering, environment management, etc., and, due to their compact size, they are convenient, cost-effective, quick, and sensitive response. Moreover, nanomaterial has to boost up their working potency and unique properties. Nanobiosensors are revolutionizing the era of biosensors by reducing laboratory load and complicated protocols, sensitivity and robustness of results, quick response, and portability. Nanobiosensors work on the atomic scale and can be efficiently used to detect environmental contaminants. However, few limitations such as low storage and stability of bio-components restrict their broad range of performances in the real field. The inclusion of hi-tech approaches such as nanobiosensors coupled with robotics and GPS could impart smart delivery practices to target specific areas before or during the onset of symptoms. Moreover, using more and more bioactive components to know their efficacy and to design more potent nanobiosensors. The utilization of nanobiosensors is not limited but can be applicable everywhere to reduce inputs and increase outputs. Among all, one of the important tasks is to develop nanobiosensors in extreme conditions such as acidophilic and alkaline environments, thermophilic and psychrophilic conditions, saline and organic solvents. The advances in nanotechnology to improve the efficiency of biosensors have enhanced technological outputs in the given field and can be evident from the number of patents, projects, and publications focused on nano-based biosensors.

The health concern growing globally due to the environmental toxicants including lethal microbes and pollutants, development of a detection method is critically required. Detection devices with high sensitivity, selectivity, and quick response are an immense need to screen these toxicants in correspondence with safety prescriptions at clinically significant levels. Undoubtedly this will encourage to upgrade the public health and life quality. However, a lot more research is required to integrate and enable current technologies to reach the optimum level of performance and open doors to realize on-site/in-situ/online measurements.

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Chapter 4
Utilization of Nanobiosensors for Wastewater Management

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Abstract Contamination of water is a burning issue of modern civilization, and the issue has been an ever-increasing concern in the present global situation. The accessibility of fresh water has been constantly diminishing in recent years although the necessity of water, especially in the dry and semi-dry climate, is growing. This associated with territory exhaustion causes scarcity of water and results in declining oceanic biodiversity. Monitoring contaminants in wastewater spillovers has rendered imperative, and it has been crucial to recognize regions of water contamination and take appropriate measures for remedial action. The development of material science and nanotechnology, particularly the innovation of biosensors based on nanomaterials, has paved the way to detect bioanalytes with very high sensitivity, lower detection limit, and improved selectivity replacing the conventional methods and strategies, which more often suffer from poorer sensitivity and consumption of time. The rapid expansion of nanomaterials-based biosensing devices has generated a surge of interest due to their high affectability, selectivity, dependability, simplicity, low cost, and consistent response. This chapter gives a general overview of the development of recent nanobiosensors, focusing on their use in wastewater management.

Keywords Water pollution · Wastewater runoffs · Nanobiosensor · Wastewater management

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4.1 Introduction

Scarcity of water and declining oceanic biodiversity are major concerns of the modern world. These are caused by contamination of water and degradation of natural environment and pose increasing threat to the mankind. Wastewater comprises a variety of harmful compounds that are often generated unintentionally and released through domestic, commercial, mechanical, or agricultural activities (Cornish et al. 1999; Henze et al. 2008; Ejeian et al. 2018). Wastewater is characterized by the nature of the contaminants, their physical shapes, chemical constituents, and and plenitude of microbes. The categorization of components of wastewater is crucial to develop strategies for determination and handling wastes and planning and implementation for their treatment to establish efficient means to recycle wastewater. The concentration of contaminants in wastewater varies with the course of time and depending on location. This has been key to the development of new technologies to monitor side effects based on techniques and methods relying on the utilization of cost-effective and continuous systems (Michael-Kordatou et al. 2015; Rehman et al. 2015).

Numerous markers find their uses in quantifying various attributes of recycled water Most of them depend on lab strategies, which require successive inspection and accumulation of information and pretreatment and are subsequently moderate and expensive (Chong et al. 2013; Yang et al. 2015). The need for developing novel approaches has generated interest in more delicate, versatile, and effective systems for on-site detection of toxins of many different kinds comprising range of components (Asadnia et al. 2016; Biswas et al. 2017). Recent bias has been significantly directed toward the improvement of biosensors for identification of substances detrimental to the environment. Such sensors have been extremely useful to detect trace amount of a pollutant in multifaceted frameworks, like wastewater. Biosensors are likewise scaled-down frameworks for the advancement of compact sensors for on-site detection (Ejeian et al. 2018; Tsopela et al. 2016).

There are a number of techniques widely used for biorecognition of specific chemical compounds. The techniques used, especially as part of the immune system, include: receptors: enzymatic, non-enzymatic, immunochemical, microscopically engraved polymer, entire cell, and components of DNA. Various kinds of biosensors: (a) electrochemical, (b) optical, (c) piezoelectric, and (d) thermal have been developed (Turner 2000). A rapidly developing arena of study began to develop fascinating nanomaterial-based biosensors that show striking features for identifying and quantifying chemical substances, reagents, and organic species (Li et al. 2013; Zhang et al. 2009). The effect of biosensors on the assessment of ground and stream water quality has been investigated in recent years (Biswas et al. 2017; Gumpu et al. 2015; Leonard et al. 2003; Sharma and Sharma 2014). There have been a few attempts to detect contaminants in wastewater using conventional frameworks but significant drives have also been made for continuous biosensing of sources of wastewater. A good number of works placed emphasis on the advancement of nanotechnology-based methodologies in the treatment of wastewater (Berekaa 2016; Cloete et al. 2010; Henze et al. 2008).

This chapter overviews the most recent commercialization and innovative developments in the biosensor-based monitoring of pollutants in wastewater, with an extraordinary spotlight because of nanotechnology in this field. Critical issues in assessing wastewater based on the novel approach are identified, and the prospect of biosensor-based systems for wastewater treatment has been discussed.

4.2 Nanobiosensors

Nanotechnology deals with the development and modification of substances to dimension down to nanoscale (Raghu et al. 2020; Sagadevan and Periasamy 2014). The traditional techniques comprise both quantitative as well as qualitative approaches and are often limited to widespread use due to the relative longer period of estimation of contaminants in composite frameworks. These strategies depend on the total partition of test segments followed via the recognition and quantification of the marked analytes. Nonetheless, high cost involved in the estimation, problems associated with the investigation of complex trials for low sample concentrations, and partition techniques restrict continuous assessment and impede further progress (Bhattarai and Hameed 2020; Thompson and Krull 1984).

As of now, a simple and appropriate method for fabrication of biosensors for the instantaneous recognition and assessment of naturally pertinent analytes provides significant advantages over traditional procedures (Lowe 1989). These biosensors can outperform the significant restrictions of traditional sensors like affectability, rate, and responsiveness. Biosensors of this kind normally consolidate (i) a biomolecular unit that appropriately detects the biochemical response and can be identified with ease and (ii) a transducer capable of change over the concentration of the target analytes into a quantitatively detectable signal. Biosensors of different kinds, such as, electronic, electrochemical, piezoelectric, and optical containing numerous bio-identifiable atoms like antibodies (that perceives a specific antigen) (Kim et al. 2008), aptamers (that shows an attachment for a distinctive objective, or a protein that explicitly interfaces with another natural particle) (Kim et al. 2016), deoxyribonucleic corrosive (DNA, equipped for perceiving its corresponding strand) (Li et al. 2010), enzymes (Zhao et al. 2017), and entire cells (Han et al. 2017) have been developed. These have been generally useful in medical services; involuntary strategies in the development of biosensors have enormously increased the selectivity, reproducibility, solidness, affectability, and linearity. In addition, advancement in the manufacture technology along with electronic components has guided scaling down of such devices to result in a surge in the biosensor industry. Conspicuously, the use of nanosized materials in the development of biosensors has realized nanobiosensors, and significant attention is now focused on these intriguing devices (Bhattarai and Hameed 2020).

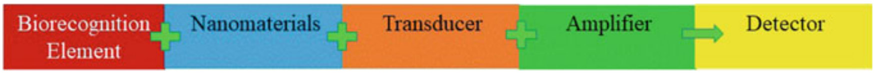

Fig. 4.1 Block diagram of nanobiosensors

Nanobiosensing is a growing due to the outstanding advancement in the areas of development of materials, innovation, designing associated with their potential clinical, ecological, food, drug, electricity, and safety applications (Mun'delanji et al. 2015). Nanobiosensor is a sensor based on nanomaterials and is capable of sensing nanoscale events and activities (Malik et al. 2013). A particular identifiable component may be assembled to wrap around the nanowire/nanotube rendering the tool explicitly sensitive to a specific purpose. The presence of a distinct functionality on the nanowire exterior provides the devices with extraordinary specificity and binding to achieve the goal (Fig. 4.1) (Raghu et al. 2020). The use of nanomaterials for biosensors has expanded the scope of the development of nanobiosensor technology. The specific properties of nanomaterials used for sensing make them a very unique since their vital component located at or close to their exterior enhances the interaction of biomolecule with the target substance. Nanomaterials may play critical role for nanobiosensors once they possess necessary physicochemical properties (Gatoo et al. 2014) including dimension, outward region, exterior characteristics, the scattering medium, and capacity to accumulate. For researchers and scientists working in the field of biosensors, metallic and inorganic nanomaterials with nanoscale sizes may present new study opportunities (Raghu et al. 2020). Nanomaterials based on metals and other inorganic materials with nanodimension may thus provide new opportunities for study and research in the area of biosensors (Raghu et al. 2020).

The unique properties of nanomaterials used in nanobiosensors have not just assisted with overcoming difficulties dependent on the specificity and recognition breaking but also improved interfacial response attributable to the improved immobilization of biorecognition atoms (Bhakta et al. 2015; Soleymani and Li, 2017). Furthermore, techniques based on nanomaterial combined with a framework in the microscale allow investigation of biomolecular species with a significant degree of specificity due to specific interaction with activities as in the case for circulating tumor cells (CTCs) (Bhattarai and Hameed 2020; Kelley et al. 2014). Nanomaterials reveal a kind of features in terms of accumulation of molecules, photoemission, magnetic, electric, and iridescent action, thermal conduction, and synergistic effect (Rai et al. 2012). These have been exploited in various areas including detection of foodborne microorganism, recognition of biomolecules, separation of samples, sanitization and transduction, and intensification of signals (Jain 2007). The nanomaterials also improve range of identification, sensitivity, deprivation, effectiveness of recovery of compounds during microbial tests (Raghu et al. 2020).

4.3 Nanomaterials for Wastewater Treatment

The nanobiosensors have nanomaterials as the key component. Nanomaterials used for this purpose may be: metal based, carbon based, dendrimers, and nanocomposites. Metal-based nanomaterials may be of different kinds including quantum dots, 1D nanoparticles, naorods, and nanowires and may also be oxides as well as unique metals. Carbon-based nanomaterials cover a range of carbon allotropes like carbon dots, graphenes, carbon nanotubes, and fullerene. Dendrimers, as the name implies, are nanosized radially symmetric polymers with distinct, monodisperse, and a homogeneous core with certain degree of symmetry and inner and outer shells; while nanocomposites have nanosized particles incorporated into a standard matrix (Table 4.1).

Gold and silver nanoparticles have extraordinary thermal, electrical, and optical properties to make them suitable for application in various nanobiosensors. Particularly, their optical properties have been exploited for development of nanobiosensors (Hiep et al. 2009). The photophysical properties of nanomaterials, especially Au nanoparticles, were assessed by Huang et al. 2011. When light is incident on nanomaterials, four phenomena occur, which are as follows: absorption (2) fluorescence, (3) Rayleigh dispersing, and (4) upgradation of the nearby electromagnetic field.

Table 4.1 Description the outline of nanomaterials used for enhancing biosensor technology

Nanomaterial used in biosensors	Significant features	References
Nanoparticles	Assistance in immobilization, permit improved stuffing of bioanalyte, and have good catalytic properties	Katz et al. (2004); Luo et al. (2006); Merkoci et al. (2005); Wang (2005)
Nanorods	Great plasmonic substances which can combine detecting mechanism and size tunable energy guideline, and initiate explicit field reactions	Kabashin et al. (2009); Ramanathan et al. (2006); Rahim, (2010)
Nanowires	Profoundly adaptable, great electrical and detecting features for bio-and substance detecting; good charge conductor	Cui et al. (2001); MacKenzie et al. (2009); Stern et al. (2007)
Carbon nanotubes	Further developed chemical stacking, capacity to be functionalized, advanced viewpoint proportions, and improved electrical correspondence	Davis et al. (2003); Sotiropoulou et al. (2003); Zhao et al. (2002)
Quantum dots	Great fluorescence, quantum control of charge transporters, and size tunable band energy	Huang et al. (2005); Wang et al. (2002); Zhu et al. (2003)

Spectroscopic signals from the particles at the exterior thus may expand and experience surface-enhanced Raman scattering. Metal nanoparticles, due to their interaction with light, exhibit significant enhancement in such properties (Huang et al. 2007). In addition, metal nanoparticles have certain unique electronic properties.

High conductivity associated with synergistic effect has made Au and Ag nanoparticles suitable for constructing “digital wires” to facilitate electron transfer between redox cores in proteins and exterior of the electrode. They at the same time serve as catalysts for enhancing electrochemical responses (Kerman and Kraatz, 2009; Lan et al. 2011; Selvaraju et al. 2008). Au and Ag NPs have been successfully used for fabrication of chips based on localized surface plasmon resonance for biorecognition (Endo et al. 2006; Haes et al. 2004; Vestergaard et al. 2008). They display explicit variations in their absorptivity in the visible range of electromagnetic radiation due to the different extent of interactions with species like proteins or nucleic acids. Au and Ag NPs, for their specific electronic properties, have thus been used as markers for protein along with recognition of other target species (Ambrosi et al. 2007; Chen et al. 2007; Kerman et al. 2007). Semiconducting nanocrystalline quantum dots have intriguing optical properties for use in nanobiosensors. They are fluorescent and emit radiation of longer wavelength with different colors. The wavelength of the light may be tuned by controlling the size and dimension of the nanocrystals (Bailey and Nie 2003; Banin and Millo 2003; Gaponik et al. 2002).

The size of dendrictic nanoparticles is comparable to a typical protein. Dendrimers have a bifurcating structure with a huge exterior surface capable of binding a range of materials like therapeutic agents and dynamic particles of this kind (Buhleier and Wehner 1978). A dendrimer may comprise a particle that detects targeted malignancy cells, a therapeutic agent to destroy those cells, and an atom that perceives the signals of cell demise. Researchers aim at controlling dendrimers to deliver their substances just within the vicinity of trigger atoms related with malignancy. Following the discharge of the drug, the dendrimers may assess whether they are effectively destroying the targets. They have also been used for adjustment of different nanoparticles (Hasanzadeh et al. 2014).

Nanomaterials based on carbon are integrated from graphite that possibly maximizes utilization of natural resources. They exhibit superior mechanical strength, thermal, and electrical conductivity in addition to desirable optical properties. Consequently, they have been widely used for different applications including nanobiosensing (Cha et al. 2013). Carbon nanotubes have, so far, been the most extensively used carbon-based nanomaterials. They were first reported by Iijima (1991) and have since then been attempted for a wide range of applications. Graphene has been integrated from graphite (Novoselov et al. 2004). Graphene oxide (GO) derived from oxidation of graphene has emerged as an even more fascinating carbon nanomaterial. GO is easily dispersed in the fluid environment and has hydrophilic functional groups that enable essential covalent functionalization of GO with biomolecules to develop contracted nanobiosensors (Cha et al. 2013; Dreyer et al. 2010; Mun’delanji et al. 2015).

4.4 Application and Importance of Nanobiosensors for Wastewater Treatment

Environmental pollution has been an increasing concern around the globe. This can significantly affect human health and living bodies. Different sorts of biosensors have been used to forestall climate-actuated risks in the everyday life. A delicate and profoundly explicit biosensor that can be fabricated in a simple, easy, and cost-effective manner to assess ecological risks including wastewater run-offs and other normal sources has remained a challenging task (Zhang et al. 2018). As of now, biosensors dependent on chromatographic, electrochemical, spectroscopic, or other techniques have been generally accepted for such applications. Nonetheless, recent trends have been directed toward the fabrication of biosensors based on aptamers for applications related to nature (Bhattarai and Hameed 2020; Malik et al. 2013; Raghu et al. 2020).

Nanomaterials-based biosensors scaled-down the size of instruments (Lv et al. 2018). The small size of the sensor helps integrating a few cycles into a single device to allow continuous monitoring of range of materials, to expand the scope advantageously for treatment of wastewater. Also, the use of nanomaterials influences their physical and chemical properties in a desirable fashion and enhances the selectivity and consistency, reduces investigation time, and shows prospect for identification of high-throughput multiplex (Ansari, 2017; Kim et al. 2015; Rodriguez-Mozaz et al. 2006).

The efficacy of nanobiosensors relies upon the selectivity of small biomolecules for biorecognition and sensitivity of nanostructures to sense the contaminations (Ghasemzadeh et al. 2014; Kaittanis et al. 2010). A new intriguing utilization of nanotechnology in the realm of biosensors is the use of nanomaterials to change and functionalize cathodes, especially in microfluidic stages to give high-throughput screening.

The use of nanomaterials in combination with the freeze-drying technique can help to improve advanced maintenance time for convenient biosensors for microbials (Lim et al. 2015). Recent finding shows that nanomaterials have significant prospect in the recognition of toxic contaminants in water (Dasgupta et al. 2017). The recent advancement in blending nanotechnology with biosensing systems has been extremely useful for monitoring environmental pollutants (Justino et al. 2017; Reverté et al. 2016; Stoytcheva et al. 2018). Indeed, several biosensors based on nanomaterials have recently been invented and accounted for detection of toxic contaminants in wastewater with high accuracy and precision. In such manner, there have been a few instances of successful set up and application of nanobiosensors for identification of natural segments (Aragay et al. 2012; Atar et al. 2015; Zehani et al. 2015), microorganisms (Abbaspour et al. 2015; Güner et al. 2017; Wu et al. 2012a, b), and substantial metals (Chen et al. 2011a, b; Huang et al. 2016; Tan et al. 2016; Zheng et al. 2013). In general, nanotechnology may be consolidated with biorecognition components and different transducing methodologies to monitor wastewater and our ecological environment. There are huge scopes of research in this intriguing avenue and concerted efforts may lead to the development of highly efficient nanostructure-based biosensors in conjunction with solid-state signal processors or microfluidic devices.

Tables 4.2 and 4.3 summarize different types of nanobiosensors used for monitoring of hazards materials such as heavy metals and organic contaminants. Regardless of the success of biosensing techniques, a few key challenges should be faced to realize marketable value and advancement of biosensors for wastewater treatment. Nanobiosensor devices developed, till now, cannot detect numerous organic substances, for example, pesticides and herbicides present in agrarian sewage (Nanekar and Juwarkar 2015). Therefore, improvement of current nanobiosensors by fabricating appropriate and suitable biorecognition components and biosensor standardization for organic substances in a cost-effective manner to detect a wide range of toxic organic pollutants from wastewater is the most important goal to be achieved. It is also alarming that trace quantities of a few harmful organic substances are progressively being added to waterbodies. For instances, wastewater from pharmaceuticals has different sort of chemicals, analgesics, anti-infection agents, lipid controllers, and other drugs (Das et al. 2017; Radjenovic et al. 2007; Tschmelak et al. 2005; Yuan et al. 2013). Many heavy metals in wastewater are reported to form stable complexes with organic substances. But, development of biosensors till now has been focused to detect free heavy metal ions. Furthermore, wastewater samples might comprise particulates, or organic substances may intervene with the detecting signals (Koedrith et al. 2015; Li et al. 2013). Thus, the samples might require pretreatment prior to detection. Most of these biosensing frameworks are in the rudimentary stages, and a few of them are commercially available for monitoring of large-scale water and wastewater run-off (Ejeian et al. 2018).

4.5 Conclusion

Water quality largely influences all aspects of human life, which interalia include: health, food, energy, and finance. Aside from the rapidly expanding area for wastewater treatment, numerous problems are associated with the use of conventional methodologies for treatment of water, like low efficiency, high cost, and consumption of huge time. These have direct links with the technical limits of regular monitoring frameworks and inadequacy to giving monetary limit over them. Nanobiosensors are considered as a viable option for monitoring environmental pollutants through continuous and in situ operations and may guide to develop efficient means for their removal to ensure adequate supply of clean water.

To accomplish the objective of the identification of wide variety of contaminants from wastewater, it is important to plan novel detecting frameworks with a wider recognition capacity and ability for simultaneous detection. Despite the fact that the majority of the recently announced investigations have focused on

Table 4.2 Biosensors used in the detection of heavy metals

Heavy Metals	Bio-receptor	Transducer	LOD[a]	References
Cr^{6+}	Urease	Conductometric	0–50 ppm	Nepomuscene et al. (2007)
	Bacteria	Electrobiochemical (Potentiometric)	1 mg L^{-1}	Liu et al. (2014)
Cr^{4+}	Carbon dot/Ascorbic acid	IFE	10^{-4} M	Zheng et al. (2013)
Hg^{2+}	Urease	Optical	10^{-4} μM	Prakash et al. (2008)
	DNA/GNPs	Piezoelectric (QCM-D)	4–7 nM	Chen et al. (2011a, b)
Cu^{2+}	DNAzymes/(CdSe-ZnS)	Fluorescent	0.5 nM	Wu et al. (2010)
	Cysteine/CNT	ASV	0.015 mg L^{-1}	Li et al. (2013)
Pb^{2+}	MWCNTs/guanine-rich (G-rich) aptamer/GNPs	Electrochemical (Impedance)	4.3×10^{-15} M	Zhu et al. (2014)
	L-cysteine/rGO	DPASV	0.416 μg L^{-1}	Muralikrishna et al. (2014)
Cd^{2+}	AuNPs/Phosphatase and esterase/BSA	Conductometric	10–20 M	Tekaya et al. (2014)
	Peptide/MWCNT	Electrochemical (Potentiometric)	2.749×10 M	Rahman et al. (2012)
Ag^{1+}	DNAzyme/GO	Fluorescent	5.0×10^{-9} M	Wen et al. (2011)
	GO/Cytosine rich aptamer/QD	FRET	0.1 mg L^{-1}	Chang et al. (2014)
As^{3+}	AgNPs/GSH	SERS	0.00076 mg L^{-1}	Li et al. (2011)
	GNPs/Aptamer	Colorimetric	0.0006 mg L^{-1}	Wu et al. (2012a, b)

[a] LOD, limit of detection; IFE, inner filter effect; GNPs, gold nanoparticle; QCM-D, quartz crystal microbalance with dissipation monitoring; CNT, carbon nanotube; ASV, anodic stripping voltammetry; rGO, reduced graphene oxide; DPASV, differential pulse anodic stripping voltammetry; BSA, bovin serum albumin; MWCNT, multi-walled carbon nano tube; GO, graphene oxide; QD, quantum dot; FRET, fluorescence resonance energy transfer; AgNP, silver nanoparticles; GSH, glutathione; SERS, surface enhanced Raman spectroscopy

the recognition of heavy metal ions, there are growing interest in monitoring other inorganic components. Since a good number of investigations have already been performed using nanobiosensors for the detection of contaminants in groundwater and waterway, further research should be focused and devoted on exploiting these ideas in wastewater, primarily for natural and heavy metal contaminations as well as microorganisms. The advancements are encouraging, and nanotechnology offers

Table 4.3 Detection of organic pollutants by biosensors

Organic Pollutants	Bio-receptor	Transducer	LOD[a]	References
Octane	Whole-cell: Escherichia coli DH5	Light emission/bioluminescence	0.34 μg L^{-1}	Sticher et al. (1997)
Phenol	Whole-cell: Acinetobacter sp. DF4	Light emission/bioluminescence	2.5 mg L^{-1}	Abd-El-Haleem et al. (2002)
Naphthalene Phenanthrene	DNA/Sol–gel array	Fluorimetry	–	Doong et al. (2005)
Atrazine	Labeled antibodies	TIRF	0.002 μg L^{-1}	Tschmelak et al. (2004)
Bisphenol	Labeled antibody	Fluorimetry	0.014 μg L^{-1}	Rodriguez-Mozaz et al. (2005)
	Enzyme (tyrosinase)	Amperometry/MWCNT modified	2.2 ng L^{-1}	Zehani et al. (2015)
PBDE-100	Enzyme (peroxidase)/immobilised polyaniline- modified Pt electrode	Amperometry	0.014 μg L^{-1}	Nomngongo, et al. (2012)
Paroxon	Enzyme (organophosphorous hydrolase)	Amperometry/carbon nanotube modified	0.041 mg L^{-1}	(Deo et al. 2005)
Carbofuran	Enzyme (acetylcholinesterase)	Amperometry/multi-electrode	0.2 μg L^{-1}	(Bachmann and Schmid 1999)

[a] LOD, Limit of detection; PBDE, Polybrominated diphenylether; MWCNT, Multi-walled carbon nanotube; TIRF, Total Internal Reflection Fluorescence

extraordinary promises in working on nanobiosensors and may open up new possibilities for comprehensive treatment of wastewater with high efficiency at a low cost (Aragay et al. 2012).

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Chapter 5
Nanobiosensors for Environmental Risk Assessment and Management

Cansu İlke Kuru, Fulden Ulucan-Karnak, and Zeynep Yilmaz-Sercinoglu

Abstract Environmental analytes have become important, where harmful pesticides and water pollutants are present. When environment or human health damage have occurred, the recovery processes could be impossible or very expensive. Using sensing systems such as sensors, biosensors, or nanobiosensors and biochemical responses such as biomarkers or genes are required for the development of control and precautional strategies. Environmental risk assessment (ERA) investigates the environmental risks and informs about handling of these risks. Subtitles of ERA are hazard identification, risk assessment, risk management, risk communication and monitoring, and feedback. These are all interpreted as main sustainability concepts. Risk assessment and management policies include quantitative and qualitative analysis of potential and/or specific chemical and/or biological pollutants. Accurate, fast analysis methods are required for the detection of these environmental pollutants. Biosensors can be used for analysis of these substances since biosensors are portable, have low cost, and can be tailored according to specific needs. They can also be combined with nanomaterials for increasing surface area and for enhancing specificity and selectivity. They have the potential to detect analytes at very low concentrations in a solution. These technical improvements can be applied to detect infections, medicines, heavy metals, and other contaminants that have yet to be discovered. Thus, nanobiosensors are perfect tools for hazard identification and environmental monitoring. In this chapter, nanobiosensors for ERA and management will be highlighted.

Keywords Environmental monitoring · Nanobiosensors · Risk assessment · Risk management

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5.1 Introduction

Industrialization increased the annual production capacities of chemicals, but unfortunately, it also causes uncontrollable wastes. Almost 6 million chemical compounds have been produced since the beginning of mankind (most of them in the twentieth century), and thousands more are being produced every year (Gadzała-Kopciuch et al. 2004). Increasing amount and types of waste, e.g., metals and its compounds, are difficult to deal with so that the harmful effects of them are clearly seen (Baby et al. 2011).

Numerous initiatives and legal measures for environmental pollution management have been introduced in recent years, with a special focus on water quality. (Rodriguez-Mozaz et al. 2006). This introduction is being carried out in collaboration with scientific and social groups. Environmental risk assessment (ERA) is a scientific initiative that uses scientific facts and assumptions to predict potential adverse effects of specific pollutants or toxic substances on human health and the environment after short and long-term exposure (Meent et al. 2007). The need for disposable, fast, cheap, and on-site environmental monitoring systems or tools has prompted the creation of new technology and methodologies to track a growing amount of environmental analytes (Hayat and Marty 2014).

Risk assessment studies allow to emphasize the effect of anthropogenic actions on environment and to determine relevant quality management as transparent policy, regulatory, or other decisions (Siontorou et al. 2017). Environment is facing more contaminants, metal residues, drug residues, pathogens, and other pollutants as time goes by. Increasing industrialization, human population, human-related pollutants, climate changes, and evolutionary effects such as viral pathogen transmission animals to the humans can be pronounced as the reasons of the contamination (Kraemer et al. 2019; Landrigan et al. 2020). Nanobiosensors are known as the best solution for diagnosing and monitoring of environmental pollutants, pathogens with the aid of their unique constructions (Mohammadi Aloucheh et al 2018). We basically aimed to emphasize nanobiosensors for the environmental risks, risk assesments and management.

5.2 Environmental Risk Assessment

Physical, chemical, and biological pollution in the environment can be classified as air, water, and soil pollution, but they can turn into each other very quickly. Deterioration of one part of the ecology adversely affects the structure of the whole system (Alley 2007). Environmental pollution can be regional, country-wide, or international (Gupta 2007). Pollutants do not stay in a specific environment but spread throughout the biosphere due to actions of organisms, fluid mechanics, and transport phenomena. They infiltrate into the organisms, participate in the material cycles in nature, and inevitably accumulate in the food chain (Brevik et al. 2020; Manisalidis et al. 2020).

Another problem is the unknown interactions of these chemical compounds. Due to the toxic effects of these chemicals, various harmful, neoplastic, mutagenic effects are seen in all organisms in the environment. Since these compounds are accumulated in tissues and organs of living things (Laumback and Gochfeld 2011), they might cause tissue-organ damages and dysfunctions. Although individual effects on living creatures can be seen following the exposure to certain chemicals, population-wide changes, such as change in population ratio, decrease in species diversity, can be seen in a longer period of time (Saaristo et al. 2018). If the necessary preventive measures are not taken, it is obvious that biological balance will deteriorate, and the Earth will become uninhabitable in the not-too-distant future. For this reason, many studies have been carried out to monitor environmental pollution (Adedeji et al. 2012). Until now, chemical analysis methods have been accepted as golden standard methods for monitoring environmental pollution. Although most of the obtained data reveal the presence of pollutants in the environment, they hardly give an idea about the possible toxic effects on living creatures on the same environment (Sumampouw and Risjani 2014).

Remarkable interest and awareness about the possible effects of environmental pollution on the ecosystem has been emerging (Jianping et al. 2014). As a result, ecology and ERA have become two of the most popular sciences all over the world, and researches on this subject tend to increase. Continuous generation and discharge of contaminants by human activity necessitates environmental monitoring. The key method for tracking environmental health and prioritizing conservation and restoration activities is ERA (Fig. 5.1). Chemical, physical, and biological pollutants, among other stressors, are investigated by ERA in order to see if they pose a threat to an environment (EPA 1998).

5.3 Nanobiosensors for Environmental Risk Assesment and Management

Air, water, and soil pollutions are the main types of environmental problems. In environmental monitoring, the system should have the ability of determining low concentrations of contaminant selectively and sensitively (Hashem et al. 2021). Numerous environmental pollutions can be determined by the novel biosensors. Resulting data can contribute to our understanding the reason of several diseases and health issues associated with these pollutions. Thus, one of the most important issue is rapid detection and monitorization of these hazards, so we can control them (Gupta and Kakkar 2020).

Water and soil quality can be monitored by biosensors in terms of (a) physical parameters, (b) organic contaminants, (c) biochemical hazards, and (d) biological contaminants. Therefore, pollutants in aquatic environment, drinking water, and soil can be traced (Halilovic et al. 2019; Srivastava et al. 2020; Su et al. 2020). Biosensors

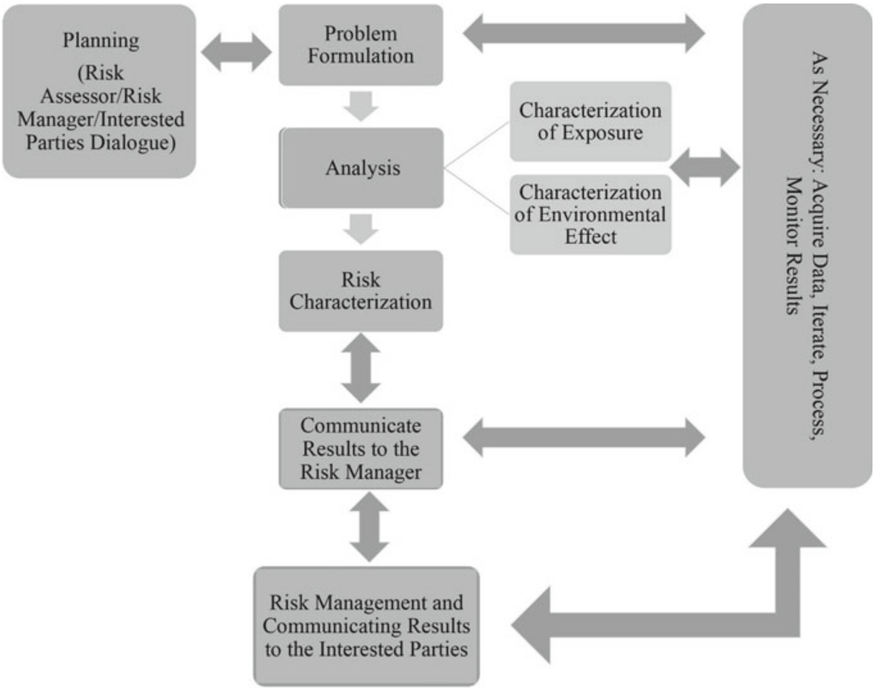


Fig. 5.1 Environmental risk assessment flowchart, redrawn with permission from reference (EPA 1998)

benefitting from nanomaterials can lead to a sustainable agriculture, aquaculture, and livestock (Griesche and Baeumner 2020).

The most encountered environmental contaminants are phenolic compounds, plastics, pesticides, antibiotics, gas pollutants, and pathogens (Mei et al. 2020). Among the chemical pollutants, heavy metals are the main toxical hazards. Development of biosensors for detection of heavy metals is a prominent topic (Odobašić et al. 2019; Tovar-Sánchez et al. 2019).

Undoubtly, there are many other studies about this topic. But, due to space constraints, the most important and up-to-date examples of utilization of nanomaterials for the environmental risk monitoring applications were summarized in this section. Heavy metal pollution is a worldwide environmental issue. Pesticides and fertilizers are the main sources of heavy metal waste, and even extremely low concentrations are a severe threat to environment and human health. In addition, they are non-biodegradable and accumulate in the food chain. Chromatographic and spectroscopic techniques are already available for determining metallic ion levels in water, but they require extensive technical knowledge due to their time-consuming and complicated procedures. It is critical to design novel biosensors for monitoring heavy metals, particularly in water. Chemical sensors with functionalized nanomaterials have been used widely. On-site sensing capability and increased device performance (due to high selectivity, sensitivity) are advantages of these sensors (Bereli et al. 2021). Utilization of biomolecule-functionalized surfaces increases the selectivity of a detection system, but it can also lead to issues such as reproducibility and complexity. A variety of nanobiosensors were designed based on functionalized nanoparticles, nanotubes, nanowires, and nanofibers (Bellan et al. 2011).

We attempted to emphasize the definitions, basics, and requirements of the ERA and management processes. It is preferable to quickly discuss the main definition of biosensors, their types, the immobilization procedure, and nanobiosensors in the following section.

5.3.1 Hazard Identification

A hazard is any potentially harmful source or a situation in terms of human illness or injury, damage to property or environment, or a combination of these. Risk, on the other hand, is defined as the probability of hazard and the likelihood of the unwanted event. Both the occurring probability of the event and the magnitude of its consequences are included in this definition. Unlike the term "hazard," it includes both danger and its potential to occur. Danger can arise without augmenting risk, where risk arises if there is a potential route for dangerous exposure (Aven 2016; Theodore and Dupont 2017).

The concept of risk has been constantly considered by environmental politicians throughout its history. However, in the early days, risk assessments were based on intuition, rather than scientific principles such as toxicology, chemistry, transport, and modeling of pollutants. Scientific risk assessment has played an important role on the formation of environmental policies in recent years. To emphasize this distinction, risk analysis based on scientific methods referred to as numerical risk analysis (Kasperson and Kasperson 2013).

A vast range of manmade and natural substances and creatures influence marine habitats, which can have negative consequences for human health and ecosystems. To properly monitor these hazards, manage the repercussions, and understand the processes driving their magnitude and distribution, real-time measurements of pollutants, toxins, and infections across a range of spatial scales are required. In recent years, significant technological advances have been made in the detection and investigation of such marine dangers. Sensors installed on a range of mobile and fixed-point observing platforms, in particular, provide a helpful means of assessing dangers (Zielinski et al. 2009).

For detecting hazardous algae and their associated poisons, biosensors are a clear priority. To meet the sensitivity requirements to replace the AOAC mouse bioassay, techniques such as membrane-ion channel biosensors, surface plasmon resonance-based biosensors, and molecular and biochemical diagnostic methods (e.g. immunoassays) must be improved (Zielinski et al. 2009).

The variety of biosensors available for seafood toxicity screening enables for the detection of phycotoxins at low levels of sensitivity, but their restricted availability, mostly as research tools, prevents their widespread use in monitoring programs. Combining existing expertise in interdisciplinary fields such as nanoelectronics, bioelectronics, micromachining, and microfluidics could improve commercial utilization (Campàs et al. 2007). This would also make it easier to integrate these devices into deployable measurement platforms.

In several natural systems, phytoplankton cells are effective biosensors for environmental monitoring and toxicity assessments. This is noteworthy in light of the fact that Cu contamination in coastal areas has increased dramatically over the last century, wreaking havoc on marine ecosystems. Unfortunately, there is no high-throughput technology for detecting Cu toxicity in seawater in the environment. High-throughput screening was done on five luminescence reporter lines generated in the green algae Ostreococcus tauri RCC745, to investigate potential uses as Cu pollution biosensors. The iron storage ferritin protein fused to luciferase reporter line was the most sensitive, responding to Cu values in the *M* range. These findings show that biosensors may be used to undertake high-throughput laboratory investigations of seawater in coastal areas with metal disturbances in a reliable, cost-effective, and automated manner (Henríquez-Castillo et al. 2018).

Consuming such impure food that made with adulterations can result in a variety of adverse health effects. Although government regulatory agencies have detection techniques, they are not only costly but also inaccessible to everyday consumers. As a result of this condition, there is a clear need for a portable and simple detection method for domestic users to avoid major health problems (Khaliq et al. 2019).

Khaliq et al. present all-dielectric meta-biosensors that use refractive index sensing to identify dangerous adulterants in water and milk. With a numerically computed sensitivity of 500 nm/RIU, a change in transmittance per refractive index unit of 8 (800%), and a figure of merit (FoM) of 14.7, the platform offers a highly efficient and innovative technique of analyzing water and milk. The suggested single-wavelength transmittance-based measurement could significantly reduce the cost and size of the sensing platform while maintaining sensitivity. These silicon-based designs pave the way for the development of a variety of highly efficient and cost-effective sensors for the qualitative and quantitative monitoring of various food toxins (Khaliq et al. 2019).

By giving data on early biological effect induction, biosensor technology has the potential to revolutionize the field of air pollution monitoring. Such information can be used to enhance routine pollution monitoring, improve exposure estimation, including potential effect estimation, and raise public awareness about air pollution. Especially, considering the complicated biological impacts of air mixture exposure, such as mutagenic, genotoxic, and other proven effects, using genetically modified microbes, a basic biosensor was created. A first sample session was done utilizing the biosensor in one of Italy's most polluted cities, collecting data from the standard monitoring system, including PM2.5, PM10, NOx, PAHs, and metal concentrations. The biosensor responds to a dirty air mixture, indicating a potentially harmful genotoxic effect. Monitoring with a biosensor requires a small sample size and direct contact with the microbe, making it an excellent option for determining air genotoxicity (Traversi et al. 2020).

5.3.2 Risk Assessment and Evaluation

Different institutions have implemented different standard approaches to ERA. Although these efforts generally result in a consensus on the ERA process, yet there are differences during the application of the process to specific situations. The risk assessment method consists of four commonly accepted stages: hazard identification, hazard exposure assessment, toxicity assessment, risk characterization (Corburn 2002).

Toxicity and exposure to the pollutant should be considered by risk assessment. It is crucial to investigate both the current and probable exposures and the possible exposure routes. Potential ecological impacts and other environmental impacts are substantial (Damalas and Eleftherohorinos 2011). Risk assessment explores the acceptable degree of risk. A "zero degree" risk standard cannot be mentioned, but a negligible risk can be mentioned. This is the stage where researches are done in order to understand at what extent there is no serious health or environmental risk and at which points sufficient safety margins can be implemented to protect the public health and the environment (Environmental Health Risk Assessment, 2002; Fournier et al. 2014). ERA is a systematic environmental decision-making process. Data, forecasts, and uncertainties are evaluated and organized to determine and foresee the relationships between stressors and ecological impacts. Chemical, physical, biological elements can be evaluated to reach a holistic understanding (Hope 2006; Lawrence 2015).

The total levels of metals, i.e. amounts determined after digestion of soils with strong acids, are commonly used to estimate the environmental risk of heavy metal-polluted soils and sediments. Almost every scientific study dealing with heavy metal bioavailability in soils has acknowledged the flaws of this approach, namely the difficulty to distinguish between hazardous and non-hazardous portions of metals. It is important to support the development and refinement of existing heavy metal whole-cell biosensors. These sensors should be particular and sensitive enough to be employed as bioassays for early warning. Risk assessments based on total metal levels in soils should be reexamined in light of evidence gained from bioassays. Biosensors (great tools for mechanistic research and signaling hazard at subtoxic levels) should be developed and used more (Kahru et al. 2005).

Nanobiosensors have been proposed as a potential method for monitoring pollutants at the micro and nanoscale. In 2020, heavy metals (such as Zn, Pb, Ni, Cd, Co, and Al) in river water were detected using cantilever nanobiosensors with alkaline phosphatase in the study of Rigo et al. They used N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), 16-mercaptohexadecanoic acid, alkaline phosphatase enzyme, and N-hydroxysuccinimide (NHS). The surface was modified by self-assembled monolayers (SAMs). Detection limit of the nanobiosensor is at ppb level with high sensitivity, and nanobiosensor has fifteen-day stability. The developed sensor was straightforward method for detecting pollutants in river water (Rigo et al. 2019).

Cadmium (Cd) is abundant in the ecosystem because of industrial and agricultural operations and can accumulate in drinking water and food. Because of this accumulation, serious high levels of contaminant can be seen, and this level can seriously threat human and animal life. Exposure to Cd (II) ions would be limited if a trustable method is developed, and this will help also support current detection methods.

Alodhayb et al. developed a DNA-based electrochemical technique which use multi-walled carbon nanotubes (MWCNT) and ethyl green (EG) to detect Cd (II) ions. In the presence of immobilized dsDNA on the working electrode, the indicator dye EG prefers to bind to ssDNA, and this leads to substantially lower peak current than control. After engaging with dsDNA, the Cd (II) ions unwind the dsDNA to ssDNA, which the EG molecules bind to, resulting in a larger decrease peak current. In the presence and absence of Cd (II) ions, the difference in reduction peak currents is proportional to their concentration. This approach achieves a linear detection range higher than 2 and lower than 10.0 nM. Sensitivity and detection limits of the biosensor are 5 nA nM^{-1} and 2 nM, respectively. This is less than the permitted limit of exposure to Cd (II) ion. This research expands the range of applications for MWCNT-modified electrodes in biosensors. This expansion suggests the possible utilization of MWCNT-modified electrodes in biosensors detecting environmental samples with suspected contamination of other heavy metals (Sreekanth et al. 2021).

Carbon nanotubes (CNTs) and gold nanoparticles (AuNPs) can be simultaneously used in both nanosensors and nanobiosensors as nanostructure. Ben Amran and colleagues polymerized AuNPs functionalized with electropolymerizable thioaniline onto glass surfaces with metal ions. Ion-imprinted bis-aniline-bridged AuNPs composites were formed, and ion binding site is selective and can sense ions at femtomolar concentrations (Ben-Amram et al. 2012).

Recently, utilization of graphene-based nanomaterials gains increasing interest in the field of sensors. In 2021, surface reversible addition–fragmentation chain-transfer (RAFT) approach was used to develop a heavy metal nanosensor using graphene oxide (GO). First, graphene was used to make GO, which was then modified using L-phenylalanine (LP). The surface of graphene oxide- L-phenylalanine (GO-LP) was then chemically bonded with a RAFT agent. Following that, polymerization of methacrylamide (MAM) monomers on the GO surface resulted in GO-LP/polymethacrylamide (GO-LP/PMAM). Photoluminescence intensity of GO-LP/PMAM was higher than spectra of GO-LP and GO. Cupper ions can be detected as low as 0.25, and 2 mM is the highest concentration to be detected. Monitoring the shift in fluorescence intensity was the marker for high selection of Cu (II) ions, after the addition of other metal ions. This study showed that surface modification with GO by RAFT approach contributed to selective detection of Cu (II) ions (Barzegarzadeh et al. 2021).

Economic growth of a developing country's is based on the food, agriculture, and their related industries. Controlling the production processes and checking the quality of products are critical aspects. Detection of food contaminants, such as microbial load, pathogens, antibiotics, preservatives, heavy metal ions, and toxins, is possible with nanobiosensors. Monitorization of soil physiology, plant's pathological state, and soil's microbiological microenvironment, detection of residual chemicals, such as pesticides, are some of the significant implementation of biosensors in agriculture (Srivastava et al. 2018).

Extensive and intensive utilization of organophosphates such as chlorpyrifos and carbofuran as insecticides in agriculture brings along the monitorization of trace amounts of these pesticides for the sake of public health and security. Sun and friends produced an AuNP and functionalized its surface with L-cysteine. Thanks to Au–S chemistry, chitosan is electrostatically assembled to the surface of AuNP by cysteine, and this structure is used to cover glassy carbon electrode. Electroactive molecule thiocholine is formed when immobilized acetylcholinesterase (AChE) reacts with acetylthiocholine chloride. Presence of chlorpyrifos and carbofuran inhibits the activity of AChE. Linear relationship between inhibition percentage and pesticide concentrations was utilized for quantification. Detection limits were 0.08 g dm^{-3} and 0.06 g dm^{-3} for carbofuran and chlorpyrifos, respectively (Sun et al. 2013).

Another AChE biosensor was developed by Yang and colleagues, where an electrode modified with Au-polypyrrole-reduced graphene oxide (Au-PPy-rGO) nanocomposite was used to detect OPs. The introduction of gold nanoparticles and reduced graphene oxide with pyrrole formed a nanohybrid. This nanobiosensor has a strong affinity for thiocholine and also a high conductivity. When the concentration of acetylthiocholine chloride was increased, the electrochemical response increased, while presence of AChE inhibitor-OPs decreased the response. Paraoxon-ethyl at concentrations ranging from 1.0 to 5 nM with a detection limit of 0.5 nM was possible with this nanobiosensor (Yang et al. 2014).

To detect chloramphenicol (CAP), a broadspectrum antibacterial veterinary medication, Govindasamy designed a molybdenum disulfide nanosheet-coated functionalized MWCNT hybrid electrochemical biosensor. The nanocomposite-modified electrode operated well in a broad linear concentration range of 0.08–1392 M and demonstrated perfect electrocatalysis of CAP. The electrode's practical applicability was demonstrated in food samples with agreeable recoveries, which shows its practical viability in food analysis (Govindasamy et al. 2017).

Azo dyes, which account for approximately 70% of all dyestuffs consumed, are the most commonly used synthesized colorants in the clothing, food, cosmetic, pharma, and other industries. Because of their potentially adverse effects on environment and health of living, efficient monitoring of these dangerous chemicals and their associated metabolites is of paramount importance. Using a fluorescence quenching technique, quantum dot (QD)-enzyme hybrid system was developed to detect methyl red (MR) in aqueous solutions by Hajipour and colleagues. The catalysis of the reduction reaction of azo group of MR by azoreductase lowered the FRET between QDs and MR molecules. The development of a fluorescence quenching-based sensor was achieved by the integration of QD photoluminescence recovery and MR enzymatic decolorisation at the neutral phosphate buffer. Accurate detection of MR was possible in a linear calibration of MR concentrations between 5 and 84 M. LOD is 0.5 M, and response time is three minutes (Hajipour et al. 2021).

The use of electrochemical DNA biosensors as a screening instrument for quick bio-analysis of environmental contamination and DNA–drug interaction research is proposed in this paper. The binding of tiny molecules to DNA immobilized on disposable screen-printed electrodes was evaluated using square-wave voltammetric scans of the electrochemical signal of guanine. The biosensors were able to discriminate low, medium, and high contaminated soils in 11 min, with good correlation with well-established procedures such as FF, comet, and genotoxicity assays. The same biosensors were used to assess DNA's interaction with anti-proliferative metallo compounds, and the electrochemical responses reflected the type of contact (Bagni et al. 2006).

Sensors for monitoring environmental characteristics are not easy or straightforward to design. Only, one side of the problem, which may be solved by technological improvements, is the development of field instruments to suit a target pollutant at the required detectability. The other side of the equation is determining which pollutant derivative to target in order to make ecologically relevant and trustworthy conclusions. Pollutants entering the environment may take a variety of metabolic pathways led by a complex, overlapping, and multilayered synergy of biotic and abiotic characteristics, resulting in a wide range of ecosystem elements damaged and degrees of negative reactions. Each ecosystem is unique in this regard, demanding a thorough understanding of the micro- and macrostructures that are at risk, as well as custom-designed sensors to address the threats (Siontorou et al. 2017).

5.3.3 Risk Management and Communication

Risk management is decision-making process to identify and assess a hazard, to examine population exposed to it and its possible adverse effects on both environment quality and human health (Covello and Merkhofer 1993). Following the assessment, mitigation of adverse effects and monitorization of consequences are conducted and reported accordingly. Comparative risk analysis and economic analysis are risk management activities (US 1983).

ERAs are developed within framework of risk management to estimate and evaluate unwanted anthropogenic activities. In this evaluation phase, negative impacts and pressure factors are emphasized. Negativity of risk is important to be identified since it could have a negative impact on one of environmental components, while being beneficial for other components. Fundamental and functional components of ecosystems are corrupted as a consequence of unwanted changes. Assessment of negativity may include assessment of the type, severity, scale of the impact, and the potential for improvement. The acceptability of negative effects is determined by risk managers (Chen et al. 2013). Environmental risk management combines the evaluation of weighting policy and improvement alternatives and integration of the risk assessment results. Socioeconomical and political data are gathered and used for this assessment.

Trend determinations of adverse effects can range from qualitative considerations to quantitative probabilities, which are not always easy to calculate (Van Gerwen and Zwietering 1998). Risk assessment provides a basis for comparing, rating, and scaling risks. Assessment outcomes constitute a basis for analyses of cost–benefit and cost-effectiveness, which helps to inference alternative management options (Linkov et al. 2006).

The extensive use and significant advantages of ERAs should not be regarded as the sole consideration of management decisions. A broad range of factors should be taken into consideration by risk managers. Legal obligations, social and economic values may cause risk managers to make less or protective decisions. Minimizing risk can be expensive or technically infeasible. For this reason, low-cost and efficient methods for risk identification, assessment, and management are crucial (Vermeire 2007).

Once risk assessment is completed, assessment results are used to modify and optimize the design of projects and policies, to determine the rate of safety in environmental property protection, to set new standards, limits, recommendations, and to limit existing technologies or to develop new technologies (Koller et al. 2000). Communication of risk needs to emphasize on how scientists, especially those in the medical domain, evaluate the risk and risk assessment. Risk can not always be a purely scientific issue. Risk is regarded as depending on characteristics of the hazard, risk holders, and the social context (Miller and Solomon 2003). Effective communication should be the part of the risk analysis process and is a requirement of information management about the potential hazards. The main purpose of the risk communication is to sustain more useful, relevant, and accurate information with an understandable language and format (Fitzpatrick-Lewis et al. 2010). Communication of environmental risks can be caterogized into two main parts:

I. Circumstances that might happen in the future when you focused on prevention,
II. Possibility of emergency situations where an event has happened (Abkowitz 2002)

Industry should be engaged with several regulations and challenges for large-scale productions. Environmental risk communication procedure is very important as a guidance for industry in order to understand risk management plan which is prepared with the aid of governments, local news, and media (Sadar and Shull 2019).

The role of information and communication technology (ICT) in lowering air pollution, which has damaged various aspects of human existence in recent years, is one of the most pressing concerns in environmental economics today. The use of information and communication technologies can assist environmental research and its dissemination to legislators and the general public. The use of information and communication technology (ICT) can help to minimize reliance on natural resources and hence reduce pollution caused by improper use of natural resources. The paper presents applications of ICT systems in environmental protection in order to increase the quality level of environmental monitoring and pollution protection systems (Dastres and Soori 2021).

By collaborating closely with authorities, scientists, and industry, Taiwan has launched an integrated and interdisciplinary initiative titled Civil IoT Taiwan for better disaster risk management and risk communication with all stakeholders. The goals of this project are to promote public risk awareness in order to prevent disaster damage and loss while also increasing the social, economic, and environmental consequences in a sustainable manner. In the following phase, the substantial changes and repercussions on society, economics, and the environment will be analyzed, as Civil IoT Taiwan has just completed the first development stage in constructing infrastructure for monitoring and sensing. This ongoing effort will also involve additional stakeholders in the future creation of more sustainable and resilient environmental governance (Lin et al. 2022).

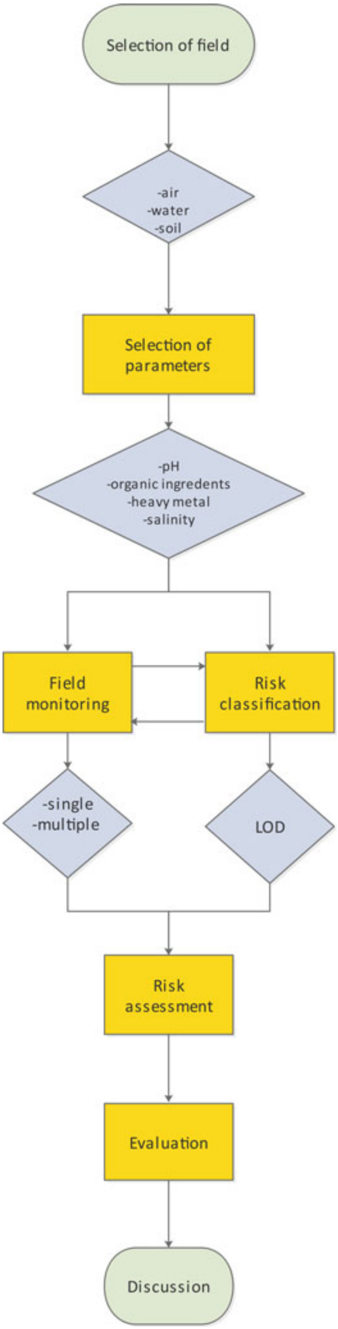
5.3.4 Monitoring and Feedback

Environmental monitoring for the identification of contaminants, especially which are harmful to human health, or the environment, is prevalent for general public, regulated sector, and regulatory agencies. Legislation is largely responsible for monitoring toxic pollutants in the environment around the world (da Costa Silva et al. 2011). Growing number of potentially harmful contaminants in the environment necessitates the use of fast and low-cost analytical techniques in large-scale monitoring programs. Most of the conventional analytical methods have time and expense constraints which are often a significant impediment to their use on a regular basis (Rodriguez-Mozaz et al. 2005).

Biosensors for environmental parameter measurement must be designed to suit a target pollutant at the required detectability, and this design may be solved by technological developments. Choosing the pollutant derivative and pollution area is also important to focus on, in order to get ecologically meaningful and valid results. Invasion of pollutants into the environment may take a variety of metabolic pathways, which are led by a complex, overlapping, and multilayered synergy of biotic and abiotic characteristics, and these paths result in a wide range of damaged ecosystem elements and degrees of negative response. Each ecosystem is unique and requires a thorough understanding of the risky areas, as well as custom-designed nanobiosensors to address those risks. Consequently, Fig. 5.2 depicts a risk assessment approach for designing cost-effective biosensors to monitor the most crucial ecological parameters. (Siontorou et al. 2017).

Designing of an environmental nanobiosensor process include some critical steps. According to Fig. 5.2; firstly, field profiles are selected, and then, the most important parameters of these fields, i.e. soil pH, salinity, heavy metals content, are selected. ERA is performed due to the measurements and comparation with limit of detection (LOD) values of pollutants that are identified and assessed by regulations. This type of methodology helps and realizes the accurate evaluation for the improvement of the biosensors that can determine these demanding environmental parameters (Bartzas and Komnitsas 2020).

Fig. 5.2 Framework of designing a biosensor system for environmental risk assessment and management



With the world's population growing at an exponential rate, reducing energy consumption has become a must for the environment's long-term survival. The use of sensing and environmental monitoring technologies in buildings is one of the most essential features in this respect, as they provide important information on the energy performance, safety, and cost-effectiveness of buildings. By 2021, the global sensor industry is expected to exceed $190 billion, with the number of sensors installed worldwide exceeding one trillion by 2025. It is highly desirable to have a wide range of communication technologies to complement them. Numerous studies examine diverse sensor technologies, as well as critical selection characteristics and strategies for optimum sensor placement, to provide insight into the various sensing and environmental monitoring technologies typically utilized in buildings (Hayat et al. 2019).

The importance of recording personal environmental conditions ("human exposure") and examining the alternatives and repercussions of individuals' adaptive and defensive behavior is becoming more widely recognized. Wearable sensors that capture environmental and spatio-spatial data while accompanying a person have been established thanks to major breakthroughs in smart technology. Wearable sensing has two aspects: First, it records an individual's exposure, and second, it allows individuals to behave as urban explorers. Wearables can be used to evaluate environmental characteristics due to current technological improvements and the fact that a huge number of people own smartphones. Wearables are reasonably inexpensive, and many individuals already own personal devices. The trend toward improved precision and lower pricing for wearables will likely allow researchers to enroll even more people in future studies of air pollution and heat stress, allowing them to collect more data and improve the reliability and generalizability of their findings (Helbig et al. 2021).

Wearable sensors or sensors deployed at home or at work that provide feedback on personal exposure to air pollution, noise, or high temperatures can provide information that can inspire people to reduce their exposure. As personal measurement, devices become more widely available, it is critical to assess how such sensors affect human perception and behavior (Becker et al. 2021).

5.4 Conclusion

Risk assessment involving a biosensor includes three basic steps: (a) determination of the necessary physicochemical status for evaluation of effect of pollutions on ecosystem to be analyzed and health, (b) ERA parameters should be proper for identification of the environmental impact at a reasonable cost, (c) designs must be based on environmentally safe disposal strategies to minimize the pollutants for a sustainable environmental health (Siontorou et al. 2017).

Numerous environmental analytical methods are now being developed or are commercially available in response to the growing need for faster and more cost-effective environmental monitorization (Bilitewski and Turner 2000). Nanobiosensors are regarded as one of the powerful candidates, compared to the existing analytical techniques, due to their high sensitivity, real-time monitoring. Moreover, they do not need extensive sample preparation (Kuswandi 2019). For a cheap, quick screening, and monitoring of a wide range of contaminants, multifunctional miniaturization of nanobiosensors is possible and required in growing attention. Many environmental nanobiosensors can be converted into single-sample or continuous configuration for screening and monitoring. Impact of environmental pollution on the human health accelerated the investigations about monitorization systems, as nanobiosensors being one of them. These monitorization systems will enhance the sustainability of the society and environment (Justino et al. 2017). Nanobiosensors are useful for detecting pollutants and monitoring the environment and will find many unique applications among the other conventional methods, which are currently competing for this growing market.

5.5 Future Remarks

Environmental monitoring has different steps such as sampling, handling, sample transfer to a laboratory and determination of the toxic effects. All of these steps are expensive, time-consuming, and in need of well-experienced personnel. Therefore, effective and rapid results are momentous for the risk assessment of potential residues. It is obvious that fast, reliable, selective, and cheap monitoring systems are and will be required for the environmental monitoring (Sadik 2014). In Rodriquez-Mozaz et al. 2005, declared that miniaturized systems and nanostructures would be trending in construction of environmental monitoring biosensors (Rodriguez-Mozaz et al. 2005). These predictions are still valid. In addition, cell-based biosensors, using cells and organisms as bioreceptors, are gathering attention (Khanam et al. 2020). All of these developed systems can be integrated with high-throughput technologies and automatization in order to reach holistic results (Tian et al. 2021).

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Chapter 6
Challenges and Scope in Nanobiosensors Utilization for Environmental Monitoring

Ekta Poonia, Jasbir Sangwan, Narender Ranga, and Vijay Kiran

Abstract Nanobiosensors have shown the potential to complement laboratory analysis methods in environmental applications. There is an urgent need for fast, reliable, and inexpensive systems to detect, monitor, and diagnose pollutants in the environment and agriculture. In the past few decades, health has received a lot of attention. Nanobiosensors have received potential applications in industrial use and monitoring, pollution control, and agricultural and food industries. The most important aspects of nanobiosensor marketing are selection, sensitivity, stability, reproduction, and low concentration. Therefore, this chapter focuses on the application and scope of nanobiosensors in environmental monitoring.

Keywords Nanobiosensor · Virus · Environment · Pesticide · Microorganisms · Microbial toxins

6.1 Introduction

A sensor is a device that detects changes in body movements and converts them into measurable or recorded signals. The most widely accepted definition of a biosensor is: “an analytical device that combines bioactive elements with an opposite objective transducer device to produce assessable signals comparative to the

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absorption of chemical species in several types of sample" (Arnold and Meyerhoff 1988). These nanoscale materials have doubtless helpful applications within the social and environmental fields and are utilized in a large range of industrial applications, including semiconductors, storage, display, optics, energy, and health care. They are also used for energy conservation, production and conversion, additional agricultural production, water treatment and regeneration, disease identification and testing, drug delivery systems, food and storage systems, pollution and rehabilitation, construction, health storage, and pesticide and control icons.

Nanotechnology plays an increasingly needed role in development of nanobiosensors. Use of nanomaterials has permitted the beginning of more signal transmission technology to nanobiosensors and, in turn, improved their sensitivity and performance (Jianrong et al. 2004). Due to the size of their submicron, nanosensors and nanoprobes transform the field of quality analysis and transform the rapid analysis of many substances within the field of food poisoning and environmental pollution. Recent advances in engineering science have made it possible to mass-produce inexpensive devices and integrate them into marketing programs for conservation or food poisoning (Bal et al. 2017). Automatic sensors mimicking natural chemical composition have already begun to emerge, supporting the functional integration of functional biomolecular units. The implications of engineering science in environmental use have been largely focused on current analysis with special emphasis on pollution prevention and therefore the removal of environmental pollutants from large amounts of contaminated soil, barriers, solid waste, air, and water (Pan and Xing 2012). The disintegration layer used for nanomaterials has been used to produce water breakers with low electrical power to provide electronic solutions.

The manufacture of nanobiosensors, their materials, distribution devices, and energy reduction techniques requires analysis in many fields of chemistry, biology, and engineering. The materials used in biosensors are divided into three groups that support their mechanisms: a catalyst group consisting of enzymes, a bioaffinity group with antibodies and nucleic acids, and a microorganism based (Metkar and Girigoswami 2019). This chapter describes the role of various nanobiosensors in environmental monitoring. In addition, the technical principles of on-site rapid detection of various heavy metals, viruses, toxins, and pesticides are introduced, and future challenges are discussed in detail.

6.2 Importance of Nanobiosensors for Environmental Monitoring

The latest practice of environmental monitoring and bioremediation is to use advanced technology based on genetic engineering and artificial environmental science to classify microbes through precise signals for release, sensitivity, and selectivity. The biological constituent in a molecular nanobiosensor is a composite plasmid. It has a specific developer, whose presence is sensitive to target molecule and uses the reporter system to produce a signal. Developers can be turned on or off by certain molecules, which is why they provide the precision needed to produce a signal. Signal production is directly related to the developer's expression. Special recombinant plasmids can be genetically modified to lead to nanobiosensor. In some cases, it is broken down in such a way that the host chromosome plays a part in the supporting function of the developer's expression. In this type of application, an additional requirement is a support system for the survival and duplication of these designed sensor molecules. For example, living cells with enzymatic activity that decomposes xenobiotic compounds will be more widely used in bioremediation (Ali et al. 2021). Nanobiosensors based on fuel have also been developed to observe chemical oxygen demand and environmental toxicity. Microbacteria can destroy organic substrates and produce fossil fuels; this technique involves use of bio-electrochemical devices to control bacterial respiratory energy and to exchange organic substrates directly into electrical energy. Despite all these capabilities, microbial nanobiosensors have limitations in manufacturing and operation due to their low power density cost. They significantly increase productivity and reduce costs through a new systematic approach, which provides a platform for the development of self-powered microbial nanobiosensors (Vigneshvar et al. 2016). An additional region of microbial nanobiosensors has shown their prospective for pesticide and heavy metal detection, where microorganism that belongs to eukaryotes has advantages over prokaryotic cells mainly due to the advantages of emergent entire cells. Nanobiosensors have sensitive and selective behaviors that can prove the toxicity of pesticides and heavy metals. In addition, higher eukaryotic microorganisms may be more sensitive to various toxic molecules, which may be related to natural world. Interestingly, the use of microbial biosensors is different (Gutiérrez et al. 2015).

In the future, these small microbes' nanobiosensors will be widely used in the detection of metal pollution and sustainable energy production (Bollella and Katz 2020). The invention of electrochemical sensor with high-throughput technology, which focuses on discovery, testing time, and portability, creates high consumption of expensive glucose and nanobiosensor pregnancy using chorionic gonadotropin fixation straps, and the market for lateral motion technology is key to improvement of sensitivity and discovery limit (Grieshaber et al. 2008).

Similarly, optical nanobiosensors are the next important technology in nanobiosensors using optical fiber chemistry. Due to the advantages of high load-bearing capacity and hydrophilicity, it is best to use hydrogel-based cross-linking to detect single molecules, such as DNA or peptides. Subsequently, an optical nanobiosensor for DNA measurement was developed which has been widely used in biomedicine and forensic medicine (Jiang 2018). In addition, microorganisms, animal or plant cells, and tissue components can also be included in the nanobiosensor system. Based on cellular optoelectronics, they even proposed a biometric optical system. The advantages of optical nanobiosensors include faster and faster analysis, signal intensity of electrical or magnetic disturbances, and spectral power of information (Chen and Wang 2020). However, the disadvantages are high costs due to certain equipment requirements. Other technological difficulties include the difficulty of preparation, especially for biological production, and the needs of the sterile environment must be strictly considered in order to make full use of optical nanobiosensors.

Mechanical production of organic products provides better results of quality nanobiosensors. In fact, both electrochemical and optical biochemicals make full use of this technology to create excellent nanobiosensors. Significant advances in micro-nano technology have encouraged the development of repair machines (Hasan et al. 2014). The capability to create such configurations using semi-conductor processing technology combines the principles of biophysics and bioengineering to develop nanobiosensors of active micro-nano electromechanical that can be made in huge amounts (Arlett et al. 2011). Nanobiosensors are biosensors used or genetically modified which are used to analyze the molecular mechanisms of biological processes (Rajpoot 2017). In the case of organic matter, fuel-based nanobiosensors also have features of high sensitivity and selectivity, but large-scale production methods and genetic engineering to create microbes require complex processes and high costs (Reshetilov et al. 2010). However, another advantage of microbial nanobiosensors is that they can be used as tools for bioremediation, which is very important from an environmental monitoring perspective.

Nanobiosensor devices use a biological recognition object directly connected to the transducer. They contain an organic sensory element (enzyme, tissue, living cells, etc.) that provides selections, as well as a transducer that converts physicochemical variants into processed signals. Specifically, a nanobiosensor consists of three components: The first element is a biomediator (biomimic or biologically available material, e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, antibodies nucleic acid, and biologically complex elements (genetically engineered); second is transducer element (physicochemical, optical, piezoelectric, electrochemical, etc.) that converts a signal caused by an analyst's interaction with an organism into a measurable signal and measured; and the third element is a compatible electronic or signal processor, which is responsible for the user's friendly way of seeing results. Some nanobiosensors require a biomediator immobilization process on the surface of the sensor (metal, polymer or glass, and other materials) using physical or chemical methods. The basic principle of nanobiosensors is shown in the Fig. 6.1, and the classification of bioreceptors and transducers is represented in Fig. 6.2.

6.3 Scope of Nanobiosensor in Environmental Monitoring

Nanobiosensors are a type of molecular sensors that have recently been used with great success in the field of clinical diagnosis. Recent developments indicate that they will now have a similar impact on environmental monitoring practices. The use of chemical reactions, combined with modern sensors and technology, opens up new opportunities. It quickly and economically measures the various statistics that are harmful to the environment.

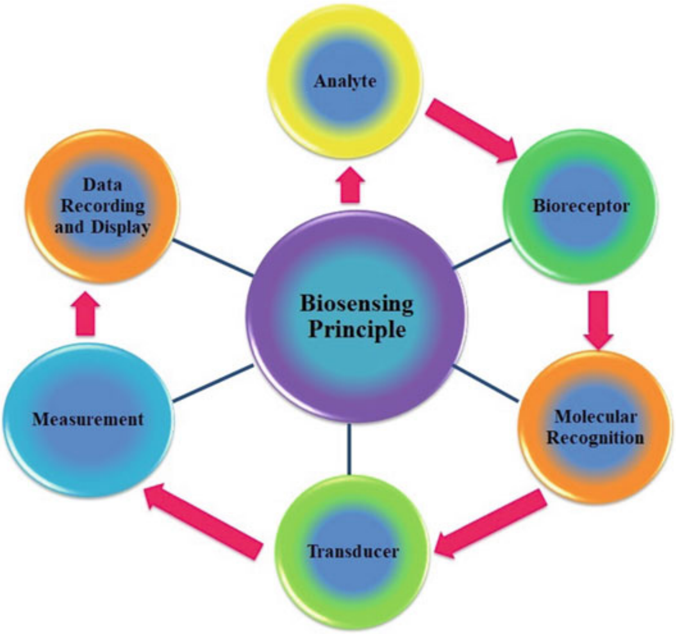

Fig. 6.1 Basic principle of nanobiosensors

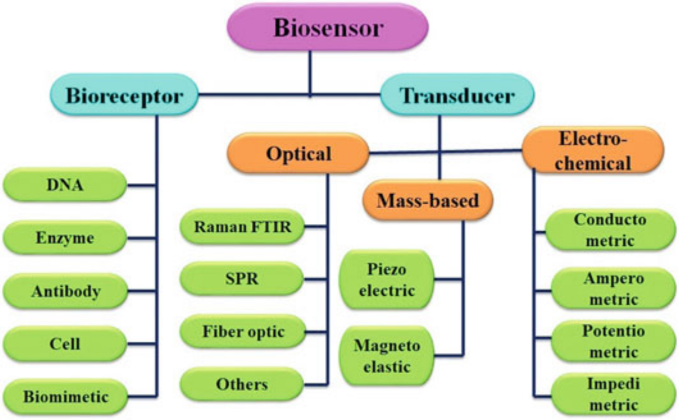

Fig. 6.2 Classification of bioreceptors and transducers

6.3.1 *Detection of Heavy Metals*

Metals having a size more than five and the size is more than 4.5 g/cm^3, iron can generally be described as heavy metals having cadmium, copper, gold, mercury, silver, iron, lead, etc. Copper, zinc, chromium, mercury, cadmium, and arsenic are difficult to decompose and therefore tend to accumulate. These heavy metals and other heavy metals enter the water system through human activities, industrial resources, and agricultural activities, creating inequality throughout the environment. A lot of heavy metals have harmful and poisonous results on our surrounding environment, and they can be absorbed into living things. If heavy metals accumulate to a certain extent in the body, it will cause chronic toxicity. These metals often contain cadmium, mercury, chromium, lead, metalloid arsenic, and other heavy toxins that are essential for pollution. Heavy metals are difficult to integrate with biodegrade, and they are easy to accumulate in the body due to the food chain's biomagnification effect (Jaishankar et al. 2014). Heavy regenerative metals can ground permanent damage to the human being since they can cause persistent toxicity and dysfunction of enzymes and proteins in human body. With rise of human activity, heavy metal ions have become a major environmental obscurity and this cannot be ignored. Consequently, it is important to build up an efficient, fast, sensitive, precise, and extremely selective method for extraction of heavy metal ions (Tian et al. 2019). Lead ions are one of major noxious waste of iron, which can accumulate in the food chain and have many negative effects on human health (Dusengemungu et al. 2020). Because of the public health risks associated with lead ions, several nations and organizations have stringent regulations regarding sanitation to lead ions in drinking water. Environmental Protection Agency of the U.S. has set a highest ion pollution stage in drinking water at 72 nM, while the Global Agency for Research on Cancer has stated that safety ions lead to food and drinking water at 48.26 nM (Kumar et al. 2020). It is very important to develop a new analytical approach to find the most common and effective ion-leading process in the environment.

The area around the food chain is highly toxic and endangers human health and the environment. Therefore, researchers must develop a faster, simpler, less expensive, and more efficient way of monitoring and detecting quantities of cadmium ions in our surrounding environment (Mohamed 2020). Because of its ease and cost-effectiveness, nanosensors of electrochemical have been repeatedly used to detect ions of cadmium over the past five years. Researchers have proposed a new sensor of electrochemical cadmium ions based on reduction of graphene oxide, gold-nanoparticles, and nano-conjugates of tetraphenylporphyrin ions (Singh et al. 2020). Improved electrochemical sensitivity can be enhanced by introducing Au nanoparticles. Arsenic ions are also considered to be a form of toxic pollutants, as their presence can cause a variety of physical condition and ecological problems like kidneys, skin, lungs, and bladder. Because of the need to create a simpler, faster, and more efficient way of analyzing the values of arsenic ions in nature, instead of traditional visual techniques electrochemical techniques are more appropriate for monitoring arsenic ions (Gumpu et al. 2018). It is easy to use, little expenditure, and elevated sensitivity. For instance, Gumpu and his team produced an excellent nanosensor for determination of arsenite and arsenate based on converted oxide of ruthenium-graphene-bipyridine nanocomposite. This advanced sensor has excellent arsenic (III) and arsenic (V) detection features, with detection restrictions of 21 and 34 nM, correspondingly.

6.3.2 Detection of Microorganism

Microbial biosensors are a rapidly developing research field, and many related publications have been published since 2005. In the 1980s, biosensors were mainly used in sensitive, short-term, and accurate measurement fields. Researchers speculate that this period may have dominated hospitals and home diagnostics in the 1990s. Lastly for a long time, there have been significant improvements and symptoms within the biosensors of the detection of rare local viruses such as Vibrio cholerae, Treponema pallidum, and Mycobacterium tuberculosis (D'souza 2001; Alberts et al. 2002). Small animals have always been an important part of our lives on earth. Bacterial reproductive factors are rapidly being used in the manufacture of various products. However, some microorganisms can cause devastating diseases in human beings and can be a reason of serious harm (Cavazzana et al. 2004). Rapid development of other microorganisms permits them to acclimatize and nurture under traumatic situations. Due to the nature of microorganisms and progresses in hereditary engineering, it is impossible to eliminate the possibility of using these harmful insects intentionally to damage health and property. In this case, the controls we currently have appear to be slow and ineffective. The best new technology to solve this dilemma is the use of nanobiosensors, which gives us a device to quickly identify the existence and number of microbes in a given atmosphere (Sheridan 2011; Dwivedi 2016).

Biomolecules such as enzymes, antibodies, receptors, organelles, microorganisms, and cells or tissues of animals and plants are used as biological detection elements. Among them, the advantage of microorganisms is that they can detect a wide range of genetically modified chemicals and a wide range of pH and temperature, making it an ideal biological detection material (Patel et al. 2016). Microorganisms are incorporated into various sensors, such as current, potential, calorimetry, conductance, colorimetry, luminescence, and fluorescence, to make biosensor devices.

6.4 Nanobiosensors in Health Care

Nanobiosensor plays a major role in the field of health care. According to World Health Organization (WHO) figures, one third of world's population is contaminated with Mycobacterium tuberculosis, and 55% of Asians are reported to have been infected with Mycobacterium tuberculosis (Sharmila and Thomas 2018). A variety of biosensors are used to detect tuberculosis, such as surface plasmon resonance and nucleic acid nanosensors. Cholera is a common disease that occurs in areas with small laboratory equipment, so we need fast, low-cost portable equipment. It is used to detect Vibrio cholerae at a rate of 105 cells/ml V.Cholera O139 in the form of an O1 serotype (Ogawa) (Ali et al. 2020). Using this method, the contaminated sample can be obtained directly without looting and is not disturbed by the contaminated matrix. The large amount, caused by binding to the antigen and antibody, is a change associated with the abundance of crystal resonance. One of the most significant uses of nanobiosensors is quick recognition of viruses in blood samples stored in blood banks. Immunosensing-based immunosensors have been described in syphilis tests. However, this sensor does not detect precursors in the bloodstream, although it detects the existence of antibodies, indicating presence of the Treponema pallidum pathogen (Malham et al. 2014; Landers et al. 2012).

6.4.1 Nanobiosensors for Detection of Food- and Water-Borne Microorganisms

Microorganisms such as bacteria and viruses are widely present in the environment, food, ocean and estuary waters, soil, and the intestines of humans and animals. Many of these organisms play important roles in nature, but some are potentially harmful microorganisms. It may have serious adverse effects on animals and humans, causing millions of dollars in losses to the food industry (and indirect consumers) every year (Priyanka et al. 2016). It is estimated that of the approximately 50 million deaths worldwide each year, approximately 40% are caused by infectious diseases. Pathogens cause 100,000–20 million deaths each year, and another 200 million non-fatal infections (Zulkifli et al. 2018). Specific methods were used for detection of these microbial contaminants. *Escherichia coli* is famous for originating food poisoning. It can contaminate pullet, vegetables, and dairy foodstuffs, and it is reported that *Escherichia coli* bacteria were detected in less than 1 h using a swing cantilever. Evidence is provided by measuring the change in the resonance frequency of cantilever matrix, which is due to enlargement in mass caused by adsorption of pathogens in cantilever. Reference arm is used to eliminate unwanted environmental changes (Labbé and García 2013). To detect the occurring trophic layer and gas phase, the use of sensors can be extended to the detection of various microorganisms. Drinking water is often polluted by a number of pathogen and its noxious waste; therefore, there is a great need for biosensors designed to detect them simultaneously. The use of commercially available assays to detect water-borne pathogens has been successful, with a detection limit of 10–1000 organisms per milliliter (Hernández et al. 2017). The research based on nucleic acid biosensors has increasingly become the focus. Even at low concentrations, nucleic acid biosensors can provide the required sensitivity, especially for detecting water-borne pathogens. Figure 6.3 shows the detection of specific pathogens in environmental matrices in different types of assays and the gradual development of corresponding biosensors.

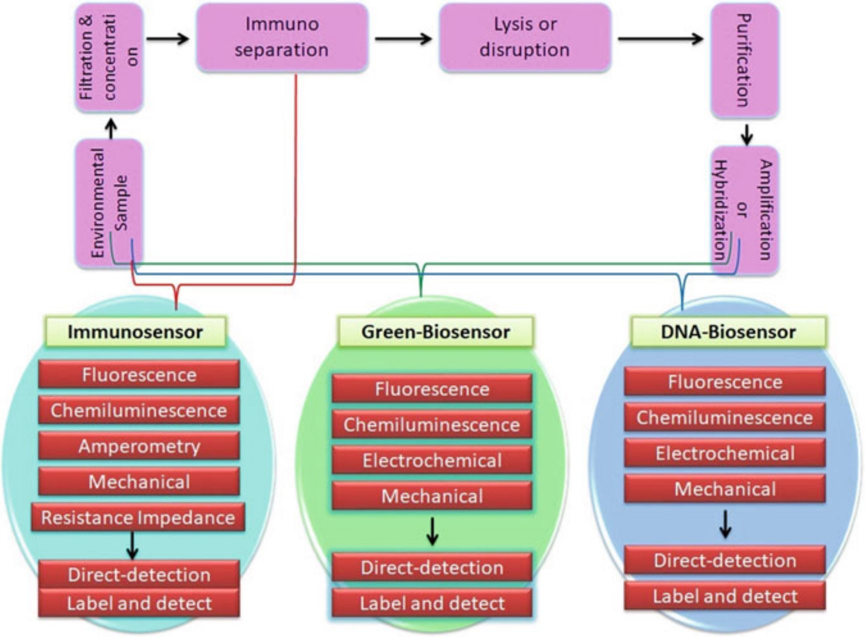

Fig. 6.3 General procedures for the detection of specific water-borne pathogens in environmental matrices in different types of assays and the gradual development of corresponding biosensors (including immunosensors, DNA-based sensors, and green biosensors)

6.4.2 Nanobiosensors for Detection of Microbial Toxins

Food safety is a major concern worldwide. When food is contaminated by organisms called food-borne pathogens (such as fungi, bacteria, and viruses), many food-borne microorganisms occur, leading to food-borne diseases. They are the culprits of food and water poisoning. Some bacteria are a major cause of food-borne illness; the most important are Escherichia coli, Salmonella enterica, Campylobacter jejuni, Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus (Xu et al. 1999). People with food-borne illness will experience symptoms such as vomiting, nausea, and other neurological disorders. These symptoms may appear within a short time or within 48 h, indicating the severity of the disease. It is produced by bacteria and should be tested for food safety. Some toxins produced by common substances such as Staphylococcus aureus grow in food and produce Staphylococcal enterotoxins A and B, leading to toxicity (Ahmed et al. 2014). *Escherichia coli* O157: H7 produces toxins similar to Shiga 1 and 2, leading to diarrhea and hemolytic uremic syndrome. Listeria monocytogenes produce an exotoxin called Listeria hemolysin O, which acts as a hemolysin. Lipopolysaccharides (LPS) are also considered toxic, and the outer membrane is irritated by gram-negative bacteria including cyanobacteria called endotoxins (Kirsch et al. 2013). Various biosensor detection methods have been developed in the food industry and have been widely used for some time. By all means, weight conversion, electrochemical, and optical transitions are sensitive and precise. When using biosensors, the location of bioreceptors is required to identify and measure various viruses. The aptamer beacon lamp that responds to botulinum toxin was developed by Bruno et al. (2015). They have identified several sequences that can create the necessary barriers to the blurring effect of cellular bacon. Three of these sequences showed fluorescence when exposed to elevated levels of botulinum toxin A. They found the best sequence. The buffer is limited to 1 ng mL^{-1}, but responds only to natural samples, not to botulism in human serum. The beacon is suitable for real-time analysis of environmental samples containing a variety of biotoxins, including staphylococcal enterotoxin B, and other aptamer lamps developed by his team are highly suitable for clinical use of biosensors. The researcher reported a non-labeled biosensor acquisition verrucarin-A at concentrations of only 2 pg/mL^{-1} in the buffer and 6 pg/mL^{-1} in powder samples. Its sensors are derived from Fourier Transform Infrared Attenuation (FTIR-ATR) and sensitivity to antibodies. When interacting with ATR materials, FTIR-ATR can perform spectral analysis on chemicals in solution, while FTIR can provide measurement details in addition to spectral features and accurately identify interacting molecules (Hennekinne et al. 2012). Staphylococcus aureus can cause food poisoning in Staphylococcus aureus because it can produce seven different toxins that can contaminate food. These bacteria are widespread and are found in nose and skin of 25% of well-being persons and animals. An unsafe cooking method is used by people with infections. As bacteria grow, they release heat-resistant toxins and do not rot during cooking. Staphylococcal food poisoning is usually rapid and can cause minor intestinal symptoms. Excessive doses of staphylococcal enterotoxin B can cause adverse health effects as well as sensitive and light processes (Sergelidis and Angelidis 2017).

6.4.3 Nanobiosensors for Detection of Viruses

Virus encephalitis, viral hemorrhagic fever, smallpox, and further infectious diseases have been identified by the Centers for Disease Control as potential weapons of mass murder or bio-terrorism. This type of drugs can be complicated to distribute; however, due to the limited vaccination and treatment options available, when these drugs are used in most people, the risk increases significantly due to infection. Like anthrax, smallpox is one of the two most common diseases. Because of the high risk, stability when transporting aerosols, and high production capacity, the power of weapons increases. The availability of a vaccine for smallpox is limited because the effectiveness of the stored drug is not guaranteed (Berche 2001). It is hard to come up with a small virus, but because of the infection, its effects can be very dangerous, and less than 20% of people are immune to previous vaccines. There are many viruses that can cause bleeding, including Lassa fever and Rift Valley fever, Ebola hemorrhagic fever, and Marburg disease. These pathogenic organisms can be active ingredients due to the high aerosol infection, killing, and possible recurrence in tissue culture (Cleri et al. 2006).

Existing diagnostic methods include culture, cell, immunosorbent assay (ELISA), and PCR. These methods are not suitable for care points other than exclusive infrastructure, and these methods are time devastating and work intensive. Antibacterial antibodies and peptides provide biorecognition options. These substances are used as biosensors and sensors in critical optical, electrochemical, and mass-sensitive processes (Sakamoto et al. 2018; Mokhtarzadeh et al. 2017). Researchers have synthesized a photofluidic nanobiosensor for recognition of viruses based on plasma nanopores. Its advantage is that it is able to detect many viral particles that do not change without damaging the formation or charge of nucleic acids, thus allowing for extensive research. The antibody is bound to the nanoporous matrix, and light transmission is measured by resonance, comparable to the occurrence of surface plasmon resonance (Gupta 2005). Gupta et al. recommended use of antibody-bind antibodies to detect viral particles by modifying changes in microcantilever resonance frequency. They rated the average dry weight of one vaccinia virus to be 9.5 but no identification factor was used. Microarray is a new and evolving method of detecting animal viruses, the basis of which is the integration of compatible DNA molecules (Dinh et al. 2014). In a biosensor connected to a microchip, thousands of DNA samples can be moved to the upper electrode. Specifically, viral RNA must be converted into complementary DNA. The DNA molecules can be color-coded or fluorescent for easy access. A microarray-based biosensor containing synthetic dextran was developed to directly detect flu viruses. Synthetic glycans and their free amines are a screen printed on glass slides to create microarrays with small focus points (Dalal et al. 2020). The nanobiosensor can sense and separate H1N1 and H3N2 with a recognition limit of 10 PFU/mL. It can also be used to obtain antiretroviral drugs such as oseltamivir, zanamivir, and other chemicals and antimicrobials in these compounds. Through the ELISA method, the antigen virus is connected to a robust interface, usually a microtiter plate. An immovable antigen is then expressed in the action of the anti-HIV antibody. Detection of HIV antigens by antibodies has been assessed by indirect enzymatic processes, using second-line antibodies or proteins of enzyme linked to detect (Čavić et al. 1997). However, multi-step process and sufficient revitalization of the acquisition area require the improvement of consistent methods of perceiving and measuring viral load of immunodeficiency human type 1 (HIV1). In the field of nanobiosensors, use of SPR-based optical methods can be very helpful in better understanding the functional characteristics of HIV contamination and its control. First commercial use of optical nanobiosensor technology is occupied in epitope-mapping of antibody monoclonal anti-HIV capsids. Cavic et al. tested real-time detection of peptides bound to HIV1 TAR RNP inactive in piezoelectric quartz crystals. With diverse peptides extracted from the surface of the nerve, different responses were obtained (Gill and Garg 2014).

6.4.4 Nanobiosensors for Detection of Pesticide

Pesticides are mainly used in agriculture to increase crop yields because they are very profitable. Due to its insecticidal properties, various types of insecticides are designed to stay in the environment long after the introduction of high performance. In addition to its stable structure and agricultural benefits, pesticides have also had serious toxic effects on certain species. If it is installed at a higher concentration, its accumulation in living systems may be harmful. Therefore, its fast and accurate analysis is essential. Conventional methods such as chromatography (GC, HPLC) used to detect insecticides are subject to diverse precincts, such as low sensitivity and efficiency, labor-intensive, expensive, and well-trained personnel, technical equipment, and many other equipment. A reduction method that can identify all these neuro-toxic complexes by sensitivity, selectively, quickly, and effortlessly in the field is required (Kumar and Kalita 2017).

The basic need for greater use and development of pesticides is to achieve high yields on farms. It is anticipated that about 45% of food production is vanished through pests each year. The best way to increase the yield is to have an effective pest control program. The pesticides are divided into organochlorine, carbamate, organophosphorus, synthetic pyrethroid, and pesticides that do not react according to their chemical composition. Pesticides can also be classified according to their intended use, such as fungicides for nematodes, fungicides for fungi, and herbicides for weed pests. There is also a class of pesticides made of natural materials called biological pesticides (Dincer et al. 2019).

Nanobiosensor is an efficient analysis tool that can solve the problems related to traditional pesticide detection methods. Biosensors have opened the way for simpler and more effective high-precision detection of pesticides. Detection of very small amounts of target substances, incessant monitoring, and inexpensive and decentralized on-site examination are the basic characteristics of nanobiosensors. A close relationship between organic procedures and signal production creates opportunities for the development of compact, easy-to-use, and investigative instruments with high specificity and sensitivity. Due to their biological basis, they are very suitable for toxicological measurement (qualitative) of pesticides, although traditional methods can simply appraise pesticides quantitatively (Shruthi et al. 2014). In twenty-first century, the development of biosensors is advancing by leaps and bounds. Different biosensors are used to manufacture biosensors for the determination of pesticides. With many differences, various fixing methods have been used. The development of immunosensors has also played a role quickly. Several cost-effective aspects of nanotechnology are also related to biosensor technology (Xu et al. 2018).

The simplest and most innovative biosensor method for enhancing enzyme biosensors to detect pesticides is based on the inhibition of the enzyme cholinesterase. Two types of enzymes are known: acetylcholinesterase and butyrylcholinesterase. Biosensors are derived from activities designed to detect pesticides (especially organophosphorus pesticides). Peroxidase-based biosensors are another options that can be used to make enzyme-based biosensors. In some cases, biosymymatic and triencymatic methods are used to develop biosensors. In addition to enzymes, ALP biosensors and tyrosinase biosensors have also developed various microbial/whole-cell biosensors for pesticides, such as Paraxon, carbamate, malathion, and thiodicarb (Audrey et al. 2012). As a critical factor in the development of biosensors, microorganisms have many advantages contrast to enzyme systems. Microbes have the capability to combine many chemical substances, which are inaccessible to enzymes. In the case of a complete cell system, expensive enzyme purification processes are not required, and they themselves act as a source of intracellular enzymes (Xu and Ying 2011).

The immunosensor has shown large potential and has become a small, inexpensive gadget for monitoring the in-situ nature samples. Compared with enzyme-based biosensors, they can test overall toxicity because immunosensors are specific to certain chemical elements. In addition, many enzyme-derived biosensors are used for detection purposes and are unable to differentiate certain pesticides (Mehrotra 2016). Enzyme biosensors can only estimate the total number of pesticides and cannot provide specific information about specific pesticides. Immunosensor is another way to get pesticide, and it uses antibodies or antigens as certain biosensor substances. In recent years, the use of insect antibodies has become increasingly important, leading to the introduction of various insecticide-targeting immunoassays. Pesticide residues have made significant progress in the twenty-first century. Immunoassay based on antibody-hapten reaction has evolved into an alternative method of analyzing agricultural chemicals and pollution due to its specificity, economic efficiency, high yield, and attractive environment for the acquisition of small molecules/pesticides in food and other environmental samples (Herdman et al. 1988).

6.5 Nanobiosensors for Environment Safety and Security

For new technologies such as nanobiosensors to be successful in the environmental market, the development of these nanobiosensors must fill gaps in the field of monitoring and acquiring matrix technology, which will have a significant impact. This can be achieved in a variety of ways. These include (i) providing new opportunities that have not previously been achieved and (ii) providing clear advantages over existing site analytics methods such as cost, time, sensitivity, or details of specific applications. The current trend of biosensors is achieved with the help of little, easy-to-use, and quick sensors, called smart systems. A major challenge that will be solved in future is the growing need for increased sensitivity and selection, so that molecules can be considered in real time at very low cost. Future nanobiosensors will operate on a lab-on-a-chip basis, where all key components are micro-built into the chip to facilitate and increase reliability monitoring. However, real success lies in the development of a reliable nanobiosensor for clinical and clinical research. Environmental use relies on concerted efforts of scientists from many fields, including chemists, biologists, and engineers. In this perspective, significant advancement is anticipated in field of bioelectronics, making it easier to transfer signals from biometric components to electrical or optical devices, and vice versa.

The future nanobiosensors will entail the upgrading of the latest steady gadgets or construction of these that are in the process of allowing for advanced transmission, amplification, processing, and natural signal modification. Active nanobiosensors will no longer be seen as a stand-alone tool, but will now become a critical part of the analysis process. Integrated and compatible devices will represent any other end-of-life creation of in-depth studies of various disciplines. Significant improvements are being made to genetic engineering in the production of robust bioreceptors and selected as additives for visual acuity, as well as enzymes. The nucleic acid proteins and synthetic peptides will persist to promote to the achievement of sensible nanobiosensors. Finally, outstanding nanobiosensor software in the environment can be used as testing equipment for more than one analyzer. Advances in biosensor studies will allow for the elimination of automated diagnostic devices in clinical trials.

Also, the use of nanotechnology in biosensors introduces novel capabilities to enhance the brand new generation of biosensor era. Nanomaterials are primarily based entirely on nanobiosensors that set the main features and are commonly performed in medical examinations, dietary pattern tests, and environmental tracking internally next to the complete path. New types of features must be developed in an area of nanotechnology so that the nanobiosensor and nanotechnology interaction process shows to be more prolific. Use of the nanobiosensor era in an ecological guidance has so far not been fully explored in scientific experiments. Most commercially available nanobiosensors are associated with the application of scientific instruction, while the most effective are designed to follow environmental samples. Therefore, the great value of the effort continues to be needed to develop large and reliable advanced devices that allow for the acquisition of highly pesticides as limits below. Improving the balance of a biocomponent garage requires attention.

6.6 Conclusion

There is now a clear and growing need for faster, more efficient, and less expensive methods of environmental analysis on site. Because of its versatility, nanobiosensors can find many specialized systems in many other ways and technologies that counteract this expansion. In fact, it is reported that various enzymes, antibodies, and microbial nanobiosensors using optical, electrochemical, and acoustic transducers can measure significant levels of environmental pollution from a variety of chemicals. However, consideration should be given to issues such as the needs of potential environmental applications, the ability to customize laboratory models, and whether there is sufficient market to ensure the investment required to sell these devices. Although the development of environmentally friendly nanobiosensors is no small feat, there seems to be ample evidence that biosensors can be adapted to have lower selectivity, sensitivity, and lower production costs. In addition, if designed, tested, and marketed, nanobiosensors can have a significant impact on cost savings and improve the effectiveness of certain environmental monitoring applications.

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Chapter 7
Role and Significance of Nanobiosensors for Environmental Remediation

A. Manjceevan and K. Velauthamurty

Abstract Environmental pollution has become a global challenge due to non-environmentally friendly activities and scrutiny of novel techniques for environmental remediation. The detection and quantification of pollutants is a prime task for environmental remediation. The use of nanobiosensors has become one of the hot topics in the detection of environmental pollutants. Nanobiosensors are currently used for rapid detection and identification of environmental pollutants including herbicides, pesticides, polychlorinated organic compounds, heavy metals, and pathogens. This chapter mainly focuses on the principle of nanobiosensors and the role of nanobiosensors associated with environmental remediation and summarizes the recent and future trends of nanobiosensors.

Keywords Nanobiosensors · Heavy metal · Detection of pollutant · Environmental monitoring · Remediation

7.1 Introduction

Environmental remediation is a challengeable task to sustain the living condition for the future generation due to the continuous generation of pollutants in the environment by non-eco-friendly activities mostly by human beings. The overgrowth of population, urbanization, industrialization, and change in lifestyle complicates the environmental balance. The enhanced production of goods leads to consuming more natural resources and disposal of industrial byproduct as waste damage the environment continuously. Further, in conventional agriculture practices, farmers use excessive synthetic fertilizers, pesticides, and fungicides, to harvest a better yield. The excessive use of fertilizer may enhance environmental toxicity (Kumar and Guleria 2020). As a consequence, the excess of such fertilizers, pesticides, and fungicide usage may reach the reservoirs by leaching, and it leads to the algal bloom, accumulation of phosphate and nitrate, and heavy metal toxicity. Industrial wastewater

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may consist of numerous harmful toxic organic and inorganic materials including dyes, heavy metals such as arsenic, chromium, nickel, lead, cadmium (Kalita and Baruah 2020). The toxicity of heavy metals arises from the ability to bind with the proteins, displacement of essential elements, and bioaccumulation (Ferrari et al. 2020). Heavy metals and polychlorinated organic compounds may be persistent in the environment without degradation and get into bioaccumulation. Further, these chemicals even in low concentration may lead to harm to humans. These elements get into the body, and the concentration increases through the food chain. Particularly, based on the WHO guidelines, the maximum acceptable levels of heavy metals in drinking water are 3 ppb, 50 ppb, 10 ppb, 1 ppb for Cd, Cr, Pb, and Hg, respectively (Beyene and Berhe 2015). Therefore, the contaminants in the environment such as wastewater from industries, drinking water, soil, air should be monitored carefully and take proper clean-up action. There are several detection methods available to detect harmful chemicals. These include atomic absorption spectroscopy (AAS), inductively coupled plasma MS (ICP-MS), atomic emission spectrometry (AES), X-ray fluorescence (XRF), and so on (Ferrari et al. 2020). However, those instruments are highly expensive, time-consuming methods, need highly qualified technicians and may get a change of properties of samples during the handling and storage (Ferrari et al. 2020). Change of properties of samples may lead to error measurements. Therefore, fast, low-cost, user, and environmentally friendly methods are essential for environmental monitoring. Usage of nanobiosensors is one of the best methods to environmental monitoring of pollutants such as heavy metals, toxic intermediates, pathogens in wastewater, reservoir, soil, and in other vital features, etc., (Salouti and Derakhshan 2020). This chapter mainly focuses on the applications of nanobiosensors for environmental remediation.

7.2 Role and Significance of Nanobiosensors for Environmental Monitoring

Nanobiosensors could be used as an environmental quality monitoring tool. It provides a fast, reliable response with higher sensitivity, and the devices could be fabricated miniaturized. The nanobiosensors are used to analyze the biological quality, ecological quality, and organic and inorganic chemical monitoring (Salouti and Derakhshan 2020; Duhan et al. 2017). Specifically, the number of nutrients releasing from fertilizer, herbicides, and pesticides, as well as insecticides' amounts, presence of pathogens, pH, and moisture contents could be measured by using nanobiosensors. Further, it could be used to measure the parameters continuously, and it could be connected to the Global Positioning System (GPS) and perform the monitoring of parameters in real time. Further, the aforementioned technologies use in intelligent farming such as continuously monitoring environmental conditions, infected disease well in time, pest and weed, etc. By careful monitoring of the required nutrients and health of plants in agriculture leads to prevent the excessive use of fertilizer and carefully control the pest and weed on time. As consequence, we could protect the environmental damage (Duhan et al. 2017).

Nutrient delivery to the crops could be controlled by using the thin layer of nanomaterial coating and limited delivery of nutrients to the crop on the requirement. Nanotechnology synchronized the release of fertilizer and uptake of nutrients by crops. Biosensors exist with nanofertilizer monitor the release of nutrient-based on the environmental and soil nutrient content. Smart delivery systems release the fertilizer based on plant signals (Salouti and Derakhshan 2020).

Furthermore, the nanobiosensors used to detect environmental pollutants such as pesticide, herbicide residues, heavy metal pollutants, and other toxic substances evolved through industries and microorganisms. The quantitative and qualitative estimation and determination of pollutants is the first step to perform environmental remediation.

7.3 Nanobiosensors for Environmental Remediation

7.3.1 Fertilizer Residues

Nitrogen, phosphorous, and potassium are essential elements for plant growth. Depletion of such elements in the soil during the cultivation needs to be refurbished by using fertilizer. The greater amount of nutrients in applied chemical fertilizers were lost as waste into the environment such as 70% of nitrogen, 80–90% phosphorous, and 50–70% potassium enter into the environment without utilizing the crops (Duhan et al. 2017). The significant loss of such nutrients may enter into the reservoirs and air, through surface runoff, leaching, volatilization of gasses such as NH_3, N_2O, NO, and NO_2 (Mejias et al. 2021). The expel of such gasses may lead to environmental issues including algal blooming, greenhouse effect, and global warming. Therefore, the careful monitoring of such fertilizer supplements by precisely adding such fertilizer on the requirement of nutrient by plants. This could be achieved by using nanotechnologies, such as nanofertilizers and nanobiosensors.

Nanofertilizer increases the nitrogen usage efficiency by slow release of nutrients and as consequence suppresses the loss of nutrients into the environment. Nanobiosensors precisely tracking the nutrient release into the environment acquire the data of nutrient content in an environment with seasonal variation (Salouti and Derakhshan 2020). There are several nanobiosensors reported successfully to monitor the nutrient/fertilizer content up to now (Table 7.1). Interestingly, there are several nanobiosensors introduced to monitor the potassium, nitrate, phosphate, urea, and carbon content in the environment.

In smart agriculture, sensors are required to monitor the nutrient requirement on time. Normalized difference in vegetative index (NDNI) sensor is a tool to detect the nitrogen requirement by the plant. Nitrogen exists in deoxyribonucleic acid (DNA), and protein is essential for photosynthesis. NDVI indicates the biomass and speaks

Table 7.1 Nanobiosensors used to detect the pollutants from agriculture

Analyte	Bionanosensor	Transduction method	Sample	LOD	Sensitivity	References
NO_3^-	Co-immobilized nitrate reductase and poly(3,4-ethylenedioxythiophene) (PEDOT)	Amperometric response	Water-soluble contaminant associated with the increasing use of nitrate-based fertilizers	0.16 ppm	92 µA/mM	Gokhale et al. (2015)
NO_3^-	Nitrate reductase (NR) from the fungus *Neurospora crassa* entrapped within a polymeric chitosan matrix on glassy carbon (GC) electrode	Electrochemical, rotating disk cyclic voltammetry, or constant potential amperometry methods	Nitrate concentrations in the lake and river water samples without any pre-treatment			Kalimuthu et al. (2021)
Urea	Ag-NPs-functionalized nitrogen-doped single-walled carbon nanotubes(Ag–N-SWCNTs	Potentiometric response	Detect urea in tap water and milk samples	4.7 nM	141 μAmM^{-1} cm^{-2}	Kumar and Sundramoorthy (2018)
Urea	Urease/ZnO–MWCNT/ITO bioelectrode	Electrochemical impedance spectroscopy (EIS) and cyclic voltammetric (CV) techniques	Urea concentration	66 nM to 20.6 mM	43.02 µA mM^{-1} cm^{-2}	Tak et al. (2013)
Urea	Lipase from porcine pancreas, glycerol kinase(GK) from *Cellulomonas* sp. and glycerol-3-phosphate oxidase(G	Colorimetric method	1.0 µg/ml	20 µM	1.0 µg/ml	Pundir and Aggarwal (2017)

up the plant nitrogen requirement. In this method, red and infrared light were used to calculate the NDVI (Duhan et al. 2017). Red light falls into the visible region of the spectrum particularly in between 630 and 700 nm (Sorbellini et al. 2018). Chlorophyll mainly absorbs red light during photosynthesis. The healthy plant absorbs infrared light and scatters the near-infrared light (Duhan et al. 2017; Salouti and Derakhshan 2020).

7.3.2 Pesticide Detection

Pesticides cover a wide range of toxic materials including insecticides, fungicides, herbicides, etc. (Aktar et al. 2009). Among them, organochlorine (OC) insecticides successfully controlled the number of diseases such as malaria and typhus. The OC includes dichlorodiphenyltrichloroethane (DDT), endosulfan, endrin, heptachlor, lindane, and others. But these chemicals are banned or restricted in most of the countries after the 1960s (Aktar et al. 2009). However, these residues are still present due to their persistent nature (Guo et al. 2019). Later, organophosphates, carbamates, pyrethroids, herbicides, and fungicides contributed greatly to pest control. These pesticides are toxic and need to monitor the pesticide residues in plants products and the environment. This is essential for environmental protection (Aktar et al. 2009). Detection and quantification of a contaminant in the environment is the prior step of environmental remediation. Monitoring of pesticides needs a simple, inexpensive, rapid, sensitive detection method. Nanobiosensor is one of the best methods to monitoring such pesticide residues since it consists of most of the desired characteristics. Biosensors incorporated with nanomaterials improve the sensitivity due to the higher surface area and facilitate the electron transfer and electron activities (Fang and Ramasamy 2015). The transduction process in nanobiosensors in the detection of pesticides is mainly based on electrochemical detection, fluorescence, optical, electrochemical impedance measurements, electrochemical amperometric measurements, cantilever, and others (Salouti and Derakhshan 2020). Table 7.2 summarizes the recently reported nanobiosensors for pesticide monitoring.

7.3.3 Heavy Metal Detection

Heavy metals enter into the environment mainly due to the activities of the metal industrial process, rapidly growing agriculture, excessive use of fertilizers and pesticides, improper waste disposal (Briffa et al. 2020). Mercury, arsenic, lead, cadmium ions are toxic heavy metals and potential carcinogenic at trace levels. These heavy metals accumulate in the food chain and could lead to adverse effects on living beings. Therefore, the careful monitoring of heavy metals in the environment, food, drinking water, and biological fluid is essential (Li et al. 2013a). Heavy metals could be detected by conventional methods including atomic absorption spectroscopy (AAS),

Table 7.2 Nanobiosensors used to detect pesticides

Pesticides	Transduction methods	Nanomaterial used in nanobiosensor	Bioreceptor	LOD	References
Methyl parathion	Electrochemical based, cyclic voltammetry	Au nanosphere	Acetylcholinesterase	–	Jiang et al. (2016)
Paraoxon and methyl parathion	Amperometric biosensor	Carbon nanotube	Organophosphorus hydrolase	0.15 μM paraoxon and 0.8 μM	Deo et al. (2005)
Methyl parathion	Chronoamperometric measurement	ZnSe quantum dots, graphene-chitosan nanocomposite	Acetylcholinesterase	0.2 nM	Dong et al. (2013)
Paraoxon	Potentiometric method, fluorescence properties	Gold nanoparticles	Organophosphate hydrolase	20 μM	Simonian et al. (2005)
Paraoxon and methyl parathion	Fluorescence	Silica nanoshell on silver nanoparticles	Organophosphorus hydrolase with a histidine tail (OPH_{6His})	20 ppb to 2 ppb and 50 ppb to 10 ppb	Thakur et al. (2013)
Paraoxon	Fluorescence	Mn:ZnSe d-dots	Acetylcholine esterase	1.31×10^{-11} mol/L	Gao et al. (2012)
Paraoxon	Fluorescence	Nanomagnet-silica core–shell	Organophosphorus hydrolase (OPH) enzyme	5×10^{-6} μM	Khaksarinejad et al. (2015)
Paraoxon	Fluorescence	Gold nanoparticles	Organophosphorus hydrolase	5×10^{-11} M	Karami et al. (2016)

(continued)

Table 7.2 (continued)

Pesticides	Transduction methods	Nanomaterial used in nanobiosensor	Bioreceptor	LOD	References
Methyl parathion	Square wave voltammetric responses	Gold nanoparticles on silica particle mixing with multiwall carbon nanotubes	Methyl parathion hydrolase	0.3 ng mL^{-1}	Chen et al. (2011)
Methyl parathion	Square wave voltammetry	Nanocomposite of gold nanoparticles decorating a magnetic Fe_3O_4 core	Methyl parathion hydrolase	0.1 ng mL^{-1}	Zhao et al. (2013)
Methyl parathion	Amperometry signal	AuNP-modified GC electrodes	Methyl parathion hydrolase	0.07 ppb in 0.1 M phosphate buffer at pH 7.0	Liu et al. (2014)
Methyl parathion	Linear scan voltammograms	Au nanoparticles (AuNPs) on silica nanoparticles (SiNPs) mixing with multiwall carbon nanotube	Methyl parathion hydrolase	0.3 ng/mL	Ye et al. (2016)
Paraoxon	Optical Detection	Single-walled carbon nanotube films	Organophosphorus Hydrolase	5 μM	Kim et al. (2015)
Parathion-methyl, monocrotophos, and dimethoate	Fluorescence resonance energy transfer	$NaYF_4$:Yb,Er upconversion nanoparticles (UCNPs) and gold nanoparticles (AuNPs)	Acetylcholinesterase	0.67, 23, and 67 ng/L	Long et al. (2015)
Methyl parathion	Optical detection	Silica nanoparticles	Functionalized with polyethyleneimine (PEI), integrated with Sphingomonas sp. cells	0.1–1 ppm	Mishra et al. (2017)
Chlorpyrifos	Ultrasensitive electrochemical	Polyaniline (PANi) and carboxyl functionalized multiwalled carbon nanotubes (fMWCNT)	Acetylcholinesterase	8.8 ng/L	Nagabooshanam et al. (2020)

(continued)

Table 7.2 (continued)

Pesticides	Transduction methods	Nanomaterial used in nanobiosensor	Bioreceptor	LOD	References
Carbamate	Electrochemical	Gold nanoparticles	Acetylcholinesterase (AChE) and acetylthiocholine (ATCl)	1 nM	Song et al. (2016)

inductively coupled plasma mass spectrometry (ICP-MS), and UV–Visible spectroscopy. However, those methods require expensive instruments, high sophistic techniques, and properly trained personnel. Furthermore, these methods are time-consuming and difficult to measure the quantities of pollutants on-site (Li et al. 2013a). Therefore, the use of nanobiosensors is a good alternative to heavy metal detection. The use of nanobiosensors brings several benefits such as the possibility to detect multiple heavy metals simultaneously, high sensitivity, and reproducibility.

Fluorescence sensors are based on the chemical and physical properties of fluorophores, fluorescence intensity, lifetime, and anisotropy (Li et al. 2013a). Fluorescence occurs based on charge transfer or energy transfer process. The development of fluorescence sensors for heavy metal detection is mainly based on the Förster resonance energy transfer (FRET) process. This FRET process occurs due to spectral overlap of emission band of donor and absorption band of the acceptor (Li et al. 2013a).

Hg^{2+} ions were detected by using the FRET process in which organic dye molecules as fluorophore and quencher were used (Ono and Togashi 2004). Presence of Hg^{2+} ions forms the T-Hg^{2+}-T bonds and forms hairpin-like sandwich structure**.** As it forms the sandwich structure, fluorophore and quencher are brought closer and enable the FRET process and quenching of fluorescence to occur. The measurement of the quenching ability of fluorescence may facilitate the estimation of Hg^{2+} concentration.

Later, in FRET process instead of organic dye molecules, quantum dots (Q-dots), graphene oxide, metallic clusters were used as fluorosphere due to their attractive properties. Q-dots show higher extinction coefficients (Manjceevan and Bandara 2016) in which carbon Q-dots and graphene Q-dots are well suited in this process due to being free of heavy metals, excellent biocompatibility, and surface functionalities (Li et al. 2013a). Replacement of organic quencher by the gold nanoparticle, the SPR plays a major role in Förster resonance energy transfer mechanism. If the gold nanometal does not show the SPR, then the energy transfer mechanism is called nanometal surface energy transfer (NSET) (Li et al. 2013a).

Q-dots/ DNA/ Au nanoparticle-based nanobiosensor was used to detect the Hg^{2+} ions explained based on the FRET process (Li et al. 2011b). As shown in Fig. 7.1**,** oligonucleotides are linked to the Q-dots and Au nanoparticles. In the presence of Hg^{2+} ions, selectively binding of Hg^{2+} ions with thymine in oligonucleotides brought Q-dot and Au nanoparticles closely. As a consequence, energy transfer process occurs from Q-dots to Au nanoparticles by NRET process and quenches the fluorescence emission. Based on the quenching of fluorescence, the Hg^{2+} could be detected in a water sample. The limit of detection (LOD) in this method was 0.4 and 1.2 ppb Hg^{2+} in the presence of buffer and river water, respectively (Li et al. 2011b).

Based on a similar FRET process, the Pb^{2+} ions were detected by using a Q-dots-aptamer-graphene oxide sensor. Here, graphene oxide is used instead for gold nanoparticles. Surface-coated aptamer molecules bind with graphene oxide through aptamer molecules in which aptamer molecules transfer the energy from q-dots to graphene oxide. As consequence, the quenching of fluorescence of Q-dot occurs in absence of Pb^{2+}. However, the presence of Pb^{2+} induces conformational change and

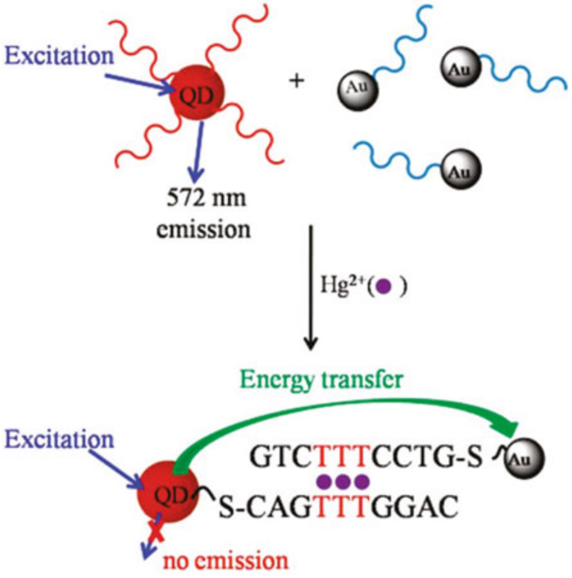

Fig. 7.1 Schematic diagram of the energy transfer process in Hg^{2+} detection. Reproduced with permission from Ref. Li et al. (2011b), Copyright 2011 American Chemical Society

forms G-quadruplex/Pb^{2+} complex. The formation of complex, followed by leaving of Q-dots from graphene oxide, turns on the fluorescence of Q-dot. The LOD of Pb^{2+} by this sensor exhibits 90 pM (Li et al. 2013b).

Hg^{2+} and Ag^{+} ions were detected by using nucleic acid-functionalized Q-dots and their use of logic gate operation. Freestanding DNA forms sandwiched rigid hairpin structure of DNA in the presence of Hg^{2+} and Ag^{+} ions. The fluorescence of Q-dots under the illumination of light is quenched due to electron transfer from q-dot to ions bound to the thymine or cytosine bases. In this method, the LOD of Hg^{2+} and Ag^{+} is 10 nM and 1 μM, respectively (Freeman et al. 2009).

Colorimetric sensor method is also used to detect the presence of heavy metal. SPR is an oscillation of conductive electrons in the noble metal in resonance with incident electromagnetic radiation. Aggregation of such noble metals leads to shifting in SPR. Based on this phenomenon, colorimetric sensor is used to detect the various analytes including heavy metals (Li et al. 2013a). Oligonucleotide-functionalized gold nanometals could be used to detect the Hg^{2+} ions. Hg^{2+} ions are selectively bound with thymine base, which leads to thiamine–thiamine mismatch, and it would lead to aggregation of gold nanometal. The aggregation of nanometals leads to change in SPR and color change. This colorimetric sensor shows a LOD value of 3 μM. Colorimetric methods are used to detect and monitor various heavy metals Hg^{2+}, Pb^{2+}, Cu^{2+}, and As^{3+}. On the other hand, aggregation of Au nanometal coupled with surface plasmon of neighboring nanoparticles provides enhancement of surface-enhanced Raman scattering (SERS). SERS is used to detect various heavy metals present in the analytes (Li et al. 2011a). Furthermore, some details of some nanobiosensors are shown in Table 7.3.

Table 7.3 Nanobiosensors used to detect the various heavy metals

Heavy metals	Transduction method	Used nanomaterial	Recognition element	LOD	Sample	References
Hg^{2+}	Optical, fluorescence emission	Quantum dot, gold nanoparticle	DNA	0.4 ppb buffer solution and 1.2 ppb in the river water	Buffer solution and in the river water	Li et al. (2011b)
Hg^{2+}	Colorimetric detection	Gold nanoparticle	Oligonucleotide	3 μM with 14 nm NP system	Aqueous solution	Xue et al. (2008)
As^{3+}	Surface-enhanced Raman scattering (SERS)	Silver nanoparticles	Glutathione (GSH)/4-mercaptopyridine (4-MPY)	0.76 ppb	Real water samples	Li et al. (2011a)
Hg^{2+} and Ag^{+}	Optical	CdSe/ZnS quantum dots	Nucleic acid	2 ppb	Water sample	Freeman et al. (2009)
Cd^{2+}	Cantilever surface to the photo-detector is proportional to the deflection	Silicon cantilever gold deposited, graphene oxide	Urease	18 ppt	River water samples	Ballen et al. (2021)
Pb^{2+}	Optical, localized surface plasmon resonance (LSPR)	Gold nanoparticles	Tripeptide glutathione (GSH)	47.6 nM	Lake water	Chu et al. (2015)
Pb^{2+}	Colorimetric sensor	Cellulose acetate (CC-CA) nanofibers	Curcumin	20 μM by visually and 0.12 ± 0.01 μM from the linear graph	Water sample	Raj and Shankaran (2016)
Hg^{2+}	Electrochemical DNA sensor, electrochemical impedance	Microspheres of cuprous oxide and nanochitosan	DNA	0.15 nmol L^{-1}	Water or the environment	Liu et al. (2015)

(continued)

Table 7.3 (continued)

Heavy metals	Transduction method	Used nanomaterial	Recognition element	LOD	Sample	References
Pb^{2+}	Electrochemical	Multiwalled carbon nanotubes, gold nanoparticles	DNA	4.3×10^{-15} M	Water samples, environment	Zhu et al. (2014)
Hg^{2+}	Electrochemical	WS_2 nanosheet	DNA	0.5 pM	Water sample	Li et al. (2017)
As^{3+}	Electrochemical	Ag–Au alloy nanoparticles	Aptamer	0.003×10^{-3} µg/L	Water from natural water courses, lakes, and wells	Yadav et al. (2020)
Cd^{2+}	Electrochemical method	Multiwalled carbon nanotube (MWCNT)	DNA	2 nM	Environmental samples	Sreekanth et al. (2021)
Hg^{2+}	Fluorescent	Graphene oxide (GO)	DNA aptamer	0.92 nM	Water sample	Li et al. (2013b)
Pb^{2+}	Fluorescent	Carbon nanotubes	Aptamer	0.42 nM	Tap water and biological sample like serum	Taghdisi et al. (2014)
Hg^{2+}	Fluorescent	Gold nanoparticles	Aptamer	16 nM	Aqueous solution	Tan et al. (2013)
Pb^{2+}	Electrochemical	Graphene (GR)	Single-stranded DNA	3.2×10^{-14} M	Real water samples	Gao et al. (2016)
Hg^{2+}	Surface-enhanced Raman scattering (SERS)	Gold nanostar dimer	ssDNA	0.8 pg mL^{-1}	Aqueous environment	Ma et al. (2013)
Pb^{2+}	Colorimetric method	Gold nanoparticles	Peptide	20 nM	Aqueous solution	Slocik et al. (2008)
Cu^{2+}	Fluorescence	Gold nanoparticles	Glutathione	3.6 nM	Environmental and biological samples	Chen et al. (2009)

(continued)

Table 7.3 (continued)

Heavy metals	Transduction method	Used nanomaterial	Recognition element	LOD	Sample	References
Hg^{2+}	Fluorescence polarization	Gold nanoparticles	DNA	0.2 ppb	Eenvironment, water, and food samples	Ye and Yin (2008)

Table 7.4 Nanobiosensors used to detect *E. coli*

Transduction method	Used nanomaterial	Recognition element	LOD	References
Resistive biosensor	Titanium dioxide nanoparticle	DNA probe	1×10^{-11} M	Nadzirah et al. (2020)
Selective membrane-based electrochemical, cyclic voltammetry	Nanoporous alumina-membrane modified electrode	Antibodies	22 cfu mL^{-1}	Cheng et al. 2011)
Surface-enhanced Raman spectroscopy (SERS)	Silver on a silicon platform	T-4 bacteriophages	1.5×10^2 cfu mL^{-1}	Srivastava et al. (2015)
Absorption properties monitored	Mesoporous titania thin-film	Specific antibodies	1×10^2 cfu/mL	Mura et al. (2012)

7.3.4 *Detection of* Escherichia Coli

Escherichia coli lives in the gastrointestinal tract of human and warmblood animals (Lim et al. 2010). Most of them are harmless, and some of their stains particularly O157:H7 may cause diarrhea, urinary infection, and inflammation (Salouti and Derakhshan 2020). The existence of *E. coli* in water is used as an indicator of water quality parameters. Because it provides the details of fecal contamination of water. If the water consists high amount of *E. coli*, there is a chance exist more pathogenic organisms in such water. *E. coli* count could be detected by nanobiosensors. Most biosensors use antibodies as recognition elements (Price et al. 2017). There are several types of nanobiosensors reported that successfully detect the presence of *E. coli.* Some of these types of nanobiosensors are shown in Table 7.4.

7.4 Conclusion and Outlook

Nanobiosensors are applicable in vast areas such as analysis of environmental organic pollutants, heavy metal detection, application in medicine, engineering, and pollutant monitoring. These nanobiosensors are fabricated in small size, and as a consequence, cost-effective and user-friendly apparatus is available for sensing applications. In nanobiosensors, nanomaterials are embedded into biosensors to enhance the sensitivity. Nanobiosensors are used to detect pathogens, pollutants, toxic substances, and so on. Furthermore, nanobiosensors are facilitating quick measurement and reproducible results, and use to monitor the spatial and temporal changes of parameters. However, these fabricated nanobiosensors are applicable in artificial mediums, tap water, and so on. Interestingly, nanobiosensors are applicable to intelligent farming such as precisely supply the nutrient on the requirement of plant, continuously monitor the environmental conditions, and monitor the requirement by using nano biosensors. Additionally, nanobiosensors could be connected to the GPS and able to map the pollutant, water condition, soil conditions, and environmental condition. However, an almost huge number of the sensors are reported as tested using in tap water or artificial medium. Still, it was not applied to the complex system. However, to achieve the full benefits of nanobiosensors, more and more researches should be performed and nanobiosensors could be applicable in a complex matrix and extreme conditions.

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Chapter 8
Bioluminescence Sensors for Environmental Monitoring

Geetha Palani, Karthik Kannan, Venkatesan Perumal, Arputha Latha Leo, and Poobana Dharmalingam

Abstract Environmental monitoring describes the activities and processes which need to take place to monitor and characterize the environment quality. By influent toxicity biological wastewater treatment plants can be adversely affected. For biologically monitoring the environment, bioluminescent analysis is one of the most promising methods because the luminescent system is highly sensitive to even micro quantities of pollutants. Fundamental to these processes is the concept of bioavailability and bioaccessibility of these pollutants at a suitable and relevant scale. Environmental analyses are still based on chemical approaches that usually require an exhaustive extraction step before to chromatographic analysis. It is widely acknowledged that modeled values may be appropriate for human risk assessment but yield little information for hazard assessment in a wider ecological or environmental context. Many authors have demonstrated that chemical analysis alone does not provide information regarding the bioavailable fraction of compounds nor about their effects on selected biological receptors. Nano-biosensor-based biological assays can complement chemical analysis by considering the effects of all pollutants, including those not detected by chemical analysis or those unable to be fitted in a model. Some bioassays are suitable for biotesting of air, the chemical substances, soil, and water

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used in everyday life. This chapter aims to highlight the experimental and fundamentals insights and to emphasize next-generation luminescent nano-biosensors the yet-to-be-reached potential.

Keywords Nano-biosensor · Pollution · Bioluminescence · Environmental monitoring · Wastewater treatment

8.1 Introduction

Human action creates expansion of new compounds measurement that is delivered in the environment without earlier information on their possible poisonousness or effect in living organic entities. A biosensor is a sort of sensor that can recognize and distinguish a component inside a cell or tissue. It is made from artificially made biomolecules acknowledgment components and different sorts of physical or compound transducers. Due to the cross-disciplinary nature of their development, research on their advancement has been distributed in the fields of biological science, physical science, and data science (Long et al. 2013a, b; Parmar et al. 2016; Rasheed et al. 2019). These biosensors can be arranged into three sorts dependent on contrasts in their sub-atomic, cell and tissue detecting segments. The sub-atomic-based biosensors utilize organic dynamic substances like catalysts, DNA, antigens, antibodies, and biofilm as the columnist components. The significant benefit of these atomic-based biosensors is their high selectivity (Starodub et al. 2012; Vigneshvar et al. 2016). Be that as it may, the usability of this kind of biosensor has been restricted by weaknesses, for example, costly macromolecule seclusion, restricted location ability, and the short useable lifetime of the distinguishing atoms. Guaranteeing the wellbeing and nature of food and water is a worldwide issue deserving of consideration. World Health Organization (WHO) indicated that the basic human ailments and infections are in a general sense identified with the absence of admittance to food and drinking water, explicitly; utilization of perilous water represents ~80% of the detailed diseases (Bahner et al. 2018; Chang et al. 2013). Hence, it is of extraordinary importance to create exact and quick identification advancements to screen these two significant assets, further shielding human wellbeing. Food and water wellbeing are regularly influenced by a few variables, including the presence of microbes, poisons, natural pollutants, metals, and other harmful substances by and largely borne to the water and food through various channels (Cunha et al. 2018), particularly farming and modern cycles. Concerning to contamination of food, it eludes various food varieties which is ruined/corrupted for human utilization because it may be either contain small organisms (microorganisms) like parasites, toxic materials, or bacteria which makes it inadequate (Ding et al. 2019). Contamination in food is broadly isolated into different (three) classifications, which include physical, biological, and chemical contaminations, considering the idea of the pollutant.

In the visible spectrum area, bioluminescence is a strange normal phenomenon. It can be found on each part of the evolution tree that the capacity to emanate light in the apparent range is normal for living creatures. Simultaneously, most of glowing life forms occupy oceanic environments (Jokar et al. 2017). The natural significance of iridescence for many of these organic entities is as yet hazy, and bioluminescence keeps on the fascination for examinations. The specific interest is the chance of utilizing this quality for tackling different applied and essential issues. The danger of increment of paces of natural tainting puts an errand to settle one of the significant applied issues—improvement of express strategies for an assessment of contamination. Fast reaction and high affectability to the activity of different specialists (in observation with other natural tests) make bacteria bioluminescence a proficient analytical tool to decide miniature amounts of different inhibitors of organic action (Kaur and Shorie 2019; Kudłak and Wieczerzak 2020; Liang et al. 2019). Environment toxicity observing is a space of utilization where microbial bioassays are broadly utilized all the while for both the coordinated assessment of contamination in some random climate just as the invention and biotic impact of explicit substances. Luminescent bacteria—makers of oxidoreductase and luciferase—are given to make different arrangements for bio testing of various substances for just about a portion of a century. Bioassays dependent on luminous bacteria measure poisonousness and are frequently faster, less difficult, more exact, and delicate than different bioassays dependent on ciliates, daphnia, green growth, fish, etc. (Malik et al. 2019; McKeague et al. 2015; Moon et al. 2015). They are applied to screen countless poisons. Microbes of two common marine genera, Photograph bacterium and Vibrio (*Vibrio harveyi*, *Photobacterium phosphoreum*, *Vibrio fischeri* and *Photobacterium leiognathi*) are broadly utilized for this reason. Different tests of bioluminescent have been created now. They depend on bacteria bearing the qualities of bioluminescent frameworks of characteristic radiant isolated chemical substrate and microorganism's frameworks (Qi et al. 2020a). Glucose and lactate are the most commercial biosensors created and are engaged in clinical applications. Biosensor markets for food, agribusiness, military, drug, climate, and veterinary are to be analyzed still.

8.1.1 Proper Organism—Application and Choice

For the recognition of pollutants and their effects, their appropriate microorganism decision in the climate and their consolidation in the improvement of an environmental biosensor which is having the transducer capability is a vital advance. Yeast and bacteria are the primarily utilized products (Rapini and Marrazza 2017; Sun and Zhao 2018). The picked microorganism should be powerful and fit for explicit pollutant location in little fixations, to guarantee cost proficient detection. As of late entire cell biosensors and microbial power modules draw exceptional consideration on the environmental monitoring and genetic engineering field (Yang et al. 2017; Zhang et al. 2018). It is not possible to control the organisms of analyte location to improve instruments or in new life forms express them. DNA section detection systems coding maybe moved with streamlined developing state into model organisms, for example, *Saccharomyces cerevisiae* and *Escherichia coli* (Farzin et al. 2017). The organism

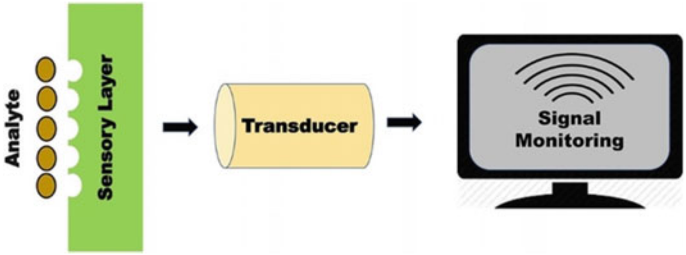

Fig. 8.1 General components of a biosensor (adapted with permission from Malekzad et al. (2017). Copyright [Malekzad et al.], some rights reserved; exclusive licensee [De Gruyter]. Distributed under a Creative Commons Attribution License 4.0 (CC BY))

and the location design ought to be consolidated appropriately to accomplish the most ideal identification of the sign.

This review manages the bioluminescence and their applications, in various areas and their luminescent system created, particularly in environmental things. The gathered data has been isolated in a few sections. The initial segment of bacterial luminescence concerns general highlights. The principal trademark and characteristics of bioluminescent bioassays, bioluminescent examination dependent on both regular and hereditarily luminescent systems and adjusted bacteria are chosen from them is in the second part. Further, it informs about the advancement of bioluminescent investigation of some ultra-structure of luminous bacteria, chemical substances on bacterial luminescence and particular consideration commits of bioluminescent assays utilization in climate observing. Also, in this review, aptamer-based biosensors for observing the quality of water, detection of bacteria, (Watanabe et al. 2015) aptamer-based biosensors for recognition of heavy metals in water, aptamer-based biosensor for monitor air and soil quality is broadly discussed, and the general components of a biosensor are shown in Fig. 8.1 (Malekzad et al. 2017).

8.1.2 Medicinal Plant—Cultivation Environment Monitoring

Restorative plant developmental climate observation incorporates pathogens identification, allergens, heavy metals, and toxins estimation. Pathogens are primary specialists for the crop production restriction there may lead to economic losses increase. Pathogens location is a significant initial step that deals with a sickness of the plant. Aptasensor is promising devices because of their quick, touchy, and exact pathogens location. Also, the following levels of assurance of pesticides, harmful mycotoxins, allergens, and heavy metals in bio drugs and restorative plant have gotten vital because healthcare organizations, and the consumers to this sort of toxins are cautious and delicate (Tchounwou et al. 2012). Hence, true insightful aptasensor development of bio drug contamination is basic for guaranteeing quick and touchy assurance. An aptamer DNA sensor was created to identify an ochratoxin A in plant item and mycotoxins, which is having greater particularity. For the identification of endotoxin Kim et al., created are used aptasensor which is made up of electrochemical gold nanoparticles from rough organic alcohol. This kind of aptasensor showed magnificent selectivity as well as affectability with a 0.01–1 ng mL^{-1} location scope (Li et al. 2017). Additionally, number of cases started to expand announcing serious hypersensitive responses to spice seeds of Lupin. To identify this issue DNA aptamer-based colorimetric detection framework is used to identify Lupin allergen levels in spice products. Endocrine disrupting compounds (EDC) structure an important type of pollutants that causes extreme wellbeing peril by upsetting typical endocrine capacities among human and oceanic organisms. One such compound is 17β-estradiol affects the male reproductive system (Liu et al. 2013a, b). Recently, fluorescence-based DNA aptasensor particularly and an exceptionally fast for recognition of 17β-estradiol in samples of low degrees (Jamdagni et al. 2016). A different type of biosensors is shown in Fig. 8.2 (Rodriguez-Mozaz et al. 2006).

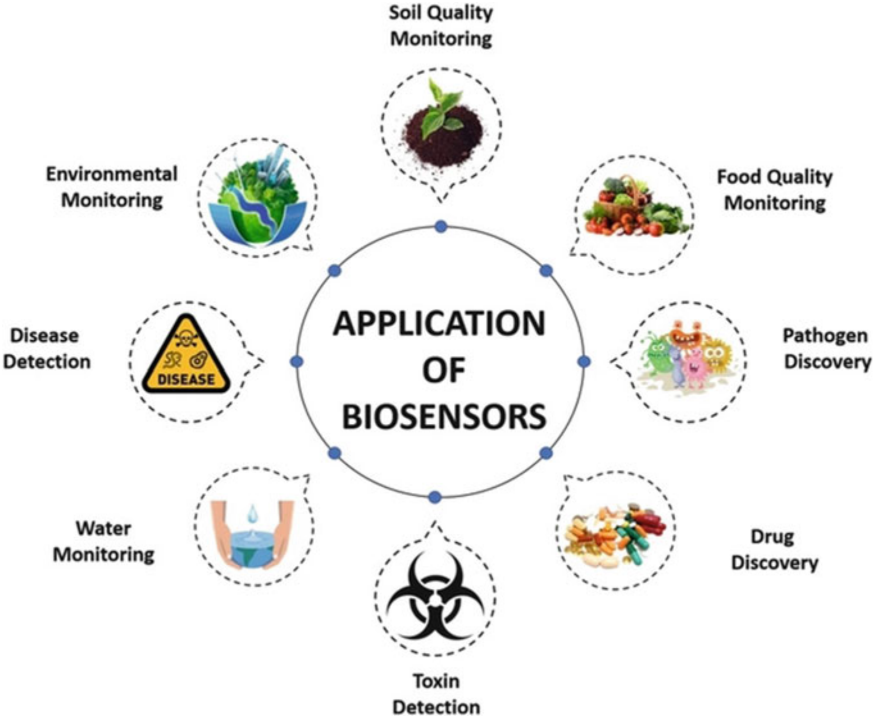

Fig. 8.2 Different applications of biosensors (adapted with permission from Rodriguez-Mozaz et al. (2006). Copyright [Rodriguez-Mozaz et al.], some rights reserved; exclusive licensee [MDPI]. Distributed under a Creative Commons Attribution License 4.0 (CC BY))

8.1.3 Infectious Disease Detection—Biosensor

A different irresistible disease spreads such as Nipah, Hendra, SARS, and avian flu have turned into a worldwide danger that requests extensive exertion their multiplication to manage. As there are different difficulties related to these irresistible infections, diagnostic tools should be developed for limiting/eradicating the odds of infection flare-up beforehand. Biosensors have arisen as an appealing apparatus for giving powerful data on these diseases (Liu et al. 2013a, b; Kim and Gu 2014). Generally, biosensors are described based on the nature of the cycle and their biological parts like immunological bodies (like antibody), biocatalytic agents (like enzyme), and nucleic acid material (like DNA). The biosensors dominant part produced for pathogens detection associated with cause's irresistible infection to depend on the electrochemical reaction principle (Li et al. 2019). This biosensor kind which holds the greater part as they of solution turbidity are of low power necessity independent, high sensitivity, simple instrumentation, and cost-effective.

An electrochemical method features such as amperometric, impedance, and potentiometric which are utilized to inspecting the progressions which happen on the detection of diseases (Wu et al. 2017). The biosensor of amperometric generally includes DNA hybridization, biosensor marker, and antibody antigen responses equal to an electrochemical transducer that enhances the sign for detection to a huge level. Glucometer is the most well-known amperometric sensor. Beforehand, associates of Gong have built up for identifying an amperometric-based immunosensor sickness of Newcastle (Shi et al. 2013). Immunosensor another amperometric likewise has been created to determine backwoods to have incredible exactness of spring encephalitis. Biosensors dependent for Japanese B encephalitis vaccine on mark free amperometric immunosensor has created. Another gathering of scientists built up to check the presence of Newcastle illness infection optical biosensor of 10 ng/mL with affectability. Severe acute respiratory syndrome (SARS) is identified by SPR-related immunosensors (Díaz-Amaya et al. 2019). Another kind of biosensor has likewise been created by the exploration gathering the mouth and food infection to survey, i.e., for a piezoelectric biosensors. Additionally, piezoelectric-related DNA biosensor likewise has created to distinguish hepatitis B infection contamination by scientists group with restricted fixation of about 0.02–0.14 μg/mL scope.

8.1.4 Bacteria as Biosensors

Bacteria are the essential makers in numerous environmental ecosystems and assume a fundamental part in the supplement cycle, and they are plentiful and universal. The microorganisms multiply quickly, are effectively distinguishable, simple to test, and react rapidly to changes naturally, such as pH, the presence of foreign substances or temperature which includes heavy metals (Geleta et al. 2018). These attributes make bacteria a great competitor as contamination biosensors. In this regard, bioluminescent bacteria like *P. phosphoreum* and *Aliivibrio fischeri* have been used to screen soil and water contaminated with HM. This bioassay is completed utilizing the normal bioluminescence by these bacteria discharge on the decline and depends on this fluorescence when the bacteria filled with examples of contaminated soil and water with various substantial metals like Hg, Cr, Cu, and Cd when compare with others (Kaur and Shorie 2019). The test has been grown monetarily because of *A. fischeri* and is dispersed under the Microtox® name. Hence, this technique is delicate to various pollutants like anti-infection agents, pesticides, poisons, and natural mixtures, for the recognition of heavy metals which makes it a non-specific method (Malik et al. 2019). Bioindicator proposed by *Vogesella indigofera* is another bacterium. This bacterium builds up a blue tone under ordinary conditions because of the Indigo dine creation. The microbes diminish the color creation when it fills within the view of Cr6, and this abatement is reliant of Cr6 upon the centralization; at 150 μg/mL, the bacteria are harsh and white (Pan et al. 2018). *Serratia marcescens* creates a red color known as prodigiosin, and it is a gram-negative bacterium; of Cd, Pb, and Cr in sub-inhibitory groupings; when the bacterium fills the pigment production diminish fast and it acts as a heavy metal contamination bioindicator.

In the environment, heavy metals presence applies extreme stress based on the living creatures. The high pace of level quality exchange and an expansion in its fixation can choose substantial metal-safe microorganisms. In this manner, detoxification genes and resistance have been utilized as biomarkers using molecular techniques for the study of polluted environments. Quantitative PCR and ongoing quantitative converse record PCR are some of the known availability (Sun and Lu 2018; Yang et al. 2017). Inside these qualities are those engaged with the protection from arsB (arsenical efflux pump), As, aioA (arsenite oxidase), ACR3 (arsenite efflux pump), arsM (arsenic methyl transferase), and arsC (arsenate reductase) those that present protection from Cu, and copA (Copper-exporting P-type ATPase), cusA (copper export system), for Zn, Co, and Cd obstruction, for hgcA (mercury methylating protein), czcA (Co/Zn/Cd efflux pump); mercuric reductase (Hg and merA) (Zong and Liu 2019); the mercury that involved in heavy metal-binding protein, and metallothionein a cysteine rich and sodA; which codes for a superoxide is mutase of toxicity against heavy metals associated with the protection. Commercially, available GeoChip (hereditary microarrays) is another procedure to find the heavy metals genes abundance and presence which involves in resistance. By utilizing this microarray, which is feasible to connect the findings of cueO, arsC (multicopper oxidase), copA and metC (cystathionine beta-lyase), terC (tellurium resistance protein), merB (alkylmercury lyase), and tehB (tellurite methyl transferase), (Rai et al. 2019; Mok and Li 2008), genes which are present in the sediments/waters contaminated with mercury, chromium, cadmium, copper, and sulfur.

Their processes and microbial populations are influenced by heavy metals. Consequently, the microbial cycle assessment addresses great exposure to biomarkers in various conditions. Inside the boundaries advance utilized are enzymatic exercises of the nitrogen and carbon cycle, biological system biodiversity, microbial mass, and the soil breathe the monitoring (Zhao et al. 2011). Microbial biodiversity is influenced by tainting with heavy metals. Heavy metal of higher concentration diminishes species of bacteria. In any case, DNA sequencing with the massive, contamination of bacterial gatherings were identified, for instance, Schneider and associates did the investigation by tracks down that the bacterial gatherings Verrucomicrobia, Chlamydiae, and γ-Proteobacteria showed a reliable reaction content across contrasting to Pb biological systems (Lu et al. 2010). The phyla Chlamydiae and γ-Proteobacteria and was available more plenty, the availability of verrucomicrobia was at high contamination less level plentiful. Thus, it is the reason that some proportions and gatherings should be taken as significant lead contamination bioindicator. In soils, contaminated with Cu bacterial richness was negatively affected, it was visible that at expanded focuses, and upgraded relative wealth of *Acidobacteria* and *Nitrospira* (Bidmanova et al. 2016) individuals of *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* of a lower portrayal proposing a promising job as bioindicator in soils and copper contamination.

8.2 The Principle of Bacterial Bioluminescent Biosensor

A biosensor is an incorporated scientific device that utilizes biological acknowledgment components, generally biomolecules, to tie a contaminator an analyte. Regarding the bioavailable part of the analyte, the binding event is caught by a transduction system. Biosensors are modest, fast, selective, and generally explicit. Because of different models, biosensors antibody receptors, organelle, tissues, cells, sub-atomic engraved polymers, liposome's just as microbial biosensors, revealed that the decision of natural reaction specialist relies upon analyte, particularity, stockpiling, and ecological steadiness (Zhang 2014).

Microorganisms are immobilized by a microbial biosensor on a transducer for the discovery of analytes. Albeit a few microorganisms could be utilized as microbial biosensors, bacteria are biotic local areas and control most environment capacities regarding biogeochemical cycling. Entire cell bacterial biosensors generally work on the rule of bioluminescence and might be constitutively communicated (Ding et al. 2019). Few bacterial biosensors with changing discovery limits have been created and effectively applied. The location furthest reaches of the bioluminescent biosensors are range from rather insignificant. Like capacity to code for a given protein presents certain capacities to different organic entities, bacteria can get by in a polluted environment if it can encode a resistance system. This is extraordinary in addition to in the constitution of biosensors. Bioluminescent detecting includes identifying change in the glow produced by the bacteria considering an objective analyte in a portion subordinate matter.

8.3 Aptamers

From the Latin word “aptus” signifying “to fit” and Greek word “meros” signifying “part”, and the term aptamer is got derived. They are single-stranded oligonucleotides or peptides (generally RNA/DNA) which may tie to the levels with greater explicitness like antigen-counter acting agent cooperation’s and partiality. The choice cycle is said to be the systematic evolution of ligands by exponential enrichment. The two independent exploration gatherings Ellington and Tuerk, in 1990 (Qi et al. 2020b; Li et al. 2019) found this interaction at the same time. To screen oligonucleotides tests, the SELEX interaction is utilized from huge oligonucleotides libraries (single-stranded DNA or RNA); by polymerase chain response (PCR) which includes in the vitro choice of an iterative cycle and resulting intensification of the chose successions. This kind of cycle is a significant examination instrument for bio-recognition test screening. In Fig. 8.3 (Li et al. 2019), DNA aptamer the essential screening steps have appeared.

On an irregular ssDNA library, DNA sequences are incubated first from the bound sequences with the unbound sequences and objective atom which is later isolated. ssDNAs bound are removed, by PCR enhancement, as the amplified library is filled in to the following SELEX round. The target for each, 6–12 consecutive rounds are carried out, and ssDNA library the final amplified is sequenced and cloned (Aravind and Mirroshandel 2017). From the studies, it has been seen that this kind of aptamers can be created and screened as oppose to a target bunch that went from peptides

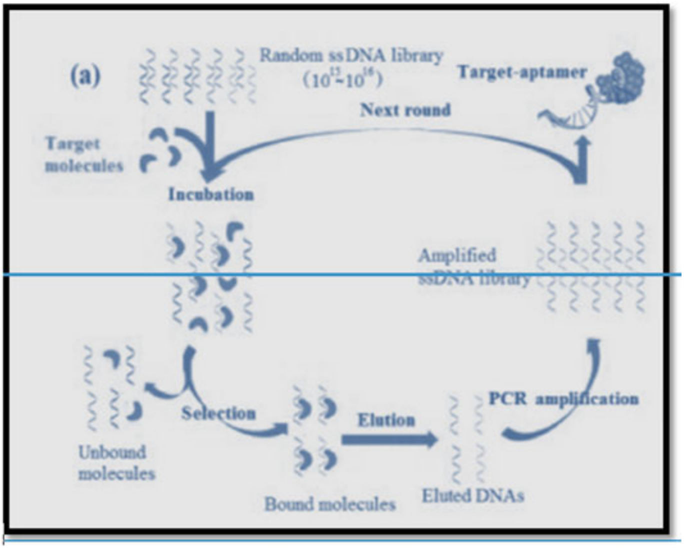

Fig. 8.3 Enrichment process—systematic evolution scheme of ligands by exponential (SELEX) (adapted with permission from Li et al. (2019). Copyright [Li et al.], some rights reserved; exclusive licensee [Elsevier]. Distributed under a Creative Commons Attribution License 4.0 (CC BY))

to metal ions, small molecules, entire cells, and proteins. Generally, this kind of aptamers is long of 25–90 bases, and its structural motifs including hairpins, kissing complexes, stems, internal loops, purine-rich bulges, G-quadruplexes, and pseudo knots. The greater part of them can tie to their targets utilizing complementary such as antibodies over other molecular recognition elements (MRE), aptamers have many advantages. Aptamers are a modest and simple ways to work and may treat with different reporter identifications, which includes electrochemical, fluorescent tags. When compared with antibodies they are steadier and can be utilized in a more extensive scope of the trial environment (such as various pH(s), salt conditions, and so forth). These beneficial attributes make them unique bio-receptors for use of E-aptasensors for the testing and observation in the plan of contaminants of water as well as food (Shi et al. 2018; Xu et al. 2012). The whole system principally comprises two sections, the signal transducer and the bio-recognition element. The transducer consists of a substrate of the electrode, an electronic detection system and a modified layer. The changed layers, which are frequently made from nanomaterials, fill in as the help material which inter connects to the substrate from the probes of aptamer. Generally, electro catalytic properties have extraordinary; which is likewise for signal amplification involves improving the electron move energy. For the E-aptasensors development, the substrates utilized are typically made of different materials of electrodes, like ITO, SPCE, AuE, GCE, and so forth (Dong et al. 2010). The detection system regularly incorporates a display screen, a processor, and a signal amplifier electronically. The E-aptasensor reaction of the detection strategy and it is significant badly affected by the decision. Bioreceptor is the recognition component of the sensor design is a similarly significant piece. In many E-aptasensor, in the example on electrode substrate aptamers are immobilized and allowed to cooperate with the interest of the analyte. By the transducer this interaction is estimated which yields a sign relative to the concentration of the target analyte in the sample (Duan et al. 2013).

8.4 Heavy Metals

In a few industry branches, like electronics, metallurgical, mining, metal finishing and electroplating were heavy metals are widely utilized. The major problem from heavy metals to human wellbeing is related where subjection to arsenic, mercury, lead, and cadmium. Standard location strategies like potentiometric electrodes, ionic chromatography, and spectrometry are tedious, costly, and require highly skilled professionals (Liang et al. 2016). In the heavy metal analysis field, there is a need for the advancement of basic strategies and should be appropriate for field application. Biosensor location is one among them. The reports of the cyanobacterium Phormidium on the consideration of an amperometrical biosensor as the biosensing component have been reported. The heat treated dead cyanobacterial biomass an electrode is formed from mixed and added to a steel rod with carbon residue (Liu et al. 2014). The detection of Pb (II) this electrode is capable in water solution. Reference electrode AgCl/Ag and as counter electrode the platinum wire were likewise the apparatus part the electric field changes that measures, were induced in heavy metal by water solution. Reports have reported great repeatability and stability of 5×10^{-8} M was set as a detection of theoretical constraint. Microbial fuel cells (MFC) got significant also. The metabolic action of the electrochemically active bacteria was hindered by the pollutant presence in wastewater, which leads to the weak current production and reduced electron transfer. For the location of single-chamber air–cathode MFC, Cu (II), enhanced with domestic wastewater real has been applied. The reaction of a biofilm, which is developed from microorganisms in wastewater at various flow rates, becomes significant. The surrounding of the MFC higher feed rate causes prompting the extracellular polymeric substances, higher shear rate, overproduction and thereby decreasing the biosensor sensitivity (Harroun et al. 2018). Utilizing *E. coli* DH5α™ an oxidative stress biosensor, changed with pRSET-roGFP2 plasmid which enables the fluorescent arsenic detection incited oxidative stress. This biosensor is quick, productive, and enables arsenic detection down to 0.2 μg/L. A microorganism of same kind was utilized for the detection of Pb^{2+}, Cu^{2+}, arsenite, Zn^{2+}, and Cd^{2+}. The prescribed biosensor is considerably more enabled and delicate down to 0.001 ppm the detection of zinc and copper ions, 5 ppm lead ion, 0.01 ppm cadmium ion and 1×10^{-7} mg/L arsenite (Jamdagni et al. 2016). The latest improvement in the field of biomonitoring is a consistent progression of the analyte to the biosensor. *E. coli* DH5 was used by Kim et al. the year 2015 in a micro fluidic device, equipped for feeding of nutrients and heavy metals ions of different concentrations under continuous feed mode of Cd^{2+} and Pb^{2+} for the detection. By CadC-type transcriptional repressors the GFP reporter gene negative control, the detection system mediated that binds to Cd^{2+}or Pb^{2+} divalent ions and de repress by the GFP reporter promoters. The biosensor in the sensitivity of three–fourfold increases and great particularity to recognize elements Pb^{2+} and Cd^{2+} was noticed (Xu et al. 2019). Another method of this method for the heavy metal pollution detection in the development of a biosensor in the field enables synthetic biology. The pressure oscillation in the environment with pH, nutrients, temperature in the environment and access to the toxicants of regulatory elements influences a diverse set of the downstream to control the signal cascade. The microbial biosensors can be developed a-new, for the new genetic circuit's manufacture utilizing regulatory elements. From the previously available reports that this biosensor application will tackle the poisonous idea of the issue of feeble explicitness and the heavy metals real applications, microbial frame (Mitchell et al. 2005).

8.4.1 Arsenic

A few aptamer-based biosensors for the detection of arsenic have been explained which utilize the first reports of an aptamer. These biosensors used electrochemical and optical methods which explained limits in the nM range of detection, for certain eminent special cases reaching at limits on the request for extents of 10–6 nM and 10–3. For the aptamer-based biosensors, some of the examples are shown the utilization of environmental sources for arsenic detection (Clesceri et al. 1998). An electrochemical biosensor that utilized glassy carbon was revealed by Yadav et al. in that the electrode is altered with nanoparticles of Au–Ag combination aptamer-coated. The biosensor exhibited of 0.01–10 μg/L a linear dynamic range, As^{3+} detection of 0.003×10^{-3} μg/L detection limits. World Health Organization suggests the regulatory limit 10 μg/L where these details are fit well. To detect As^{3+}, this kind of biosensor was used which is acquired in India as well as one river sample from distinctive ground water sources. A flexible plan was evaluated utilizing differential pulse voltammetry and cyclic voltammetry. As^{3+} measured concentrations ranged from 190 to 393 μg/L were found in these water sources and from 92 to 108% the spiked recoveries ranged. Quick and specific arsenic detection was possible and accomplished in less than thirty minutes from fluorescence estimation and preparation (Abd-El-Haleem and Zaki 2006). Some specific sign was produced within As (III) compared with common ions Hg (II) and the presence of Co, Ni (II), Cu (II), Cd, Mn (II), Zn (II), Pd (II), Fe (II), AS (V), and Ba (II). LOD of 18 nM assay had revealed. At long last, from 95.6 to 101.8% the biosensor showed recoveries going when tap water and lake water were increased of As (III) with 5, 10, or 15 nM.

To conclude this segment, because of their inconsistencies in binding it is noted that a small bunch of aptamers has earned consideration, being one such example is for arsenic aptamer. Although few literature surveys revealed that for the selective and sensitive arsenic detection aptamer-based biosensors that the generally utilized Ars-3 arsenic aptamer report does not bind arsenic was explained by Liu and colleagues recently. Basically, in one test to another is that they do not always perform aptamers challenge, featuring the need for intensive portrayal before biosensor development (Boudriot et al. 2006). But it is different in arsenic aptamer, a significant number which is recently revealed gold nanoparticles used by biosensors. Liu and partners as of late introduced proof that arsenic, as As (III), dislodging DNA of the molecule which adsorbs to the outside directly, instead of through some interaction with the aptamer. similar mechanisms may explain clarify that, however, the original paper revealed same explanation to As (III) and As (V) and numerous reports on biosensors of particular to Arsenic (III). Further, these researches outline the requirement for broad assessment in aptamer-based biosensors controls, in few instances, to the target analyte the biosensor platform's response without the aptamer must be evaluated always, and proper non-restricting oligonucleotide controls ought to likewise be used (Sill and Von Recum 2008). This is significant for the research group to remember this model during the characterizing and planning of aptamer-based biosensors in the future assays to upgrade exhibition or unwavering quality.

8.4.2 Lead

Lead (Pb^{2+}) is one of the heavy metal pollutants a non-degradable toxic which is generally found in industrial waste added to water and natural water sources. By the binding of complementary DNA strand on duplex generation has green fluorescence's DNA-AgNC which is transformed to the emission of red fluorescence (Seo et al. 2011). Test samples of human serum and water consist which changes the fluorescence back to the green of Pb^{2+} induce Pb^{2+}-dependent cleavage activity of DNAzyme. The surface of the nanoporous Pb^{2+}-dependent DNAzyme was adsorbed modified with complementary DNA-AuNPs electrode that formed duplex. A gold nanoparticle which that gives more space that produces current and was measured using chronocoulometry for the attached electroactive hexaammineruthenium (III) chloride molecules. Pb^{2+} presence in the sample initiates DNAzyme (Langer 1990) to release aptamer-gold nanoparticles landfill, tap water, and river water leach ate off the electrode surface which leads in charge to decrease.

8.4.3 Silver (Ag)

In a wide range of products from medical to jewelry technologies to gym socks, silver is used extensively which led to its bioaccumulation and environmental contamination. Ag can be in small doses as a good antimicrobial agent; at the same time, it can affect human health conditions because it is a highly bioactive metal ion (Monteiro et al. 2014). Silver is also detected by emission spectroscopy, atomic absorption, and mass spectrometry as other metals are detected. It gives the same challenges for practical environmental monitoring. To detect silver ions in water samples, utilizing DNA molecular switch biosensor was designed with Ag ion as the non-canonical interactions. To the precise G–Ag^+–G interactions, silver ion binding probe was designed and this is like the mercury aptamer. The basic principle is the rotational properties changing in between the non-bound and silver-bound aptamer states. The value determined by measuring the fluorescence anisotropy from conformationally dependent interaction of the fluorophore tetramethylrhodamine and sequence guanines (Welsh and Kay 1997), within the dynamic concentration a fluorescent signal was generated with a detection limit of 0.5 nM and which ranges from 2.0–100 nM. In the presence of 14 common interfering metal ions, the probe selection was expressed. Ag^+ recovery from the river (71.8 and 83.6%) water and spiked drinking (97.6 and 98.8%) was also demonstrated (Espinosa-Urgel et al. 2015).

8.5 Soil Contaminants—Aptamer

Human activities like industry, manufacturing, agriculture, waste disposal and mining of chemicals introduce complex mixtures like pesticides, antibiotics, and heavy metals to the soil. These chemicals maybe formed on the ground water, seas, and rivals or accumulate in soils which will disrupt the ecosystems (Hou et al. 2015). Low-level soil contaminants of long-term exposure usually spoil the health of humans. During the soil contaminants, it is important to manage and control. Up to now as far as our knowledge, less amount of surveys were carried out by researchers for heavy metal on soil samples of aptasensor application, because of the challenges originate from the complexity of soil matrices in the literature, EDC and pesticides have been reported. In soil samples for acetamiprid and pesticide detection, reports have been produced (Bereza-Malcolm et al. 2004). Though, for pesticides detection in soil apart from acetamiprid, few reports have also reported. Mainly, lead detection in soil samples using the aptasensor on heavy metal detection has been reported. There was only one report available about apta-sensing of PCBs detection in the soil samples.

8.5.1 Using Aptamer-Based Biosensors for Monitoring Lead in Soil

To solve this issue, for the lead (II) detection of two aptamer-based sensors in soil samples was explained by (Horry et al. 2007). The interaction between AuNPs, G-rich lead polypyrrole screen and binding aptamer printed onto an electrode, generates an electrochemical signal. Where the biosensor showed a 0.6 ppb of detection limit, the linear dynamic range of 0.5–25 ppb and selectivity against common interfering ions is also explained (Ke et al. 2014). Recently, a similar electrochemical biosensor which had a 0.5–10 nM of linear dynamic range and 0.36 nM detection limits was explained by Ding et al. To detect lead (II) in soil, both methods were used with accuracy and for from a nearby farm from the control measurements that were not different and having good selectivity against interfering ions in common.

8.5.2 Agricultural Toxins Detection Present in Soil

For agricultural toxins detection, most of the research focuses on the mycotoxins detection mainly focuses on the utilization of aptamers. Mycotoxins, includes which are not limited to aflatoxins and ochratoxin, on exposure to them which may lead to difficult health problems. The mycotoxins as possible human carcinogens have classified by International Agency for Research on Cancer (Li et al. 2015). The portable detection and rapid sensitive of mycotoxins are used to retailers from the farmers to consumers and along the food production pathway in various points. At present, mass spectrometry and chromatography (traditional instrumentation) for sensitivity and rapid detection of mycotoxins, and immunoassay-based detection makes it, by non-experts at field settings but there are some limits present on utilization. There are relatively simple assays examples for environmental monitoring and potential which may be conducted in a reasonable period of aqueous and soil samples on-site analysis. Aflatoxin utilizing a one-pot mix-to-read assay B1is detected in less than one minute in soil was demonstrated.

8.6 Aptamers for Monitoring Air Quality

Researchers focused more on the air quality in aptamer-based environmental monitoring. On a biomarker except for some research for oxidative stress that can be seen in urine and has been connected with electrochemical biosensors optical fluorescent, colorimetric, air pollution, and 8-OHdG (8-hydroxy-2′-deoxyguanosine) resonance light scattering that focused on developing, in an literature study one report by using aptamers to monitor air quality has focused (Li et al. 2018). Radon is toxic, odorless, and colorless gas by the International Cancer Organization and World Health Organization which has been designated as a first-class environmental carcinogen. The fact is radon exposure, which presents naturally, and as a combustion product of natural gas and coal, in water and soil sources, it develops lung cancer and that is one of the strongest risk factor. Currently, there are problems in both the measuring accumulation at low doses and radon detection as available methods are highly affects the cost and human health. Hence, new technologies must be developed to detect radon. This fluorescent biosensor design consists of a 10 mL of 0.2% acetic acid containing in a Petri dish was closed to prevent airborne lead contamination which absorbed the decayed lead ion with a mixed cellulose microporous membrane. In a radon chamber at room temperature, the collection dish was placed from 2 to 84 h of time intervals ranging, of radon to cover the half-life was chosen (Luo et al. 2009). In the acetic acid solution, a small aliquot was introduced to a reaction that contains buffer aptamer (HTG) and lead. The solution of the fluorescence was measured following subsequent 10 min incubation then malachite green (MG) was added over the period after 2 h of incubation at 37 °C. The G-rich aptamer (**T30695**) lead ion presence, to a G-quadruplex underwent a structural transition. Interestingly, in the study of the G-rich sequence than the peroxidase mimic DNAzyme (PS2.M) used exhibited a higher affinity to lead in the water assays utilization, for further work consideration. A label-free fluorescent biosensor, represented schematically in Fig. 8.4 (Li et al. 2018), to detect radon it exploits the radioactive decay of ^{222}Rn–Pb^{2+} (Van Gestel 2008). The concentration of lead ion through this method measured by traditional methods detected was directly proportionate to the amount of gaseous radon. Linear dynamic range of 6.87×10^3 to 3.49×10^5 Bq h/m^3 a and a LOD of 2.06×10^3 Bq h/m^3 displayed on the biosensor. Particularly, the device's sensitivity to other common metal ions over lead was found out. These parameters correlate favorably

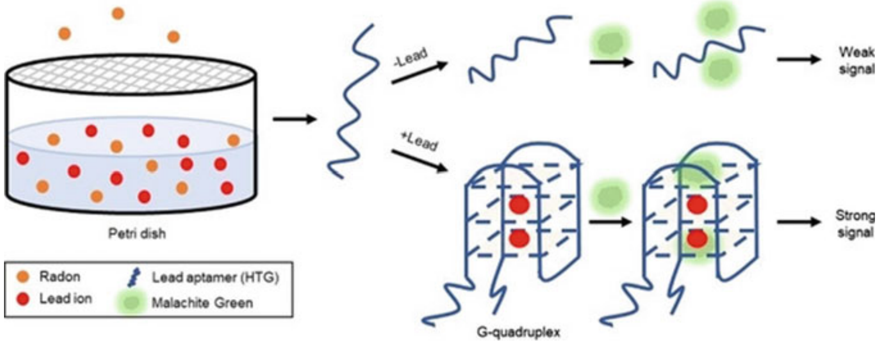

Fig. 8.4 Accumulated radon via lead ion detection aptamer-based sensor indirect detection (adapted with permission from McConnell et al. (2020). Copyright [McConnell et al.], some rights reserved; exclusive licensee [Frontiers]. Distributed under a Creative Commons Attribution License 4.0 (CC BY))

for the detection of both lead and radon ions already reported. To add on, there was no much relation or changes between the obtained measurements utilizing the described commercially available radon monitor (RAD7) and a biosensor.

Precedence recommends of the programmability of functional nucleic acid it may still be possible to take advantage to build biosensors affinity probes of toxic gases for the indirect detection. As an illustration, a recent report by utilizing a copper ion-dependent DNAzyme defined the H_2Sdetection to develop a fluorescent signal, and another research used to develop a colorimetric signal by peroxidase mimic DNAzyme of this approach (Li et al. 2018; Besaratinia et al. 2007). The H_2S detection limits for these biosensors were 410 and 200 nM. On process, usually it ranging from 91.0 to 108.0% spiked aqueous solutions were exhibited.

Aptamer-based biosensors for the generation the common toxic gases like hydrogen sulfide, sulfur dioxide, and nitrogen dioxide have interesting targets. These gases detection of some industrial processes which occurs as the natural and byproduct, that is essential for quality air observation (Throne-Holst et al. 2007). Intense exposure and long-term to this gas can dreadfully affect the wildlife, human life, and infrastructure, quality of vegetation. Currently, to monitor ambient gas levels electrochemical methods are generally used. The aptamers for gas sensing most logical incorporation may fall in these dimension aptamer-based electrochemical sensors of the common reported.

8.7 Bacterial Detection Aptamer-Based Biosensors

An international problem a bacterium contaminates the water sources are ends up in both medical and economic difficulty. By pathogenic bacteria due to this contamination, by water-borne diseases, nearly two million deaths per year are caused which is the direct result of this contamination. Contaminated ground water, wastewater, other water sources, and drinking water, may lead to worldwide death and illness (Hou et al. 2015). Also, there are some linked contacts between contaminated soil and water on the human health, aqua cultural industries, environment, and agriculture which has more impacts. Hence, for water-borne bacteria detection to generate highly sensitive biosensors there is an urgent requirement. The progress made toward the generation for the *Staphylococcus aureus*, *Listeria monocytogenes*, *Microcystis aeruginosa*, *Salmonella*, *Vibrio*, *E. coli*, and *Pseudomonas aeruginosa* (Kumar and D'Souza 2011) detection of aptamer-based biosensors. In most of the scenarios, real sample detection was explained based on beverages not in water. Hence, the contaminated water may be a cause these bacteria exist in water and infection generally in food monitoring of higher practical concern. Like many other works though these examples were included have exhibited that if complex aqueous samples detection like juice or milk is possible, environmental water samples detection may generally take place (Chan et al. 2013).

8.7.1 Listeria Monocytogenes

The food-borne bacterium *L. monocytogenes* is also contaminating the water. For the detection of this food-borne bacterium, *L. monocytogenes* to evolve an aptamer-based biosensor in environmental samples and food was desired by Suh et al. by utilizing biotinylated *L. monocytogenes* and anti-*L. monocytogenes* antibodies in their two-site binding sandwich assay, for the capture of *L. monocytogenes* that had bound to streptavidin-coated magnetic beads the first binding was accomplished. By adding LM6-116 a 5′-FAM-labeled aptamer specific for *L. monocytogenes* with a K_d of 74.4 ± 52.7 nM was accomplished the second binding of the pathogen to the solution which results in *L. monocytogenes* in the two-site binding sandwich structure was surround by the aptamer as well as the antibody (Deane and Stokes 2005). The cleaned samples were placed in a 90 °C hot water bath to discharge and LM6-116 denature after a forty five minutes incubation period, which is then collected and used as an RT-qPCR template. This assay was also tested in turkey deli meat to the bacteria detect at a 1–2 $\log_{10}$ level of meat CFU per 25 g.

8.7.2 **Vibrio** *Species*

The gram-negative anaerobic bacilli *Vibrio* species which frequently resides in aquatic environments that include surface water, freshwater and estuarine of different temperatures and salinity of biosensors for the development and environmental monitoring are of interest. Some of the most documented and analyzed of the *Vibrio* species are *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*, because of their pathogenicity toward the wellbeing of humans (Bjerketorp et al. 2006). The main mode of transmission for these pathogenic strains mostly connects the undercooked seafood consumption, contaminated water or harboring the pathogen. For *Vibrio alginolyticus* and *V. vulnificus*, recently selected aptamers were to detect the first one very less below 8 CFU/mL was assembled into a biosensor of asymmetric PCR that took more space (Bjerketorp et al. 2006).

8.8 Applications of Bioluminescent Biosensors

8.8.1 Detection of Environmental Contaminants

Numerous contaminants such as heavy metals, crude oil, and polycyclic aromatic hydrocarbons result from various anthropogenic activities. These pollutants are introduced to the environment from the industrial processes. These substances are present at various levels and generally with adverse effects on the biota. In the detection of these toxicants, use of the bacterial bioluminescent biosensors has a fruitful role even at very low concentrations (Urbanczyk et al. 2007). With a biological explanation of the effects, the detection is determined by these sensors immediately. These sensors provide a stable, definite estimate method and cost-effective, for evaluation of various contaminants. Some bioluminescent biosensors that have been used in monitoring environmental pollution include *Pseudomonas* sp., *Bacillus subtilis* and *E. coli*. Studies have successfully applied these biosensors for observation and contaminants detection in air environments, water, and soil.

8.8.2 Applications in the Food Industry

The food industry is a very responsive one with high quality forecast. Yet, the purity of the food products has been compromised independently. This has led to more conflicting effects. Unsafe contaminants in food at certain low levels are identified by various traditional testing methods. Toxins in food are detected by bacterial luminescent sensors at very negligible levels. This has been tested strongly to monitor count in food and bacterial contamination (Baumann et al. 1980). The food industry presents with a high demand for quality assurance now than forever. Biosensors offer economical and definitive methods to meet this quality assurance needs. An advanced wearable device for environmental monitoring was explained by Yao et al. could be easily formed. They were interested in the detection of the antibiotic a freestanding graphene paper-based wireless device kanamycin and they developed (Karatani and Hastings 1993; Hastings and Nealson 1977).

By aptamer-based devices rapid and ultrasensitive antibiotics detection has exhibited many times. Schematically representation in Fig. 8.5 (Yao et al. 2019) in this example it shows the malleable graphene paper device which united assisted signal

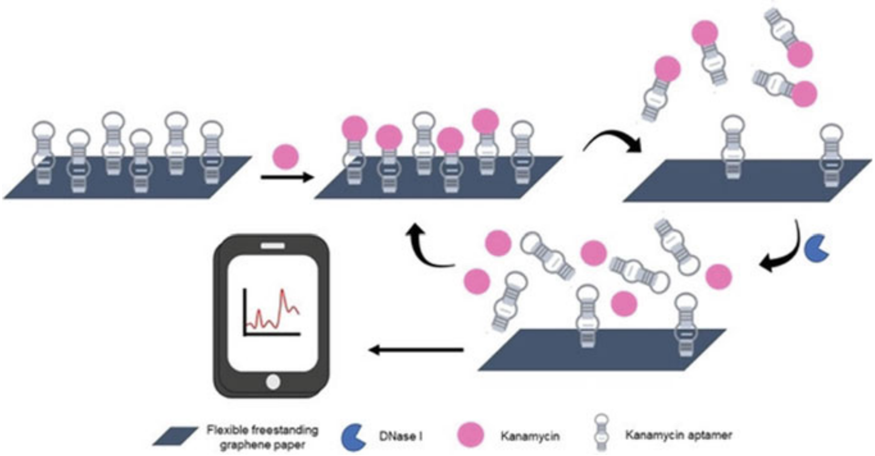

Fig. 8.5 Sensing principle behind aptamer-based schematic diagram using nuclease assisted amplification and free standing graphene paper (GNP) strategy by kanamycin detection adapted with permission from McConnell et al. (2020). Copyright [McConnell et al.], some rights reserved; exclusive licensee [Frontiers]. Distributed under a Creative Commons Attribution License 4.0 (CC BY))

amplification and aptamer-based detection were integrated into a sensing platform by using a smart phone and wireless transmitting detector Kanamycin detection for the fg/mL (Narsing Rao et al. 2020).

8.8.3 Bio Drug Delivery Systems

For transporting pharmaceutical molecules formulations, methodologies, systems into the specific tissue or a body part as required attaining effectively and safely where drug delivery system involved its desired therapeutic effect. At targeted delivery, drug delivery is very attractive approaches, controlled rate and slow delivery have been tested. Aptamers that bind internal cell surface receptors specific to deliver drugs into cells are good candidate for bio drug delivery. With specific aptamer of cancer cells for the bio drugs specific aptamers production, like integrates, as well as Taxol will be having good potential in other diseases and particularly in cancer therapy. PEG-PLGA nanopolymer some formulated with Doxorubicin (DOX)-encapsulating nanoparticles, there the surface EpCAM aptamer was functionalized. Signore reported that composition of RNA and DNA decoy aptamer a novel oligonucleotides chimera (Tu and Mager 1995). By this method, to target transfer in receptor (TfR) the RNA aptamer was utilized, where as the cell-survival factor, nuclear factor κB and DOX decoy oligonucleotides that inhibited were utilized as additional drugs. The aptamer motif promoted particular drugs under low pH value ($pH = 5.5$), DOX exhibited 100% release, delivery to pancreatic tumor cells and the co-delivery of a κB decoy oligonucleotides which are greatly shown the visibility of DOX cytotoxicity because the constitutive NF-κB activity of inhibition to targeted pancreatic tumor cells (Fisher et al. 1996).

8.9 Perspectives and Recommendations

For the detection of relevant contaminant analytes, aptamer-nanomaterial and aptamer-based biosensors growth/evolution in the past few years improved the environmental monitoring field. The portable aptamer-based devices development made more evolution in devices that provide rapid, multiple, and on-site single target analytes array detections. On account of this challenge and the progress detections in translating bench science to on-site in the field which will remain the same. For fulfilling the needs described by the WHO on ensuring their designs adhere to the guaranteed criteria researchers should focus more (Tang et al. 2001).

The presented research often concludes with biosensor detection demonstrations in contaminated environmental samples. With minimal sample preparation, it is significant to measure the function of these biosensors, to demonstrate user-friendly, in complex environmental samples detection to validate naturally occurring contaminants, and reliability and of target analytes on-site detection. In this method, this work can be translated much practical use. Water, is relatively non-complex (while comparing with, biological samples like blood), even though to most environmental monitoring the matrix relevant detection, for the on-site contaminant analytes detection it still exists as unique challenges (Rogowsky et al. 1987). The hospital or medical lab can provide permission to basic needs such as environmental samples, refrigeration and electricity, which are gathered and preserved in greatly external climates and different internal that may change quickly and affect the downstream processing condition. Nucleic acid-based access and nanomaterials from the intersectionality being used for fauna and flora monitoring examined examples proved that the researchers interested in aptamer-based environmental monitoring should pay more attention, in other fields such as personalized medicine and point-of-care devices with translatable progress. Definitely progress will come across binding these, and some inventive technologies to give end-users inexpensive and reliable biosensors for the detection of environmental contaminants (Weitz et al. 2001; Liu et al. 2019).

8.10 Conclusion

In managing environmental hazards for human health and ecosystems detection and monitoring of contaminants play an essential and important role? For the development of a sensitive biosensor for environment, monitoring aptamer acts as a promising bioreceptor and it possesses the modern plat form combination and the capability of target molecules to bind with different size. High sensitivity in comparison with the colorimetric sensors among available fluorescence-based aptasensors, electrochemical, and platforms exhibited. Aptasensors-based published literature studies revealed excellent sensor performance which can specifically/sensitively with complex matrices find the contaminants in samples. Among these, the repeated usage of aptasensor is enabled by the reversibility of aptamer which makes immensely saves the biosensor cost and possible of real-time monitoring. In addition, USA drinking water regulation aptasensors sensitivities for contaminants were close to the maximum contaminant level goal (MCLG) for environmental monitoring which displays the potency of aptasensor. It is worth saying that utilizing aptamer-based biosensor for the detection of contaminants in the environment it would be of great aid. However, the environment aptasensors are still under phase of development for the environmental monitoring to date as compared to immune assays advantages. Hence, major challenges should be addressed, which includes

1. About the length of random library there is no standard corresponds targets or study to the structure and size about which could be a key factor with most highest affinities in aptamers screening (Bhattacharyya et al. 2005; He et al. 2016);
2. Contaminants many are toxic, very diverting, so there is a very big gap between developed and contaminants aptamers;
3. Screening of aptamers in real sample conditions for the more practical use of aptamers, must be considered;
4. For newly developed aptamers, folding structures like hairpin structures or G-quadruplexes, knowledge about binding sites, and binding mechanisms should be studied in various platforms for the effective use of aptamer;
5. It requires more effort on confirming there aptasensors stability and reducibility;
6. Some studies on aptasensors application in soil samples (Mejri-Omrani et al. 2016);
7. Some study about the air sample which is another environment for human health which requires concern.

For commercialized aptasensors, there are many things to overcome while these challenges are yet to be addressed. For various contaminants present in the future and in a diverse environment, robust, reliable, and more sensitive aptasensors will be developed when these challenges are addressed.

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Chapter 9
Microbial and Plant Cell Biosensors for Environmental Monitoring

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Abstract The quest to develop more effective and cost-efficient environmental monitoring methods has efficiently improved with high sensitivity, rapidness, and selectivity, over the traditional low detecting and analytical methods such as capillary electrophoresis, potentiometric biosensor Quartz Crystal Microbalance (QCM), chromatographic techniques, Scanning Probe Microscopy (SPM), thermal and piezoelectric biosensors, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) techniques. Recent advances have led to the development of improved biological sensors that exhibit the capability to detect and analyze parameters by using bio-recognition elements. These sensitive and effective biosensors at

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nanoparticle range are becoming more efficient and cost-effective means of monitoring food analysis and our environment. Further, the use of fluorescence in environmental monitoring systems with the aid of green fluorescence protein (GFP) and yellow fluorescence proteins (YFP), and deoxyribonucleic acid (DNA)-based biosensors (genosensors) have now been engaged to provide a friendlier environment. Moreover, gold nanorods DNA biosensors (GNRs-DNA) are utilized for the identification of microorganisms. Another improvement is detecting bacterial cells and metabolites by nano/micro biosensors such as surface-enhanced Raman spectroscopy (SERS). This biosensor utilizes the spectroscopic analysis method to detect microbes, disease markers, explosives, and chemicals. In this, biosensors for food-borne diseases and environmental monitoring sensors will be evaluated and discussed and the use of genetically modified microorganisms for microbial biosensors.

Keywords Environmental monitoring · Polymerase chain reaction · Biosensor · Disease marker · Food analysis · Microorganisms

9.1 Introduction

The microbial fuel cell (MFC) is environment-friendly biotechnology that has mostly been advocated for power generation and treatment of wastewater. However, its low power output limits its uses aimed at directly powering most of the electrical strategies. Moreover, advances in its electrochemical, microbiological, and chemical features have broadened the scope of microbial fuel cells for chemical generation (methane or formate), bioremediation of polluted soils, and development of biosensors. Due to their simplicity and long-term viability, MFC-based biosensors have gained much attention in recent decades, with various applications from water quality assessment (like toxicants, biochemical oxygen demand) to air quality detection (such as formaldehyde and carbon monoxide). The current state of MFC-based biosensors was outlined in a study, focusing on biochemical oxygen demand and toxicity detection (Cui et al. 2019).

Biosensors are analytical devices that analyze biological signals and convert them to detectable electrical signals. It entails connecting organic entities like DNA, RNA, enzymes, proteins, and bacteria to electrochemical transducers that detect and notice biological analytes. Numerous categories of biosensors have been effectively engaged in the environment, food industries, and biomedical to detect and eliminate various toxins, whether non-living or living. Nowadays, the most popular sensors are optical, amperometric, enzymatic, DNA-based biosensors, SPR, and phage and bacterial-based sensors. These biosensors can analyze a wide range of biological analytes and have demonstrated improved success and response in medical laboratories, microbial detection, and food bioanalysis. The concentration of glucose in the body, heavy metals detection in soil, microbial invasion in the body and food, airborne microbes and water, pesticides in soil and water, and numerous dangerous chemicals created through the body could all be easily and quickly examined through high precision with various types of biosensors (Ali et al. 2017).

Moreover, biosensors based on genetic constructs that respond to a promoter gene coupled to Green Fluorescent Protein (GFP) are currently gaining popularity because they increase the accuracy of nitrate measurements by preventing interference from carrier ions, high salt conditions, and other variables. The current review discusses various approaches for quantifying nitrogen in plants; after that, a biosensors perspective is offered, with a focus on biosensors based on genetic modification. The review includes a list of promoter and reporter genes that might be utilized to construct various sensors and an overview of sensors that can determine nitrogen quantitatively (Raul et al. 2016). Hence, this chapter intends to provide a piece of detailed information on the relevance of Microbial and plant cell biosensors for environmental monitoring.

9.2 Types of Biosensors

Various types of biosensors like gold nanorods biosensor, ELISA, and QCM are discussed in the section below.

9.2.1 Gold Nanorods (GNRs-DNA) DNA Biosensors

The gold nanoparticle-based biosensor is considered one of the improved biosensors because of its sensitivity, cost-effectiveness, and ease of operating. This type of biosensor utilizes two strong longitudinal surface plasmon resonance (SPR) bands that are extremely sensitive to changes in the dielectric properties of the surrounding environment and processes outstanding features for biotechnological industries. These types of nanobiosensors are ideal for clinical purposes, and also, it has a very high sensitivity for detecting unknown pathogenic microorganism in environmental fields (Parab et al. 2010).

9.2.2 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a type of biosensor that uses immuno-chromatographic technique or immunoanalytical system that can accomplish these analyses different parameters ranging from food safety to environmental monitoring and clinical diagnosis. These types of biosensors have been gathering world's attention because of the ease that comes with the use, though a bit expensive to most third-world research laboratories. This is largely due to its high sensitivity, ease of handling, and multiplex capabilities. Moreover, it has been gaining the world's attention because of its use in detecting infectious agents, routine diagnosis, and treatment of several diseases, and finally on its warning against bio-warfare agents (Thakur and Ragavan 2013).

9.2.3 Quartz Crystal Microbalance (QCM) Biosensor

The application of QCM sensors has been gaining attention because of its wide array of applications in different fields ranging from material science, living organisms, and chemistry. QCM is a highly cost-effective and sensitive microbalance that can be used depending on the area of specific need. It can also be coupled with affinity biosensors such as QCM, which can be utilized biologically to detect genetically modified organisms. This type of sensor is designed such that a specific nucleotide/amino acid sequence can be detected, and to make it more effective, it is also coupled with the use of polymerase chain reaction, PCR (Alassi et al. 2017).

9.2.4 Microbial Biosensors

Microbial biosensors are investigative strategies that restrain microorganisms on a transducer for the revealing of objective analytes in addition to provide a detectable signal related to the analyte application. Integrating the microorganisms onto the transducer is the most rudimentary need for creating a dependable bacteriological biosensor. A great amount of bacteriological biosensors has indeed been developed in recent years. In addition to the investigation, this technology is perfectly suitable for environmental contamination management, biomedical uses, food industry, and so on, due to its specific, sensitive, easy-to-use qualities, and quick. The bio-elements in a microbial biosensor are microorganisms that contain a variety of enzymes. This paper presented some recent scientific improvements and trends that involves the application of microbial biosensors (Fahliyani et al. 2020).

The capacity to identify physiological and pathological linked molecules in the body through high sensitivity and specificity generates an opportunity to develop a highly potent biosensor that can treat disease with early detection. The optical fiber cable is utilized in various scientific and drug disciplines, including the optical base biosensor. Bacteriophages are viruses that can be found in any environment where bacteria live, and there are more than 1031 bacteriophages found in the world, which is more than all other organisms combined, including bacteria. Bacteriophages have various potential applications in the food business, which has become extensively recognized in recent years. They have been offered as antibiotic alternatives in animal health, food bio preservatives, and techniques for detecting harmful bacteria in the food chain. Phages are part of the typical microflora of all fresh, natural foods and serve an important role in maintaining microbial equilibrium in every ecosystem where bacteria thrive. The use of bacteriophages in different practical applications has lately gained traction, with possibly the most interest focused on harnessing them to improve food safety (Ishfaq et al. 2021).

An analytical instrument having a physically assimilated transducer that gives a quantifiable signal demonstrating the analyte application is known as a microbial biosensor. This technique is appropriate for analyzing extracellular substances and the environment, as well as sensual metabolic modulation. A complete assembly of microbial biosensor have been schematically illustrated in Fig. 9.1 (Do et al. 2020). Even though microbial biosensors have shown potential in various detecting applications, they still have several drawbacks, including poor selectivity, impractical portability, and low sensitivity. Further, microbial biosensors have been assimilated by means of several newly emerging micro/nanotechnologies and utilized for a wide range of detection purposes to overcome these limitations. This review paper describes recent achievements and applications obtained through the unique integration of nano/micro technologies through microbial biosensors. The essential innovations and improvements will be presented for future viewpoints on integrating nano/micro technologies and microbial biosensors (Lim et al. 2015). The authors created six gene circuits through reconfiguring monitoring elements as well as inserting affirmative feedback loops into the routes to increase the efficacy of a whole-cell biosensor aimed at lead detection. The pbr opposition operon codes six genes by means of pbrRT on one side and pbrABCD on the opposite side of the promoter. The lead biosensors were created using the divergent promoter it regulates, PbrR, along with GFP. To simulate the native operon, one has pbrR and gfp on opposing ends of the promoter. We rearranged it by putting pbrR and gfp on the same side of the advocate or beneath two different promoters. Lead sensitivity was 10 times higher in the one by means of gfp and pbrR on the same side than in the others. These circuits now have a positive feedback loop. The output signal was 1.5–2 periods greater in the optimistic feedback hoop designs than in the non-positive feedback loop designs. The significance of arrangement and critical outcomes as effective tactics for improving the performance of lead biosensors is demonstrated, and these techniques can be applied to the scheme of additional whole-cell biosensors (Jia et al. 2018). Although, in microbial cell factories, natural product biosynthesis, the titer of natural products, and low yield are mutual problems; therefore, designing inherited biosensors to regulate and monitor the production of specific natural yields is considered as one of the efficient techniques to alleviate such bottlenecks. Most of the current breakthroughs in creating inherited biosensors for the usual produce biosynthesis in microbes are discussed in this study. The investigator looks at genetic part selection methodologies and building concepts for the evaluation and design of inherited biosensors. (Hossain et al. 2020).

The whole-cell biosensors are a decent substitute for enzyme-based biosensors as they are less expensive and have a higher level of stability. Live cells have been used as part of biosensors for a variety of targets in recent years. This study concentrates on the application of genetically modified microorganisms with desired outputs to increase biosensor performance. Different methods for creating microorganisms by means of the requisite outputs, signal, selectivity, and sensitivity were accounted, including natural science and protein/genetic engineering (Park et al. 2013b). The

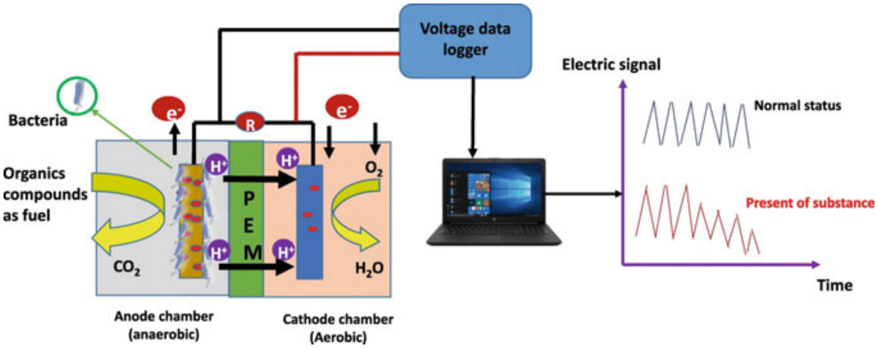

Fig. 9.1 Schematic diagram representation of electrochemical microbial biosensor (reproduced with permission from (Do et al. 2020))

improvement of biosensors as implements for identifying trace chemicals has been aided by synthetic biology. Biosensors depending on artificial biology have previously been constructed on most of the living cells, and on the other hand, the improvement of cell biosensors has been disadvantaged by means of faults like cell membrane obstruction. The emergence of cell-free synthetic biology, on the other hand, addresses these restrictions. Biosensors depending on the cell-free protein production method exhibit superior safety, low response time, and high sensitivity than cell biosensors, allowing cell-free biosensors to show a wider range of applications. Different identification mechanisms and output methods considerably increase the revealing series of cell-free biosensors. Furthermore, the paper examines the use of cell-free biosensors in pollution management in addition to health diagnostics, as well as existing flaws and areas that should be improved. The prospective of cell-free biosensors will be enhanced in the future, and their usage sectors will be enlarged, thanks to ongoing development and innovation (Zhang et al. 2020).

Food-borne infections have a negative impact on human well-being besides to identify the cause of financial losses. As a result, the rapid discovery of food-borne pathogens, in addition to the execution of steps to make certain their inactivation, is critical. Food-borne pathogens are commonly detected using immunological, molecular, and cultural approaches. These approaches have several drawbacks, including high costs, long analysis periods, and the need for specialized people. Analytical instruments include biosensors. Biosensors are being touted as a novel way to identify food-borne microorganisms along with their pollutants rapidly. Biosensors are devices that combine an organic exposure material by means of a physical or chemical transducer to convert biological, chemical, or biochemical signals into detectable electrical impulses. For the detection of harmful microorganisms, various types of biosensors are used. Biosensors are rapid, sensitive, dependable, inexpensive, and portable devices employed in various disciplines, including medicine, food safety, pollution measurement, military defense, and pharmacy. The most commonly used biosensors in the detection of food-borne pathogens are electrochemical and optical biosensors, as well as piezoelectric immunosensors (Senturk et al. 2018).

9.3 The Environmental Application of Genetic/Protein Engineering and Synthetic Biology in the Development of Microbial Biosensor

Numerous current technological improvements have enabled the application of novel technologies built using synthetic biology and genetic/protein engineering to program the activities of microorganisms, especially for certain functions because they pose specific features such as selectivity, sensitivity, and specific signal output as illustrated in Fig. 9.2. These portend this technology for different forms of application in the determination of bioremediation and environmental evaluation. It has been discovered that live-cell possess enzymes that could break down xenobiotic compounds with wider effectiveness in the rejuvenation of heavily polluted environments (Park et al. 2013a). Moreover, the application of microbial fuel-based biosensors has been identified to be applied for detection of the level of toxicity and monitoring of biochemical oxygen demand in the environment. It has been recognized that bacteria can break the organic substrate in the environment and can serve as a source of electricity, especially for fermentation. The technology entails the application of a bio-electrochemical device that could regulate the power of microbial respiration to convert the amount of organic substrates straight to the production of electrical energy. Despite all the tremendous effort, there are several challenges in applying microbial biosensors, which might be attributed to minimal power density when refereeing to the amount of money involved in the operation and electricity generation.

Several efforts have been put to develop more robust self-powered engineered microbial biosensors that could minimize the cost involved in systemic approaches (Du et al. 2007; Sun et al. 2015). The application of microbial biosensors has been noted in their utilization in identifying heavy metals and pesticides. The effectiveness of eukaryotic microorganisms in this regard has been documented when compared to prokaryotic cells, which can also be linked to the merits of developing whole-cell biosensors (Gutiérrez et al. 2015) that have several benefits such as sensitivities, selective most especially in the detection of pesticides and heavy metals in an environment. Also, it has been stated that higher eukaryotic microbes portend the capacity to be more sensitive to diverse molecules and have more application to higher animals. The uses of microbial biosensors entail their application from energy generation to the monitoring of the environment. The application of innovative techniques will generate a more robust biosensor with more selectivity and sensitivity, especially from eukaryotes to engineered prokaryotes.

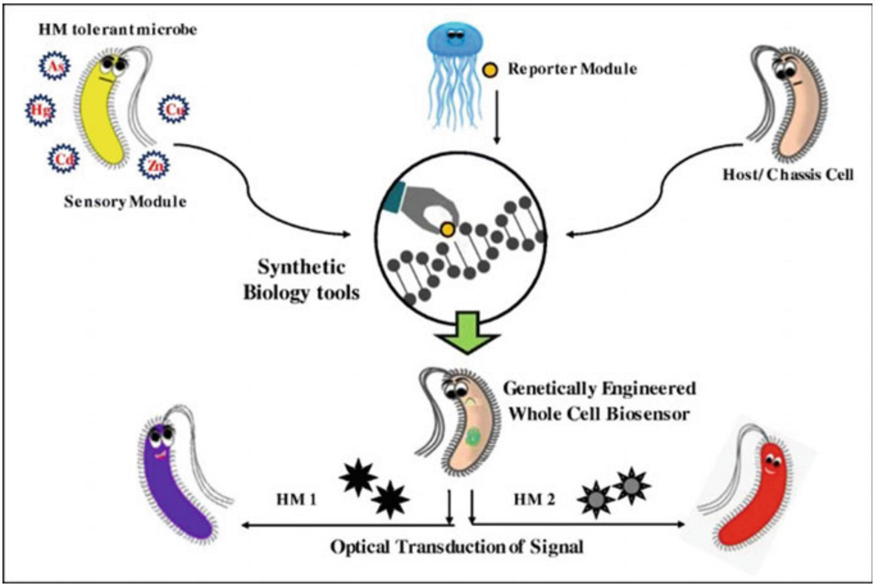

Fig. 9.2 Schematic representation of Synthetic Biology in the development of whole-cell biosensor (reproduced with the permission from Singh and Kumar (2021))

9.4 Environmental Application of Biosensor

Environmental biotechnology is the application of organisms, cells, portions of creatures, and molecular equivalents to safeguard and restore the quality of our environment through the integration of natural physics and technology. Environmental biotechnological methods have been employed for nearly a century before even "biotechnology" was coined. Environmental biotechnological tools include biotechnological strategies for treating waste before or after it is released into the environment. Biotechnology could also be used in the industrial sector to develop goods and developments that produce less unwanted applications fewer non-renewable properties, which also require small energy. Also, the biosensor is a type of systematic scheme that combines an organic detecting element (such as an antibody or as an enzyme) by means of a physical transducer (such as a mass, electrochemical or optical) to convert the collaboration among the bio-recognition molecules and target into an assessable electrical indicator.

Optical biosensors that apply fluorescence, light absorption, luminescence, reflectance, refractive index, and Raman scattering are effective another possibility to traditional methodical procedures. Several of these biosensors allow quick, real-time, very sensitive, and high-frequency monitoring apart from any pretreatment processes or time intense sample content. Optical biosensors offer many potential uses in food safety, environmental monitoring, biological research, drug development, and diagnosis, to name a few. Their application in the arenas of contamination mechanism, in addition to early warning, is still in its infancy. Biosensors are categorized based on their transduction principle, including electrochemical, depending on their recognition element or optical as piezoelectric and immunosensors, genosensors, enzymatic biosensors, and apt sensors (Bano et al. 2020).

An organic pollutant infiltrates the water, air, soil, food, and other schemes via drift mechanisms and causes a negative impact on diverse living schemes as they enter the food chain, intrusive with the ecosystem's regular biological processes. Inorganic pollutants have a reduced solubility, are largely adsorbed, and accumulate on the bottom of the sediments. Anthropogenic activities that discharge industrial and sewage effluent directly into rivers are sources of both organic and inorganic pollutants. The majority of the pollutants are hazardous, causing tumor, carcinogenesis, and mutagenesis in various life forms. Biosensors can detect these substances in the environment through transducing a signal, and they also have a variety of potential and existing applications. It also shows wide range of applications in the food business, environmental monitoring, illness diagnostics, and other regions where accurate and reliable investigation are necessary. This study also presented an overview of several biosensors, including their properties, designs, and working principles, based on the types of biological components and transduction. In most cases, biosensors have been employed in the environmental monitoring, nourishment business, clinical diagnostics, and drug delivery systems, among other uses (Singh et al. 2020).

9.5 The Application of Plant Cell Biosensors for Environmental Monitoring

In their review work, Volkov and Ranatunqa (2006) documented that some green plants can generate various kinds of intercellular and extracellular signals in response to changes in the environment. They identified one of the most observable signals reported the possibility of applying green plants for molecular recognition of the intensity and direction of light as fast biosensors, which will be relevant in detecting insects, heavy metals, and environmental monitoring (Volkov and Ranatunga 2006). Further, in their work, Tovar-Sanchez et al. (2019) highlighted the relevance of biosensors in environmental monitoring and assessment, especially in detecting various contaminants produced from anthropogenic activities. They discussed the effect on the application of plants, lichens, bacteria, and small mammals as biosensors in monitoring heavy metals and other contaminants present in the environment. They further highlighted the significance of using various biomarkers on biosensors (Tovar-Sánchez et al. 2019). Moreover, Chen et al. (2017) reported that the application of engineered organisms in detecting heavy metals within the environment has shown to be very efficient and reliable. They documented the development of biosensors for the detection of copper ions. In developing the biosensor, they screened and characterized different biological parts consisting of promoters, hosts, and output signals. They used the plant pigment known as betaxanthin to output the fluorescent signals to reduce the detection time. The resulting biosensors were developed to reveal a remarkably high sensitivity in detecting ions of copper in environmental matrices (Chen et al. 2017).

Similarly, Butnariu and Butu (2019) explained that plant nano bionics and biosensors utilize mathematical models based on qualitative and not quantitative details. Such biosensors can provide details on endogenous contents and fluxes in signaling molecules which are the plant hormones. They concluded that plants-based biosensors are of low cost, portable, and short time and allow real-time detection (Butnariu and Butu 2019). Further, Mazarei et al. (2008) documented that the utilization of plants as sentinels in the sensing of diseases and stress within the environment due to contaminants lies because plants are ubiquitous and indispensable components of most ecological systems. They highlighted the application of spectral signatures obtained from the local plants. They further explained that there is a rising trend in sentinel that are genetically engineered in plants and concluded that phytobiosensor is a rapidly growing phenomenon (Mazarei et al. 2008). In a related study, Kumar and Arora (2020) opined that nano-inspired biosensing devices have a remarkable position in improving the quality of life, especially in the area of environmental application globally. They highlighted that different nano-based biosensor had been documented, some of which involve the use of phytohormones. The nano-driven plant biosensors were revealed to be highly customized, displaying various characteristics of nanoparticles and achieving remarkable results. They, however, concluded that there are investigations on plant-based biosensors for environmental monitoring (Kumar and Arora 2020).

In another review work, Walia et al. (2018) addressed various kinds of biosensors, emphasizing calcium indicators that were genetically encoded, which have become the most outstanding regarding advancement. They highlighted that discoveries in the fabrication area of biosensors were highly dependent on characterization, engineering, and optimization needed to develop a successful and functional biosensor (Walia et al. 2018). Raul et al. (2016) highlighted the use of optical sensors, image-based sensors, and phytosensors in detecting and quantifying soil nitrate, which was more efficient when compared to other conventional approaches that were destructive and time-consuming. They also presented that biosensors derived from response promoters are gaining more ground due to their enhanced accuracy in the quantification of nitrates (Raul et al. 2016). Further, in their work, Coppede et al. (2017) presented a biomimetic textile-derived biosensor that could be introduced into plant tissue. The photosensor is capable of monitoring in vivo and real-time processes the variations in the content of solute in the cell sap of the plant. It was also observed that the biosensor had no noticeable effect on the morphology of the plant even after it was used for a duration of six weeks. The biosensor possessed a unique potential of detecting signs due to abiotic stresses in the environment, hence was recommended as a vital tool in the investigation of the physiology of plants (Coppedè et al. 2017).

Alvarez-Gonzalez and Dixon (2019) reported the usefulness of genetically encoded biosensing devices in providing a solution through transduction of targeted metabolite content into a detectable form to provide remarkable phenotypic output that permits dynamic route regulation. They observed that the fabrication and utilization of biosensors in formulation and engineering of effective biocatalytic techniques for the conversion, degradation, and valorization of lignin make a path for an economically potent and viable biorefinery (Alvarez-Gonzalez and Dixon 2019). Additionally, Rahaie and Kazemi (2010) outlined various molecules from plants and animals that have been employed in sensing receptors for the fabrication of biosensors. These include cell receptors, enzymes, antibodies, and nucleic acids. They identified lectins as a novel bioreceptor that has been shown to be highly efficient in various biosensing devices due to their specificity for cognate carbohydrates. Their work also described various biosensor receptors and mechanisms of transduction in lectin-based biosensors (Rahaie and Kazemi 2010). Therefore, with the help of microorganism, biosensors can be developed which could be further used in detecting various harmful toxins and pollutants from the environment.

9.6 Application of Plant as a Biosensor of Pollutants in the Environment and Monitoring of Pollutants

The liberation of poisonous metal contaminants into the surroundings has been identified as one of the global challenges. Pollution of the surface water, ground, and soil with such contaminants has been demonstrated to possess numerous adverse influences on all the ecosystems. Therefore, the removal of a polluted environment has become a major challenge among most scientists. The application of traditional technology entails a higher amount of money and involves a lot of time. The most common methods involved in the management of polluted soil involve management of the soil, excavation of polluted soil after the hazardous materials have been removed, and containment of the treated soil to prevent further leaching of the pollutants, especially into the groundwater. Most of these highlighted techniques are not cost-effective, and they involve a lot of time. Therefore, there is a need to search for new sustainable, cost-effective, and eco-friendly techniques for easy clean-up technologies for the rejuvenation of heavily polluted environmental. A typical example of this is the application of plant-based clean-up technologies that could be applied for the detection of pollutants from surface water and soils, which could be referred to as phytoremediation (Salt et al. 1998; Krämer and Chardonnens 2001; Clemens et al. 2002; Krämer 2005).

Different types of plants have been reorganized to possess a unique potential to build up higher levels of metals in them, especially in the above-ground tissues. This technique is referred to as metal-hyperaccumulating plants. The mode of action entails sequestration, metal uptake, translocation, and chelation. It has also provided more insight into the molecular knowledge of tolerance and metal build-up in hyperaccumulating plants. The process involved in the accumulation of metals entails mobilization, removal and sequestration, xylem movement, unloading, trafficking, and buildup (Clemens et al. 2002).

Most of these processes have an impact on the rate of the metal accumulation process. Moreover, more facts have been documented on the function of ion channels in intracellular and intercellular bioelectrochemical signaling, especially in green plants (Volkov and Ranatunga 2006; Vodeneev et al. 2015). This has also strengthened the application of hyperaccumulating plants as models while investigating the influence of transition metals as ion channel blockers in green plants. Numerous transition metal ions are recognized as the ion channel blockers for voltage-gated calcium ion channels at low concentrations (Salt et al. 1998; Decoursey 2003)

9.7 Current Research Trends, Future Challenges, and Limitations of Biosensor Technology

An integrated stratagem through the application of technologies that varies from genetically engineered microbes, electromechanical, electrochemical, and fluorescence-cumoptical-based biosensors entails the recent technology for discoveries of the biosensor. These biosensors have diverse applications in the detection of several contaminate and monitoring of pollutants in the environment. These biosensors have several merits, such as cost-effectiveness, and necessitate biofabrication permits the detection of numerous molecules which are toxic in the environment. Moreover, there should be more investment in the development of biosensors that could be applied in multiplex conditions which might entail 2D and which necessitate 3D identification of sophisticated transducers required for quantifying and targeting small analytes of interest (Dias et al. 2014).

Moreover, some biosensors have a wider application in evaluating the quality of fish and meat through applying hypoxanthine biosensors by fabrication (Lawal and Adeloju 2012). The application of piezoelectric, nucleic acid, electrochemical, and optical has been documented to detect several contaminants in the environment (Kumar and Rani 2013). Moreover, the application of polymers and nanomaterials and these biosensors has been documented to generate better hybrid devices for effective usage in detecting pollutants and their effective monitoring of these contaminants in the environment (Citartan et al. 2013; Turner 2013). Moreover, applying synthetic biology techniques will enhance the development of microbial biosensors, especially those with environmental monitoring and energy demand. It has also been discovered that biosensor development centers around certain features such as small molecule detection, cost-effectiveness, sensitivity, non-toxicity, and specificity. These features have played a greater role in overcoming all the formation challenges in the development of biosensors. Moreover, the application of electrochemical sensors together with a nanomaterial in the development of biosensors have been documented to play a crucial role in resolving diverse challenges facing mankind (Kwon and Bard 2012; Bandodkar and Wang 2014). Therefore, the synergetic combination of biofabrication and biosensing with synergetic techniques applying optic, electrochemical, or bioelectronics principles could constitute a major pathway in developing a more robust biosensor in this modern era.

9.8 Conclusion and Future Recommendation

This chapter has provided detailed information on the application of microbial and plant cell biosensors for environmental monitoring. Specific information was also provided on relevant Gold nanorods (GNRs-DNA) DNA biosensors, Enzyme-linked immunosorbent assay (ELISA), and Quartz crystal microbalance (QCM) biosensor, while detailed facts were provided on the application plant cell biosensors for environmental monitoring. Examples of Biosensors such as Optical biosensors that apply fluorescence, light absorption, luminescence, reflectance, refractive index, and Raman scattering were also highlighted. Moreover, relevant applications obtained through the unique integration of nano/micro technologies through microbial biosensors were highlighted. The chapter majorly focused on the various utilities of microbial biosensors in the environment domain as well as some other biological activities that could result into diverse sustainable development (Adetunji et al. 2019; Adetunji et al. 2013; Adetunji et al. 2021; Ukhurebor and Adetunji 2021; Olaniyan and Adetunji 2021; Adetunji et al. 2020a, b; Adejumo et al. 2017; Adetunji 2008).

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Chapter 10
Biomimetic Material-Based Biosensor for Environmental Monitoring

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Abstract Nature contains technologies that will make our lives easier. Hundreds of examples of biomimetics are now found in our daily life, one of such is biosensors. Biosensors studies have been continuously developing in recent years. The increase and widespread use of these studies are due to the fact that biosensors give correct results in many application areas in a short time. Nanoparticle-based biosensors are preferred in environmental monitoring because they are very sensitive and fast. There are high levels of potential analyte in air, water, and soil. In addition to current pollution situations, they are potential uses for farming, horticulture, and mining nanobiosensors. Nanobiosensors can detect oil spills and radioactive contamination in groundwater, as well as the concentration of toxic wastes, carcinogens, and microorganisms that get into drinking water. This chapter presents information on the use of biomimetically developed nanobiosensors in the environment.

Keywords Biomimetic · Environmental monitoring · Nanoparticle

10.1 Introduction

Human activities in the twentieth century tend to over-deplete natural resources, unlike other animals. In direct proportion to the arrival of civilization, industrialization increased, natural resources decreased, and the production and use of man-made resources increased (Gruber 2018). With the increase in human population, environmentally harmful pollutants arising from human needs have increased rapidly. Pollutants may be present in water, air, food, and day-to-day consumer products. All of these are factors that directly or indirectly affect human health (Pellizzari et al.

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2019; Shaffer et al. 2019). Environmental pollution is one of the main problems of the sustainable development of any society and of course the economy.

Heavy metals, organic pollutants, pathogenic microorganisms, and toxins of microorganisms are a serious environmental problem (Kvenvolden and Cooper 2003; Busetti et al. 2005; Duan et al. 2007). Methods that can be applied quickly, accurately, and on-site are required for controlled monitoring of the environment. The size of nanomaterials, which can be in different shapes, is similar to basic biological structures and varies between 1 and 100 nm (Xiang et al. 2014). Owing to its enormous surface area-to-mass ratio, nano-sized materials are preferred in different fields such as medicine, cosmetics, biomedicine, and the environment (Mehndiratta et al. 2013). Nanocomposite materials, interface biomaterials, nanobiochips, and nanoscale biomimetic materials have begun to be used in clinical medicine applications, industrial field, defense field, and environmental analysis (Min et al. 2009; Acharya et al. 2017; McConnell et al. 2020). Nanomaterial-based sensors are an amazing technology that provide sensitive detection, on the nanomolar to sub-picomolar level of environmental pollutants (Sadik et al. 2009; Aragay et al. 2012; Bănică 2012). In this chapter, biomimetic, biomimetic nanobiosensors, and nanobiosensors used as biomimetics are explained and their uses in environmental monitoring are summarized.

10.2 Biomimetic

The term biomimetic is derived from the Ancient Greek terms “bios,” which means “life,” and “mimesis,” which means “to mimic.” Terms such as bionics, biomimicry, biognosis, and bio-inspired design are also used instead of the term biomimetics (Vincent 2009; Gamage and Hyde 2012; Aanuoluwapo and Ohis 2017). Biomimetic is nowadays gaining overwhelming importance as a worldwide trend in design for environmentally conscious sustainable improving that often stimulates original innovations and solutions (Pedersen Zari and Storey 2007; Vincent 2009; Gamage and Hyde 2012; Pathak 2019). Biomimetic is a design discipline that examines the models, systems, formation processes, and elements of nature and produces solutions to people’s problems and needs by imitating the information obtained (Pathak 2019). Nature’s order has been designed with the aim of maximizing performance by consuming the least amount of material and resources. Since, after billions of years of evolution, nature has discovered how to use resources efficiently in order to maximize efficiency, what is useful, what is appropriate for the circumstances, and what is sustainable, and has created several lasting solutions for this (Gordon and Mattis 1985). Today, materials produced thanks to biomimetics find a wide range of uses from nanotechnology to robotics and transportation technology, from artificial intelligence to medicine and military applications. In addition, designs inspired by nature open the way to new and advanced technologies with features such as self-repair/cleaning, recyclability, environmentally friendly, aesthetic, high strength, and long life, based on the principle of the least material and the most efficiency with the least energy. The exploitation of natural resources caused by the uncontrolled global growth of industrialization causes major problems in the environment. The way to biomimetics in solving these problems is clear. In addition, many multinational companies and organizations are embracing biomimetics in terms of both efficiency and effectiveness (Rinaldi 2007; Aanuoluwapo and Ohis 2017).

10.3 Biomimetic Nanobiosensors

Over the years, biomimetics has been used to solve many complex problems. In addition, different hardware designs, software algorithms, and biosensors have been developed with the inspiration of biomimetics (Stroble et al. 2009; Wang et al. 2021). Due to their selectivity, high sensitivity, and low-cost, biosensor-based analytic devices can be exploited in situ to monitor several environmental pollutants (Mehrotra 2016; Reynoso et al. 2019; Peixoto et al. 2019). Biomimetic sensors supply an extremely selective molecular recognition taking advantage from well-known biochemical processes, such as the key–lock interaction of enzyme (Narakathu et al. 2010; Turdean 2011). Antibodies, enzymes, molecular/ion imprinted polymer receptors, proteins, and functional nucleic acids have been successfully applied in biomimetic nanosensors. The synthesis, purification, and integration of these receptors on the nanomaterial surface affect the performance and cost of the nanobiosensor (Stortini et al. 2020). In this part, the use of biomimetically produced nanobiosensors in the environment, inspired by the nature, is reviewed.

10.4 Pathogen Microorganisms

Detection of pathogenic microorganisms is very important in clinical and environmental settings (Cappitelli et al. 2014). It is very significant for a biosensor to be able to detect this low dose precisely. Sensitive and rapid detection of pathogen microorganisms is indispensable for preventing hospital-acquired infections and for the safety of food and drinking water (Steen Redeker et al. 2017). Biosensors produced for the detection of microorganisms use the enzymes of bacteria or monoclonal antibodies. These biological elements are highly selective, but their physical and chemical stability is poor. In addition, these bacteria need to be purified first, which takes more time and costs (Sapsford et al. 2008; Kryscio and Peppas 2012). Surface-imprinted polymers (SIPs), for example, can solve many of the issues associated with natural receptors while displaying comparable levels of sensitivity and selectivity. In recent years, various researchers have developed SIP-based biomimetic sensors based on optical, microgravimetric, and electrochemical detection concepts in recent years to identify pathogen microorganisms (Cai et al. 2010; Cohen et al. 2010; Wang et al. 2010; Findeisen et al. 2012; Schirhagl et al. 2012; Tokonami et al. 2013; Zhang et al. 2015).

10.5 Butterfly Wings

Among biomimetic nanosensors, nano structures found in butterfly wings have been used as models in different studies. The nanosensors can be optimized for use in a variety of environments and designed to be highly sensitive and effective (Li et al. 2016). The identification of volatile organic compounds (VOCs) or vapor gas is a valuable technology for industries such as military protection and manufacturing, which enable workers to work in hazardous and extreme conditions (Potyrailo et al. 2011; Ponzoni et al. 2012; Zhang et al. 2017). When dissolved in vapors or liquids of varying refractive indices, the Morpho butterfly's wings show a wide range of colors. This color formation changes depending on the type of gas or liquid and the immersion time (Berthier et al. 2006; Kinoshita et al. 2008; Niu et al. 2015; Wu et al. 2015; Piszter et al. 2020). Potyrailo et al. confirmed that Morpho butterfly wings give different optical responses at different vapors. They reported that this optical response performed much better than existing nano photo sensors (Potyrailo et al. 2007; Starkey et al. 2014). Butterfly wings can be mixed with a variety of materials to significantly enhance their physicochemical properties. Because of the synergistic effects of the different components, these hybrid systems have a wide range of uses, including not only the stimulus-responsive polymers mentioned above, but also materials such as inorganic magnetic materials for use in magnetooptical reaction sensors, enhanced infrared absorption, efficient electrocatalysts, photoluminescence, and water splitting catalysts (Peng et al. 2012, 2014; Zhang et al. 2014; Li et al. 2016).

10.6 Bioelectronic Nose

Various nanobiosensors using taste receptors to detect tastes have been described. The nanobiosensors could be up-and-coming devices in many industrial fields such as biomedical field, anti-terrorism, the food industry, and environmental monitoring (Moon et al. 2020).

The olfactory system in mammals can distinguish thousands of different molecules in very low concentrations. Olfactory receptors are specific for scents and can biomimetically detect the target molecule (Sela and Sobel 2010; Goldsmith et al. 2011; Oh et al. 2014; Di Pietrantonio et al. 2015). The first bioelectronic nose was discovered by Gopel et al., and olfactory receptors-based biosensors have been extensively studied in the last two decades (Gopel et al. 1998; Oh et al. 2011; Du et al. 2013). Today, many different bioelectronic noses are produced for olfactory analysis by combining nanomaterial-based devices with biomimetics. Combined with biomimetics, these devices have high olfactory sensitivity and specificity. Bioelectronic noses can be used in health diagnosis, nutritional quality management, and pollution management (Dung et al. 2018). Fast, cheap, and reliable methods are needed to detect chemical pollutants in the environment. The ability of gas sensor technology to detect various vapors (organic and inorganic) and chemical contaminants and bioelectronic nasal technology obtained by combining this technology with biological interface is an indispensable sensor. Bioelectronic nose devices can be used in many different areas such as monitoring air pollution, mapping chemical smoke distribution to track fires in chemical storage facilities, early or real-time field surveillance through sensor monitoring networks, and ensuring chemical safety at port entrances and exits (Valente et al. 1998; Hayes et al. 2007; De Vito et al. 2008). The bioelectronic nose can detect toxic substances in the industrial field and alert the facility to the accumulation of explosive fumes such as carbon monoxide (CO) or carbon dioxide (CO_2). In addition, soil pollution can be monitored on-site with bioelectronic nose technology (Dung et al. 2018). With the bioelectronic nose, different contaminants can be detected simultaneously using a gas sensor array (Severin et al. 2000; Kwon et al. 2015). Son et al. confirmed that very low levels of geosmin (GSM) and 2-methylisoborneol (MIB) in water can be found in the bioelectronic nose made with HOR and SWNT-FET (Son et al. 2015). In a different approach, the SWNT-FET sensor device manufactured using biomimetic polydiacetylene interfaced with PDA-based lipid membranes was used in conjunction with NT receptors to convert binding activities between target TNT and selective peptide receptors (Kim et al. 2011).

10.7 Nanoenzymes

Nanoenzymes with unique physicochemical properties mimic natural enzymes and exhibit enzyme-like properties. When looking at natural enzymes, they show activity at limited pH and temperature. Nanoenzymes, on the other hand, have a great advantage with their high structural strength and stability. Due to both enzyme-like activities and unique physicochemical properties, nanoenzymes are used in the medical and environmental field (Fig. 10.1). Nanoenzymes are used in the detection and improvement of inorganic and organic pollutants in the environmental field (Wang et al. 2016, 2018; Huang et al. 2019b; Jiang et al. 2019). Recent studies have reported the emergence of nanozymes for the monitoring of pesticide residues on soil, water, plants, and crop samples (Liang et al. 2013; Singh et al. 2017; Zhu et al. 2020).

Heavy metal ions (HMIs) are non-biodegradable pollutants and thus adversely affect the balance of the environment and especially biological systems (Nriagu and Pacyna 1988). It is essential to monitor heavy metals with selective and sensitive sensors, as heavy metals can cause vomiting, diarrhea, nausea, kidney dysfunction, and even death (NHMRC and NRMMC 2011). Heavy metals can be detected using a variety of techniques, including atomic absorption spectrometry (AAS) (Zhou et al. 2016), inductively coupled plasma mass spectrometry (ICP-MS) (Bua et al. 2016), and inductively coupled plasma atomic emission spectrometry (ICP-AES) (Zhao et al. 2015), spectrophotometry (Wang et al. 2020), and atomic fluorescence spectrometry (AFS) (Fernández-Martínez et al. 2015). Although these techniques have advantages such as selectivity, high sensitivity, and accurate identification, they

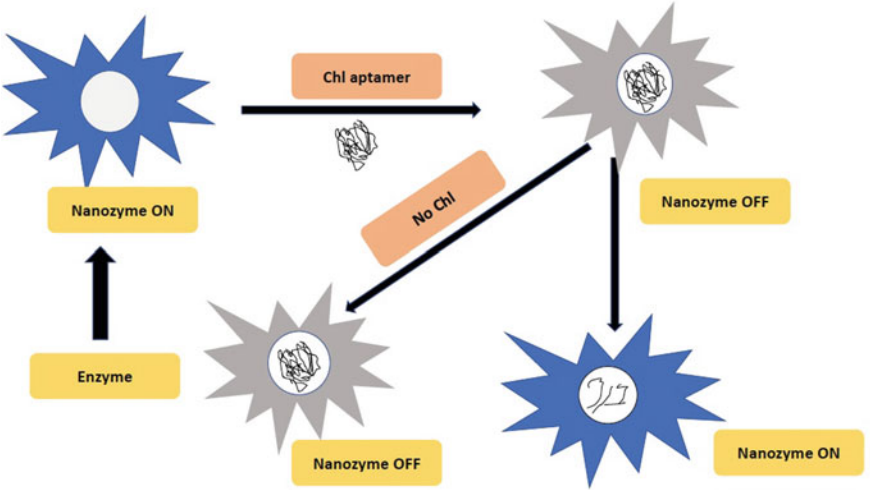

Fig. 10.1 Process of nanoenzymes

cause various problems when cost and analysis steps are taken into consideration. Therefore, portable devices are needed to detect heavy metal ions on-site. This is where biosensors are the solution (Rodriguez-Mozaz et al. 2005; Wang et al. 2014).

In the last decade, there has been a great increase in studies on monitoring heavy metal ions with biomimetic sensors (Gumpu et al. 2015; Zhou et al. 2016; De Benedetto et al. 2019; Wang et al. 2020). Several researchers have explored the use of nanozymes for the monitoring of heavy metal ions (Li et al. 2019; Meng et al. 2020; Yan et al. 2020; Unnikrishnan et al. 2021). Similar to natural enzymes, the peroxidase, oxidase, or catalase-like activity of nanozymes can be inhibited or enhanced by the presence of heavy metal ions (Li et al. 2015; Liao et al. 2017; Zhao et al. 2017; Jiang et al. 2018). The peroxidase-like activity of type I nanozymes such as cysteine-stabilized Fe_3O_4 magnetic nanoparticles, chitosan-functionalized $MoSe_2$, and Co_9S_8 can also be triggered by heavy metal ions (Mu et al. 2018; Huang et al. 2019a; Niu et al. 2019).

Every year, millions of tons of chemicals are used in farming fields, posing a toxicological danger to human health and the atmosphere (Kim et al. 2017; Storck et al. 2017). Since pesticides are sprayed on the soil or applied directly to plants, mammals, birds, and fish are also indirectly affected by means such as surface, atmosphere, and under groundwater (Rasmussen et al. 2015; Ruiz-Suárez et al. 2015; Barghi et al. 2018; Ernst et al. 2018; Koivisto et al. 2018; Zhen et al. 2018). It is very important that pesticides can be detected quickly and their compliance with legal limits can be checked (Capoferri et al. 2018).

Phosphatase-like activity is used in the detection of pesticides by using type I nanoenzymes containing nanoceria. In addition, peroxidase-like activity comes to the fore by using type I Fe_3O_4 nanoparticles or type II metal particles (Guan et al.

2012; Weerathunge et al. 2014, 2019; Biswas et al. 2016; Singh et al. 2017; Wei et al. 2019; Boruah and Das 2020; Sun et al. 2020).

10.8 Conclusion

"Biomimetics" is not a complete definition until the technology is transferred and implemented in the technical environment. The apparent need for sustainability necessitates not just the imitation of natural nature but also the method. Several of these have been discussed in this study. Simulating biological processes with biomimetic nanostructures offers a rare convenience in solving problems in chemistry, medicine, electronics, and the environment. Biomimicry will play an ever-larger role in sensor design in the future, assisting in the solution of difficult problems aimed at improving human life quality. The characterization of natural models that can be used in the production of biomimetic nanomaterials gains importance in this process. Nanotechnology can be tailored to need compared to biological structures. The use of nanotechnology in the development of biomimetic materials will pave the way for the development of different materials.

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Chapter 11
Chemiluminescence Sensors for Environmental Monitoring

Fareeda Zaheer, Muhammad Yasin Naz, Shazia Shukrullah, and Humaira Hussain

Abstract Chemical sensors are currently one of most interesting research domains because of their great selectivity and sensitivity with the small sizes for the portable application. The chemiluminescence reaction could be utilized in the analysis because intensity of chemiluminescence emission is, to some extent, proportional to reaction rate. Chemiluminescence sensors are valuable instruments because of their great sensitivity and selectivity. The instrumentations used in chemiluminescence sensor development, such as light detections and the flow injection analyses system, are discussed in this chapter. Numerous applications for detection of organic and inorganic compounds in gaseous sample and the solutions show that such sensors have good selectivity and reproducibility of analytes at the low concentration levels. This chapter also covers the practical applications of chemiluminescence sensors.

Keywords Chemiluminescence · Biosensor · Sensor · Microbial · Flow injection

11.1 Introduction

Chemiluminescence (CL) is a phenomenon that occurs when chemical reactions produce visible light. For chemiluminescence emission, there are three basic requirements:

1. Adequate energy to produce an electronically exciting state.
2. Reaction mechanism to generate energy for production of electronically exciting state.

Efficiency of creating molecule in excited states for photon release (Su et al. 2007).

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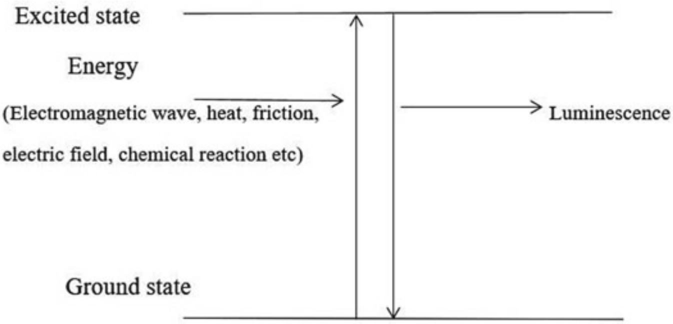

Fig. 11.1 Illustration of mechanism of luminescence process

Luminescence is the process by which electrons in matter produces light of a given wavelength without emitting heat and returns to a ground state after absorbing external energy from an electromagnetic wave, friction, heat, electric field, chemical reaction, etc. As shown in Fig. 11.1, chemiluminescence occurs when the source of the absorbed energy is a chemical reaction. Chemiluminescence does not require a light source since the energy of a chemical reaction induced by a luminescence reagent excites the material. Chemiluminescence reagents are classified into two types: direct luminescence reagents, in which the excited material emits light directly, and indirect luminescence reagents, in which energy from the chemical process excites another material.

The chemiluminescence reaction could be utilized in the analysis because intensity of chemiluminescence emission is, to some extent, proportional to reaction rate.

$$I_{\mathrm{CL}} = \Phi_{\mathrm{CL}}.(-\mathrm{d}A/\mathrm{d}t)$$

where the I_{CL} denotes the intensity of chemiluminescence emission in photons per second, Φ_{CL} denotes the chemiluminescence quantum yields, and $-\mathrm{d}A/\mathrm{d}t$ denotes rate at which chemiluminescence material is consumed (Su et al. 2007). Since the rate of reaction is the function of the chemical concentrations, the above equation can also be written as

$$I_{\mathrm{CL}} = \Phi_{\mathrm{CL}}.C_{\mathrm{A}}$$

where the C_{A} is substance's concentration.

Chemiluminescence analysis objects could be divided into 3 groups: reactant of the chemiluminescence reaction; the enhancer, inhibitor or catalyst of the chemiluminescence reaction, and the enhancer, inhibitor or catalyst of the coupling reactions. In addition, several indirect procedures can be used to examine a wide range of other compounds. Chemiluminescence is notable for its sensitivity since the lack of source of light decreases noise, and reduces Raman and Rayleigh scattering, enabling photon detector to be operate at large gain to increase signal to noise ratios (Su et al. 2007).

Chemical sensors are currently one of most interesting research domains because of their great selectivity and sensitivity with the small sizes for the portable application. Based on origin of available signals, the sensors can now be categorized into electrochemical and optical sensors, with latter primarily applying the concepts of photo luminescence, absorbance and CL detection. Because chemiluminescence-based sensors have a higher sensitivity than photo luminescence-based sensors, there was a lot of interest in developing them in recent past times. In comparison to photo luminescence detection the improved sensitivity of chemiluminescence-based sensing systems can be attributed to elimination of noise induced by scattering of light, as well as simpler set up with low background emission (Zhang et al. 2005). Moreover, the use of chemiluminescence biosensors and chemiluminescence immunosensors improved selectivity of chemiluminescence-based sensors. When chemiluminescence sensor's selectivity is compared to that of amperometric/potentiometric sensor, it is clear that chemiluminescence sensor is most selective (Aboul-Enein et al. 2000).

This chapter will discuss the instrumentation for chemiluminescence sensor development, such as light detections and the flow injection analyses system. This chapter will cover numerous applications for detection of organic and inorganic compounds in gaseous samples and the solutions. This chapter will also provide the practical applications of chemiluminescence sensors.

11.2 Instrumentation for CL Sensors

The development of chemiluminescence instrumentation and sensor architecture is linked to instrumentation utilized for chemiluminescence sensors. Majority of sensors are flow through sensors, it follows that the development of instrumentation can inevitably include flow system (valve, pump, cells, etc.). As a result, the development of chemiluminescence sensor is linked to flow injection analyses technique utilized and light detection (Aboul-Enein et al. 2000). Gold nanoparticles are also employed in the generation of reactive-oxygen species and radicals via the breakdown of H_2O_2, which leads to an increase in the formation of excited state luminol species and, as a result, increases CL signals. Citrate-capped gold nanoparticles have been used to boost CL signals in the luminol–urea hydrogen peroxide-ALP CL system by 100% via a metal-enhanced chemiluminescence mechanism. Using a dipstick-based immunoassay with detection at sub-nano gram levels, improved CL signals were used to detect aflatoxin-B1 in peanut butter and wheat flour.

11.2.1 Light-Detection

Chemiluminescence reaction is extremely sensitive, because light detection is important since its sensitivity has a direct impact on chemiluminescence sensor's sensitivity.

The development of the luminometers has recently received increasing attention. The photo multiplier tube is most common instrument for the detection of light (Aboul-Enein et al. 2000). The use of photo diode improved the sensitivity of light sensing system (Michel et al. 1999).

A video camera-based luminograph is used to enhance sensitivity. It contains a luminograph and a video system. The luminograph is high performance low light imaging systems capable of detecting any kind of the luminescent emissions over broad range of the intensities. A video system contains high dynamic range video camera that is the vidicon type tube with selenium-arsenic-titanium photoconductor attached to image intensifiers through large transmission-lenses. The luminous signal is focused on photocathode in image intensifiers, which is subsequently amplified. The image is then projected onto Saticon-tube by another lens, and final image is processed. To avoid interference from external light, the sample is kept in light-tight box (Aboul-Enein et al. 2000).

Optic fiber light guide provide a flexible solution to problem of obtaining light across instrument's optical path (Aboul-Enein et al. 2000). A reflecting flow cell with fiber optic coupled light emitting diode-based absorbance detectors is developed (Jambunathan et al. 1999). Light is carried from electronic unit to reflective flow through cell and back. Due to RI (refractive index) difference b/w core and cladding of optic fiber, the optic fiber can carry light with low loss. As a result, the cell is being placed remotely from electronic units and umbilical connections are not affected by electrical signal noise. After first warming phase, the level of noise of such detectors with LEDs of varying emissions maxima was measured within the range of 3–20 μAU, with highest short-term drifts of 4 μAU/min. The detector's response is proportional to concentration, when sample absorbance and source emissions properties are properly matched. The detection limits of chemiluminescence sensors is significantly reduced when fiber optics are used (Aboul-Enein et al. 2000).

11.2.2 Flow Injection Technique

Majority of chemiluminescence sensors, are flow through type. FIA is an important method for quantitative study of samples and is especially effective for improving reaction condition for chemiluminescence emission. Chemiluminescence signal in the flow system rise in proportion to analyte concentrations and manifest like sharp peak imposed on lower constant blanks signal, as assessed by time window in which mixtures of the analyte and the reagents passes through detector cells (Baeyens et al. 1998).

The cell geometry has a significant impact on quality of analytic data acquired with chemiluminescence flow through sensor (Valcárcel and de Castro 1993). Martin and Nieman suggested cell (Fig. 11.2) as chemiluminescence biosensor (Stewart 1981).

Three distinct modifications on positioning of the enzymes and the $Ru(bpy)_3{}^{+2}$ layer was investigated, for suggested cell. The enzymes-loaded film is almost adjacent to but up-stream from, $Ru(bpy)_3{}^{+2}$ or Nafion films in series designs (Fig. 11.3a).

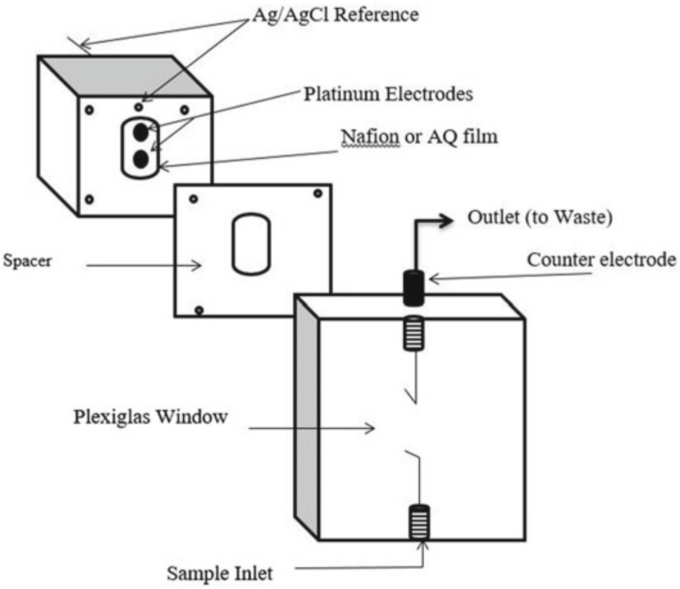

Fig. 11.2 ECL flow-cell in wider view

The stacked technique is shown in the Fig. 11.3b, stacks layers on the top of one another. In opposite technique, two layers are placed exactly across each-other in thin films channel. The capability to employ both the electrodes for chemiluminescence detection is a benefit of stacked and the opposite techniques over series. The benefits of the opposite technique over the stacked technique include short preparation period and option to reuse enzyme layers with new chemiluminescence sensor (Aboul-Enein et al. 2000).

The RI is a difficulty in optical cell utilized for flow through application. To minimize variations in refractive index, the source must be imaged on exit panel with the focusing optic and tapered cells architecture must be used. Using 2nd wavelength, as a reference can help to eliminate refractive index changes. The path of light and the path of liquid-flow are collinear in conventional *Z* cell. The path of light is perpendicular to path of liquid-flow in reflective cells design, and light is reflected again along same path, minimizing refractive index sensitivity significantly. The varying degrees of sensitivity for bubbles or the particles entrapment problem is one of the major issues with flow through optical cell. The radial way across flow tube can be used as optical route to solve this problem. For straight flow channel and large outlet bore, entrapment issues are decreased (Aboul-Enein et al. 2000).

Figure 11.4 shows reflecting cell with low refractive index susceptibility (Jambunathan et al. 1999). The cell is made up of square cross section glass/quartz tube T that is tapered on entrance end to 0.7-mm internal diameter to fit the inner diameter of conduit utilized in flow manifolds. The uniform taper allows for smooth flows transitions and appropriate wash out profile without adding noise to flow. On

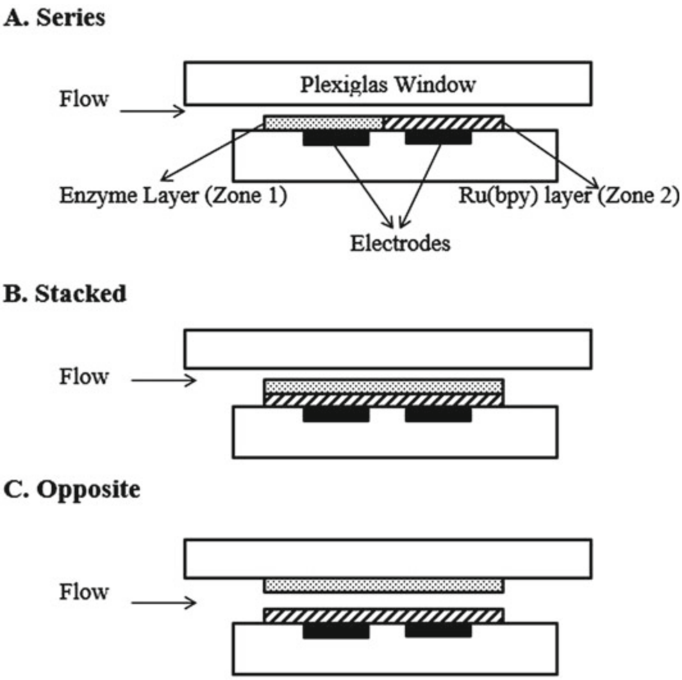

Fig. 11.3 Three electrochemiluminescence bio sensor designs for Ru(bpy)$_3$$^{+2}$ and enzyme layers

both ends, *O* rings provide essential liquid seal. F1 and F2 are two 1.50-mm core jacketed acrylic optic fibers pushed in to insert *I*. The insert fit snugly into driller holes in cell holder's body (*B*). The optic fiber bearing inserts are placed flat against cell's face, and then fastened with screw in proper orientation. The launched light is reflected by the arrangements made on other end of cell. A front face concave-mirror (*C*), with supporting nut (*N*), was put on opposite face of cell, in early design. The simple method of silvering cell body's outside provides same outcomes. To avoid silvering internal surfaces of cell, make sure to cover entrance and exit ports of cell. It was found that, silvering entire outside of cell and subsequently removing silver from optically targeted face was the most efficient method. A transparent acrylic coating protects silver coat (Aboul-Enein et al. 2000).

The material utilized in cell manufacturing has a significant role in lowering refractive index. It was found that, a cell must have lower refractive index than liquid. The refractive index of material utilized in the cell building was calculated using water as a reference. CS_2 in the glass tube or C_2H_5OH in fluorinated ethylene propylene co-polymer tubes were reported as construction material (Fujiwara and Fuwa 1985).

The excellent outcomes were achieved using amorphous fluoro polymer called Teflon AF that has lower refractive index than water. This material is suitable for the liquid cores waveguide cell. The fundamental benefit of using such cell is that it

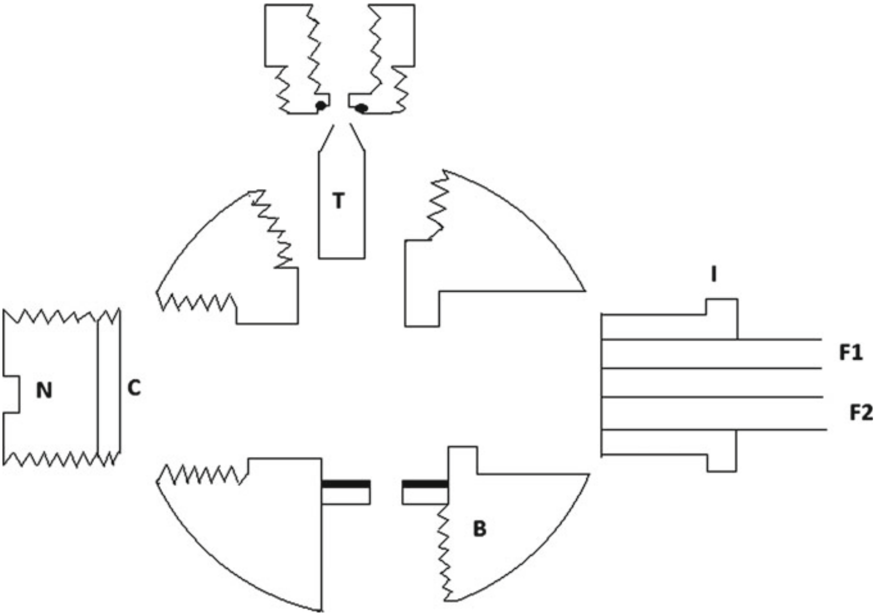

Fig. 11.4 Design of a cell. *T*, quartz cell, inserted into body (*B*) of cell, and sealed on both sides by *O* ring seals. 1/4–28 threaded are In or Out connections. Optic fibers F1 and F2 are carried in the insert and are flush against cell wall. On observe sides of cell, nut *N* holds *C* (concave mirror), alternately, except for face covered by optic fibers, cell is coated by silver than that of luminol chemiluminescence sensor. The fundamental drawback of these kinds of chemiluminescence sensors is that they are non-selective for a wide range of the substances

behaves similar to optical fibers or wave guides. Because cell is bounded to detector and the source of light, sensitivities of the measurements improve significantly (Aboul-Enein et al. 2000).

According to location on sensitive microzones where reaction occurs and connection with detector, there are 3 fundamental kinds of flow through sensors (Fig. 11.5) (Valcárcel and de Castro 1993). Two of these (*a* and *b*) depend on probes attached to instruments; sensitive micro zone can mount to probe's end (*a*) or built into flow cells (*b*). The sensitive micro zone is combined to unique flow cells in 3rd kind of sensor, which is a traditional instrument (*c*).

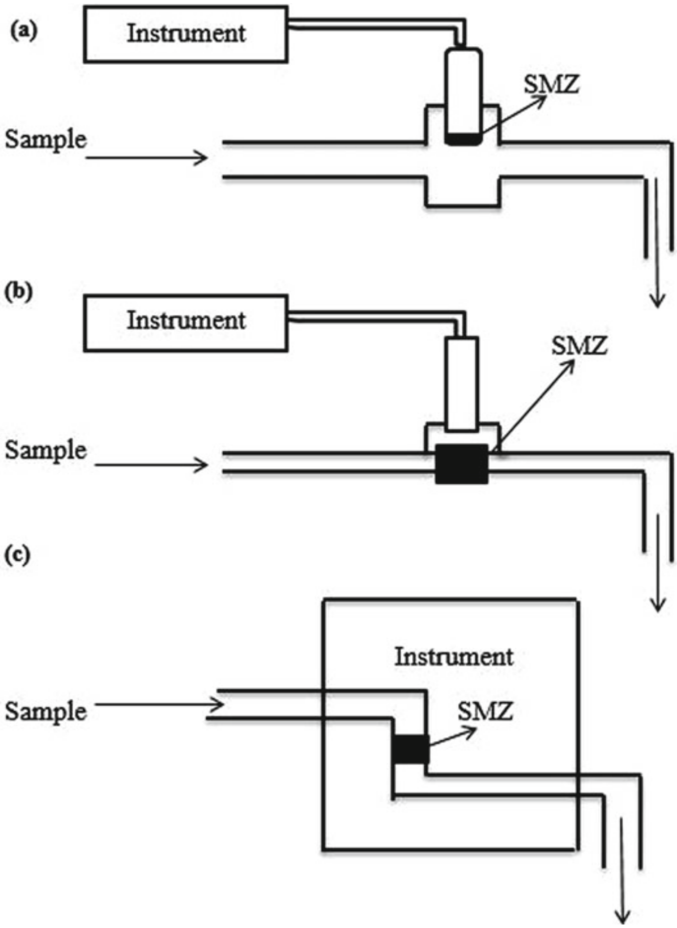

Fig. 11.5 Various kinds of flows through chemical sensors

11.3 Applications

11.3.1 Determination of the Analytes in the Air/Vapor

11.3.1.1 NO_2 Sensor

For nitrogen dioxide assay, three kinds of chemiluminescence sensors have been reported. The chip of sensor in lead phthalo cyanine thin films sensor was made up of layer of thin film produced by the vacuum sublimations of the purified lead phthalo cyanine powder and the comb like gold electrodes (Heilmann et al. 1992). The good sensitivity can obtain by using chemiluminescence biosensor (Spicer et al.

1994), the 1st of which is focused on reduction of nitrogen dioxide to nitrogen monoxide accompanied by sensing of nitrogen monoxide from chemiluminescence generated by its reactions with ozone, and the 2nd of which is focused on detection of chemiluminescence generated from reactions of nitrogen dioxide with the luminol solutions. The working range of concentration for ozone chemiluminescence sensors is greater.

11.3.1.2 O_2 Sensor

The oxygen assay (Collins and Rose-Pehrsson 1995) system includes solid phase reagents across that air undergoing analysis is pumped, which is positioned under photo multiplier tube that measures resulting chemiluminescence. The fluoropolyol combining luminol, potassium hydroxide, $Fe_2(SO_4)_3$ as catalysts yielded better result for oxygen assay with 2.40 mg/l detections limit.

11.3.1.3 Hydrazine Sensors

Two kinds of experiment with chemiluminescence sensor are suggested for assay of N_2H_4 and mono methyl and the dimethyl derivatives of hydrazine, based on ex-situ and the in-situ methods of analyses (Collins 1996). An extra device is required for ex-situ methods: a sample was diluted in the air and supplied at one milliliter/minute to chamber comprising tris ruthenium complexes in 0.10 mol/l phosphate buffers, by diffusions through cellulose membranes; the analyte was oxidized utilizing platinum electrode. Both the in-situ and the ex-situ analysis have detection's limits based on nanogram/litter concentration levels (Aboul-Enein et al. 2000).

11.3.2 Chemiluminescence-Based Sensors on Metal Ions and Non-metal Ions

Metal and non-metal ions quantification is critical for environmental control of the pollution from industrial and agriculture sources. Arsenic is most commonly found in surface and ground waters in inorganic form of arsenate As (V) and arsenite As (III), both are acutely hazardous if taken. For the detection of arsenite in the aqueous sample, a chemiluminescence flow injection procedure was presented. Arsenite sample was injected into carrier-stream containing one percent sodium hexameta-phosphate in 0.02 mol/l H_2SO_4, which subsequently merged with reagent stream containing $KMnO_4$ at *Y*-piece. Other ions of metal including mercury (II), copper (II), cobalt (II), vanadium (IV), Cr (III) and the total chromium in environment have been determined using chemiluminescence. A unique chemiluminescent flow injection analysis method relying on chemiluminescent reactions between cinchomeronic-hydrazide and H_2O_2 in strong alkaline media has been presented for measurement of vanadium (IV) in the aqueous solutions. Vanadium (IV) serves as catalyst in chemiluminescence reaction. An automatic approach for screening water sample comprising copper (II) was described in 2004 relied on peryoxalate-chemiluminescence reactions utilizing coproporphyrin *I* as fluorophor molecule to achieve selectivity and simple flow injection chemiluminescence detectors. In 2005, thiosemicarbazide-H_2O_2 was described as unique chemiluminescence reaction for detection of the copper at the nanogram/milliliter level in batch types. For non-metal ions, bromide ions detection in sea water, flow-based approach using chemiluminescence detection was suggested. The method was relied on oxidation of the bromide to the bromine through chloramine-*T* accompanied by reaction of the bromine with the luminol to create chemiluminescence emission (Su et al. 2007).

11.3.3 Determination of the Analytes in the Liquid

11.3.3.1 Enzymes-Based Sensors

Enzyme-based biosensors, which use enzymes as recognition elements and combine the intrinsic specificity of enzymes with the specific advantages of biosensors, have been widely used in a variety of disciplines. Because of their excellent sensitivity and specificity, these biosensors are continually attracting the attention of researchers due to their enormous potential for future bioanalysis.

Amino Acids

For the amino acid, a chemiluminescence biosensor was made up of PTFE tubes having amino acids dehydrogenase, peroxidase, and the NADH-oxidase mounted on the tresylated hydro philic vinyl polymers beads, which was spirally coiled in the front of photo multiplier tube (Kiba et al. 1998). NH_4^+ and proteins do not interfere with sensor. Glutathione is a reducing agent that interferes. With the detection's limit of 10.0 nmol/l, the calibration's graph appeared linear from 30.0 nmol/l to 5.0 μmole/l amino acids. The sampling rate was 75 samples per day. Values of RSD are lesser than two percent. Amino acid recovery in the plasma ranged from 98.0 to 100.0% (Aboul-Enein et al. 2000).

L-Alanine

A chemiluminescence detector was connected with packed beds flow micro reactor comprising alanine amino-transferase mounted onto sieved spongy glass bead (Janasek and Spohn 1999). Cobalt (II) and the immobilized ARP were utilized in optic fiber cell to catalyze the reaction of indicator between H_2O_2 and luminol.

L-alanine concentrations in cell cultivating media were measured in the range of 2–500 μmole/l using cobalt (II), with detection limit of one mole/litter, and in the range of 5–800 μmole/l using ARP, with detection limit of 2.0 μmole/l (Aboul-Enein et al. 2000).

Choline

The chemiluminescence biosensor detects choline by immobilizing choline ($C_5H_{14}NO$) oxidase and the fungal peroxidase on nylon or polymer. The calibration's curves were straight in concentration's range of 0.1–1 μmole/l with detection limit of 1.0 μmole/l (Zhou et al. 1993).

Ethanol

To create H_2O_2, alcohol-oxidase is utilized, followed through its reaction with the luminol in presence of potassium ferricyanide as catalysts (Xie et al. 1992). Optical fiber transmits luminescence from flow cells to detector. Ethanol may be measured in concentrations ranging from 3–70 μmole/l, with limit of detection of 3 μmole/l (Aboul-Enein et al. 2000).

Glucose

For the glucose assays, three kinds of chemiluminescence bio sensors are introduced. The 1st type relied on the use of the glucose oxidase for the enzymatic reactions and luminol for chemiluminescence reaction (Aboul-Enein et al. 1999). With limit of detection of 1.0 μmole/litter, the range of concentration is now on mole/litter magnitude scale. The 2nd kind relied on use of the enzyme glucose dehydrogenase for the enzymatic reactions, coupled with Ru (II) tris (2, 2′ bipyridyl) complex, for chemiluminescence reaction (Martin and Nieman 1993). The sensor has concentrations range of 10–2500 μmole/l. Interferences such as oxalate, NADH, tripropyl amine and proline are abundant. However, NAD^+ and the gluconic acids do not interfere. For the glucose assays, the chemiluminescence-based microbial sensors are also developed. The microbe cell was introduced onto chitosan-gel which was attached to pH sensitive FET surface, for the sensor development. This sensor can detect glucose in the concentrations range of 0.10–1 mmol/l (Aboul-Enein et al. 1999).

L-lactate

Chemiluminescence-based bio sensors for the L-lactate were made from enzyme modified graphite and silica pastes (Janasek and Spohn 1997). To produce chemiluminescence, L-lactate oxidase is combined with the luminol or Na_2CO_3. When employed in the clinical analyses, the technique is extremely selective and sensitive (Aboul-Enein et al. 1999).

Nadh

An electro-generated chemiluminescence-based bio sensor for the NADH relied on Ru (II) tris (2, 2′ bipyridyl) complex and dehydrogenase immobilized onto Eastman AQ 55S, and the Nafion cation exchange polymer film is developed (Martin and Nieman 1997). The range of concentration is 0.2–5 nmol/l. Bioluminescence-based bio sensor for NADH assay has been proposed (Blum et al. 1995). A bio active layer was created by covalently binding oxidoreductase and bacterial luciferase to pre-activated polyamide membranes. In the concentrations range of 10 pmol/l to 0.50 nmol/l, the calibration curve was straight (Aboul-Enein et al. 1999).

Hydrogen Peroxide

In presence of cobalt (II), copper (II) ions and luminol, chemiluminescence-based sensors could be used for H_2O_2 assay (Janasek et al. 1998). H_2O_2 concentrations in the ranges of 0.1–200 μmole/l and 5.0–200 μmole/l can be measured using cobalt (II) and copper (II) foils respectively. The gas dialysis cells were employed to enhance selectivity. H_2O_2 could be measured in 40 nmol/l to 10.0 μmole/l concentrations range, using cobalt (II) and luminol immobilized on highly basic anion exchange resin and the weak acid cation exchange resin, with detection limit of 12.0 nmol/l. The detection of the glucose in the blood was made possible by last system's great sensitivity; selectivity is increased by using packed-bed reactors with immobilized glucose oxidase (Qin et al. 1998).

Xanthine and Hypoxanthine

By covalently immobilizing microbial peroxidase or the xanthine-oxidase on the pre-activated nylon membrane, chemiluminescence-based enzymes sensors for the xanthine and the hypoxanthine assays are produced (Spohn et al. 1995). The luminol-hydrogen peroxide reaction was used to produce chemiluminescence. 5 μmole/l was the limit of detection (Aboul-Enein et al. 1999).

11.3.3.2 Other

Ascorbic Acids

For the ascorbic acids assay, 3 sensors depending on the luminol, and various cation's immobilization onto resins are recommended:

i. Anion exchange resin of type *D*-201 comprising permanganate and luminol immobilized.
ii. For luminol immobilization, anion exchange resin of type *D*-201 × 7 was employed, and for iron (II) immobilization, 732 cation exchange resin was employed (Qin et al. 1997).
iii. Amberlit *A*-27 anion exchange resins comprising immobilized $K_3[Fe(CN)_6]$ and the luminol (Zhang and Qin 1996).

By utilizing these sensors, the ascorbic acids can be measured in the following concentrations ranges: (1) 10.0 g/l to 4 mg/l (2) 1 nmol/l to 1 μmole/l (3) 0.010–0.8 μg/ml, with detections limits: (1) 5 μg/l (2) 0.40 nmol/l (3) 5.50 nanog/ml, respectively, although first sensor is interference-free. Uric acid, vitamin B1, thiourea and copper (II) all obstruct 3rd sensor's function (Aboul-Enein et al. 2000).

Chlorine

The pyrex tube was filled with uranine complexes immobilized onto IRA-93 anion exchange resins and photo multiplier tube was placed near Pyrex tube for the assay of chlorine (Nakagama et al. 1990). It can be used to monitor free chlorine concentrations in tap water upto 1 mmol/l with the limit of detection of 2.0 μmole/l. For concentration of 10.0 μmole/l, the variation coefficient found for free chlorine assays is 1.6%. The biggest drawback is sensor's limited life span (Aboul-Enein et al. 2000).

Copper

An ion exchange column with cyanide and luminol co-immobilized onto resin was used to make copper chemiluminescence sensor (Qin et al. 1998), and copper was momentarily maintained by the electrochemical pre concentration on gold electrode in anodic stripped voltammetric-cell. The reagents are eluted by injecting 0.1 mol/l sodium hydroxide through column, which subsequently react with copper stripped from electrodes to generate chemiluminescence signal. In 0.01–10 μg/l copper (II) solution concentrations range, the response is linear, with limit of detection of 8 nanog/l. For copper (II) assay in natural water and the human-serum, RSD value was 7.4% at a level of concentration of 40 nanog/l (Aboul-Enein et al. 2000).

11.4 Chemiluminescence for Reactive-Oxygen Species Sensing and Imaging Analysis

Reactive-oxygen species are described as oxygen containing molecules with half-lives ranging from nano seconds to hours. Many biological and chemical processes produce them as ions, radicals and molecules, such as H_2O_2, $^1O_2$, ClO^-,

$ONOO^-$,$\cdot OH$, $HOO\cdot$, $O_2\cdot^-$. Depending on kinds of reactive-oxygen species, reactions in which they take part and target molecule with which reactive-oxygen species react, the functions, they perform, differ greatly. Reactive-oxygen species have been frequently utilized in chemical process, such as the decomposition of dangerous compounds, because they are extremely reactive. Reactive-oxygen specie is by-product of the oxygen metabolism that plays a key function in homeostasis and cell signaling in biological process. However, under some conditions, reactive-oxygen species levels can continue to rise, causing oxidative-stress and a variety of diseases such as neurological disorder, cardiovascular disease, lung disease, and cancer. Therefore, one of most critical concerns in chemical, medical and biological fields is the detection, quantification and the kinetic evaluation of reactive-oxygen species in the complex materials even in cell and in-vivo. Chemiluminescence or light emissions followed by the chemical reaction, has been used in a variety of industries including food, pharmaceuticals, biology and the environment.

Chemiluminescence analysis is particularly useful for detecting reactive-oxygen species because it does not need excitation of light and all chemiluminescence reactions are relied on the redox reaction. The determination of various reactive-oxygen species has been done using chemiluminescence reagents including luminol and luminol derivative, imidazopyrazinone derivative, lophine derivative, lucigenin and acridinium esters derivative. The weak chemiluminescence systems have been created for detection of reactive-oxygen species in addition to chemiluminescence systems relied on chemiluminescence reagents. Different materials acting as catalyst or luminophors have been used to improve the methodologies and develop novel applications in reactive-oxygen species sensing and imaging. Nanoparticles, nanoclusters, quantum dots and metal organic frameworks have been used as catalysts for reactive-oxygen species in various chemiluminescence systems. Meanwhile, for reactive-oxygen species sensing, luminous compounds that behave as luminophors have been devised and manufactured. CdTe quantum dots and SiC nanoparticles have been created for selective detection of $\cdot OH$, relied on the radiative re-combination of $\cdot OH$ injected holes and electrons in luminous nanomaterials.

Various existing chemiluminescence systems are unsuitable for imaging reactive-oxygen species in-vivo. For example the maximum luminol's reactivity must be attained under the basic condition that is not present in-vivo. Furthermore luminol's maximum emission wavelength is 425 nm, which prevents deep-depth imaging of reactive-oxygen species in the organs. Therefore luminescent substances like nanoparticles based on quantum dots and luminol derivatives with large chemiluminescence intensity in the physiological condition, HRP-SiO_2@FFLuc nanoparticles and peroxalate nanoparticles that can create near infrared radiation have emerged fast. Peroxalate nanoparticles have been the most effective materials for the in-vivo imaging of hydrogen peroxide because of tunable emission wavelength and good selectivity for hydrogen peroxide over the other reactive-oxygen species. Peroxalate has recently been utilized as chemical fuels to produce electronic excitations energy for hydrogen peroxide, and semiconducting polymer rather than dye molecules, have been utilized for in-vivo hydrogen peroxide imaging (Su et al. 2019).

11.5 Conclusions and Prospects

Chemiluminescence sensor is a valuable instrument because of its great sensitivity and selectivity. The use of enzymatic/antigen antibody reaction increased selectivity of sensors. The adoption of novel reagents to increase selectivity of chemiluminescence reaction will be important in future. They are, however, highly sensitive to various environmental parameters, including solvent, pH, temperature, ionic strength and the presence of other compounds in matrices. The use of optical fiber increased the detecting quality of the light. For chemiluminescence sensor's construction, new kinds of flow through cells are proposed. Because of its high sensitivity, chemiluminescence sensor can now be used for detection of organic and inorganic compounds in gaseous sample and the solutions with good selectivity and reproducibility of analytes at the low concentrations level.

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Chapter 12
Nanobiosensor for Mycotoxin Detection in Foodstuff

Garima Rathee, Gaurav Bartwal, Jyotsna Rathee, Anil Kumar, and Pratima R. Solanki

Abstract Mycotoxin is a term coined for toxic metabolites released by certain fungi species in feed, food, and food products. The most eminent mycotoxins comprise fumonisin, patulin, ochratoxin, aflatoxins, zearalenone, and deoxynivalenol. Some of these mycotoxins, such as AFs B1, are considered highly carcinogenic agents, while a few of them are doubted to have deadly effects. The presence of such mycotoxins contaminates the food and feed to a great extent. The presence of mycotoxins can be assessed by using various traditional methods. However, these techniques suffer from multiple drawbacks. Therefore, there is a significant need to develop novel and innovative techniques to control and monitor mycotoxins. The involvement of nanotechnology in the advancement of nanobiosensors would be an alternative sensitive method for prompt recognition of mycotoxins. This chapter involves the implementation of nanomaterials in the synthesis of nanobiosensors that could be successfully applied to detect mycotoxins in foodstuffs.

Keywords Mycotoxins · Nanomaterials · Biosensors · Detection · Foodstuffs

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12.1 Introduction

Fungus is a class of organisms that can exist as single as well as complex multicellular organisms. The fungus has the ability of developing in every habitation over a variety of nutrients at distinct pH and temperature range. According to scientific estimation, about 1 million different fungal species exist (Hawksworth 2012). The fungal kingdom is very useful for human and animal life. Due to these renowned applications, this kingdom is continuously exploited by the industries to produce antibiotics and chemicals.

Additionally, the practical applicability of fungi in beverages and food, particularly for enhancing the commercial and nutritional values and usage in fermentation, has simultaneously enhanced fungal exploitation (Copetti 2019; Zhao et al. 2019). However, various studies have estimated that around 400 fungi species are present in nature and these species have the speciality of exhibiting pathogenicity to human beings. Moreover, there exist chiefly two groups of fungal infections, which are endemic pathogens (instigated by "true pathogenic" fungi species) and opportunistic pathogens (disturbs immuno-compromised patients) (Safavieh et al. 2017).

Toxins, derived from "*toxikon,*" a Greek word, are majorly the lethal constituents released by numerous micro-organisms, animals, and plant species. These toxins are generally comprised of proteins or peptides that result in severe diseases on collaborating with cellular and enzyme receptors. Mycotoxins (oriented from the Greek words *mykes*, "fungus" and *toxini*, "toxin") are minor secondary metabolites generated by various fungi species with MW approximately equal to 700. These metabolites are well-defined as teratogenic, carcinogenic, and mutagenic. These toxins are eventually more dangerous than food additives and various pesticide residues in foodstuffs, making them one of the most leading causes of food poisoning after consumption of the contaminated products in humans and animals (Turner et al. 2009). Due to the extreme resistivity offered by the mycotoxins, a wide range of crops are affected not only before but also after harvesting. These toxins are also present in certain beverages such as wines and beers, produced by using contaminated materials, for instance, juices of selected grapes, fruits, and cereals. Moreover, these toxins can reach humans as contaminated eggs, meat, and milk when the animals are fed with initially contaminated food (Becker-Algeri et al. 2016).

Aspergillus, *Penicillium,* and *Fusarium* are the three foremost fungal species responsible for the generation of mycotoxin in foodstuffs (Aiko and Mehta 2015). Some of the fungal species and their corresponding mycotoxin release are listed in Table 12.1. Based on the probable menace toward human health and the economic effect, there exist an individual set of mycotoxins, which embraces aflatoxins, zearalenone (ZEN), ochratoxin A (OTA), ergopeptine alkaloids, fumonisins, and trichothecenes (HT-2, T-2, and deoxynivalenol (DON)) (Barac 2018). Moreover, some of the mycotoxins (patulin (PAT), citrinin, cyclopiazonic acid, penitrems, gliotoxin, and sterigmatocystin) are hazardous because of their uncommon regularity in feed and food adulteration (Marin et al. 2013; Ismaiel and Papenbrock 2015; Weidenbörner 2018).

Table 12.1 List of fungal species and corresponding mycotoxin release

Fungal species	Mycotoxin
Aspergillus section *Circumdati Aspergillus* section *Nigri Penicillium verrucossum*	Ochratoxin A
Fusarium graminearum, Fusarium culmorum	Deoxynivalenol
Aspergillus section flavi	Aflatoxins B1, B2, G1, G2, M1
Fusarium graminearum, F. culmorum, F. cerealis	Zearalenone
Fusarium section *Liseola, Aspergillus* section *Nigri*	Fumonisin
Fusarium sprotrichioides, F. poae, F. acuminatum	T-2 toxin (type A trichothecenes)
Claviceps genus, Acremonium coenophialum	Ergot alkaloids
Fusarium sprotrichioides, F. poae, F. langsethiae	HT-2 toxin (type A trichothecenes)
Aspergillus flavus, Penicillium commune, P. camamberti	Cyclopiazonic acid
Aspergillus niger, Penicillium citrinum, P. verrucosum	Citrinin
Penicillium expansum, P. griseofulvum, P. carneum	Patulin
Aspergillus versicolor, *A. flavus*	Sterigmatocystin
Penicillium crustosum	Penitrems
Fusarium sporotrichiodes	Diacetoxyscirpenol (type A trichothecenes)
Penicillium roqueforti	Roquefortine
Penicillium expansum, P. griseofulvum, P. carneum	Patulin

The consumption of mycotoxins adversely affects the host cells, which results in DNA damage, oxidative stress, and cell death. The host might also suffer from diarrhea, headache, vomiting, and more adverse cases with nephrotoxic, mutagenic, immunosuppressive, and dangerous character, ultimately causing death (Chhonker et al. 2018). The International Agency for Research on Cancer (IARC) has classified mycotoxins into three main groups based on carcinogenicity. Aflatoxins (AFG1, AFG2, AFB1, and AFB2) are categorized as carcinogenic to humans (Group 1); OTA, AFM1, fusarin C, fumonisin B1/B2, and sterigmatocystin are categorized as carcinogenic to humans (Group 2B); and lastly cyclochlorotine, citrinin, ZEN, T-2 toxin, DON, PAT, nivalenol, and fusarenone X are classified as carcinogenic to humans (Group 3) (Ostry et al. 2017). The mycotoxin carcinogenicity has forced the World Health Organization (WHO), EU Scientific Committee for Food (SCF), and other organizations to introduce specific recommendations and regulations for estimating the highest concentrations in foodstuffs (Stoev 2015).

As mycotoxins are hazardous, there is an urgent demand for protecting the food materials from adulteration. To protect food products and ensure food safety, there is a need to control and monitor the presence of mycotoxins at diverse serious phases of food chain, which are the monitoring of food supply, i.e., raw materials, monitoring of the materials during the handling of foodstuffs, and one-to-one care of final products at all the stages. The execution of such monitoring and testing to maintain food safety would require cutting-edge technology for designing systems with pioneering answers to specific quality, safety, and analytical necessities. The involvement of an integrated intelligence methodology, which permits a complete inter-connection and communication of sensing systems, would play a crucial role in food traceability. Moreover, the participation of nanotechnology enthused systems would be potent in achieving these requirements.

The nanotechnology field has proven its ability to improvise food safety and quality by demonstrating consistent quantifiable and qualitative statistics. Nanotechnology is the universal grouping of chemistry, physics, biology, toxicology, engineering, and material science, all assimilated to craft nanostructured materials to yield innovative new-fangled devices to detect mycotoxins introducing treatment methods against toxic fungi. The upsurge in mycotoxin derivatives has continuously increased the importance of creating multiplexed sensors built on nanostages. Nanotechnology's inclusion would offer an efficient system in rapid on-site estimation with higher reproducibility and sensitivity. Therefore, it eliminates the need to send samples to the laboratories for analysis, which is time-consuming and requires expensive instruments. Innovative novel systems (e.g., lab-on-a-chip devices, microarrays, nanosensors, etc.) are persistently introduced by exploiting the supremacy of applied nanomaterials for estimating the presence of mycotoxin (Prasad et al. 2017).

Continuous advancement in nanotechnology has generated hope for developing effective nanosensors, which would be beneficial for detecting mycotoxins. Nanotechnology, as the name suggests, deals with nanomaterials (e.g., nanorods, nanoparticles, etc.) ranging from 1–100 nm. Nanomaterials (such as silver, iron, palladium, gold, rhodium, and platinum) are designed using various physical, chemical, and biological methods (Dar et al. 2014). In a study, Ingle et al. have successfully fabricated silver nanoparticles from *Fusarium acuminatum* by using the mycosynthesis route. The activity of designed NPs was evaluated against various human pathogenic bacteria (Ingle et al. 2008). Moreover, nanotechnology has created many practical methodologies for sensing and recognizing the concentration of mycotoxin in livestock (Li et al. 2012).

The devices established by combining the nanoscale devices and biological components belong to a new generation of biosensors titled nanobiosensors. Many specific biosensors have been introduced to directly integrate mycotoxins into the fields during harvesting, storage, and transport. Moon et al. have designed sensors that are more easy on the pocket and could be applied for on-the-spot identification of mycotoxins at distinct platforms (field to market) (Moon et al. 2013). The sensing ability of nanobiosensors is a significant part, and it must be highly sensitive and specific toward mycotoxins (even at minor concentrations).

Moreover, Gan and co-workers have introduced a novel nanocomposite sensitive for detecting aflatoxins at very low mycotoxin concentration (0.3 pg/mL) (Gan et al. 2013). Similarly, Yotova et al. have fabricated a nanosensor that can be used to detect the presence of tyrosinase enzyme in carcinogenic fungi at a minute concentration (Yotova et al. 2013). Such available nanobiosensors display a critical sensitive mechanism that offers the benefit of initial recognition of mycotoxins even before it influences animals and human beings because they are capable of sensing mycotoxins at concentrations lesser than the permitted value set by the international standards (4 mg/kg fixed by the European Union (EU) for aflatoxins in foodstuffs) (Communities 2008).

This chapter explains the involvement of nanotechnology in the synthesis of nanobiosensors for testing mycotoxins in foodstuffs. Herein, we have reviewed the progression in the nanotechnology field for the detection and monitoring of mycotoxins in accuracy. In this chapter, various existing nano-platforms available commercially for detecting and surveilling mycotoxins have also been discussed.

12.2 Categories of Mycotoxins

Mycotoxin, an important class, poses a menace to the agriculture economy as well as public health. Based on toxicities and carcinogenicity, mycotoxins are divided into various sub-groups, which are as follows.

12.2.1 Fumonisins

Fumonisins' existence is abundant in corn and is released by *Fusarium* spp. (*F. proliferatum* and *F. verticillioides* are accountable for greater creation of Fumonisins). Moreover, polluted corns are predominantly comprised of Fumonisins B_1, B_2, and B_3. Fumonisin B_1 is abundant in nature as compared to Fumonisin B_2 and Fumonisin B_3. Fumonisin B_1 represents 70% concentration in adulterated feeds and foods (Direito et al. 2009). These toxins are nephrotoxic and hepatotoxic and disturb the immune system. This class of mycotoxins has structural similarity with sphingosine, a constituent of sphingolipids composed in specific nerve tissues and myelin. Fumonisins are categorized as Group 2B carcinogens with related reported cases of oesophageal cancer in China, United States, South Africa, and Egypt (Milićević et al. 2010).

12.2.2 Aflatoxins

This category of mycotoxins, abundant in maize and peanuts, comprises aflatoxin B (AFB_1 and AFB_2), aflatoxin M (AFM_1 and AFM_2), and aflatoxin G (AFG_1 and AFG_2). Aflatoxin B transforms into their corresponding hydroxylated metabolites when consumed by the livestock as contaminated feed. The members of *Aspergillus* species mainly release aflatoxins (*A. parasiticus and A. flavus*). Milk and milk-derived products are mostly adulterated by AFM_1 and AFM_2 (Zain 2011). As AFB_1 and AFM_2 might be the reason for human primary liver cancer collaboratively with hepatitis B virus, these are classified as Group 1 carcinogens by the IARC (Mosiello and Lamberti 2011). Shreds of evidence have shown that the higher ingestion of aflatoxins by children results in growth impairment and stunting, making them more prone to other diseases. Higher exposure to aflatoxins might result in acute poisoning and even death.

12.2.3 Ochratoxins

Ochratoxins are mainly released by numerous *Penicillium and Aspergillus* species (Muñoz et al. 2010). This category of mycotoxins comprises ochratoxin C (OTC), ochratoxin A (OTA), ochratoxin α (OTα), and ochratoxin B (OTB) (Ringot et al. 2006). Among these, the most lethal one is OTA and is readily found in coffee, cereals, spices, grape juices, dried fruits, and animal feeds. OTA is nephrocarcinogenic and nephrotoxic (Milićević et al. 2010). OTA has been categorized as a Group 2B carcinogen due to their tendency of inducing oxidative DNA impairments that can cause noxiousness and immunosuppression (Ringot et al. 2006).

12.2.4 Trichothecenes

Trichothecenes (TCs) are generated mainly by *Fusarium* genus and can be sub-allocated into four groups centered on chemical features and discharging fungi. TCs are immunosuppressive mycotoxins that adversely affect the gastrointestinal tract and result in vomiting, diarrhea, and sometimes abortion (Yazar and Omurtag 2008). The utmost existing TCs are mycotoxin deoxynivalenol and T-2 toxin. T-2 toxin chiefly pollutes barley, wheat, rice, and corn crops in both the stages (field and throughout storage). It is the most carcinogenic one. T-2 toxin has damaging effects on both human and animal health, such as irreversible harm to the bone marrow, decrease in white blood cells, and inhibition of protein synthesis (Zain 2011).

12.2.5 Zearalenone

Fusarium species mainly release this class of mycotoxins (*F. culmorum and F. graminearum*) as naturally existing endocrine-disrupting chemicals and are found in wheat, sorghum, oats, and maize. Zearalenone (ZEN) might result in osteogenesis and reproductive system toxicity among animals (Milićević et al. 2010). Most of the mycotoxins are thermal stable varying from 80 to 121 °C (cooking conditions) (Hueza et al. 2014).

12.3 Orthodox Techniques for Mycotoxin Identification

The orthodox methods applied for identifying and tracking mycotoxins are based on the analytical procedures that need the involvement of various costly instruments, time, and trained workforce for the precise analysis. Methodologies have been established by the Association of Official Agricultural Chemists (AOAC) to use inter-laboratory validation techniques for improvising the reliability of the analysis of mycotoxin data. In total, nearly 45 analytical techniques are used for the recognition of mycotoxins. These analytical approaches are persistently being reviewed and updated by the AOAC (2005). Conversely, there is a requirement for effective protocols for ample security and food safety. Conventional analytical methods are restricted because the modified compounds are becoming unextractable via extraction solvents employed for parent compounds.

12.3.1 Chromatographic-Based Methods

Chromatographic-based methods such as liquid chromatography (LC), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC) are the most frequently used methods for effective detection of mycotoxins with high sensitivity. These methods are most frequently coupled with a fluorescence detector (FLD), mass spectrometric (MS), and ultraviolet (UV) detectors for the detection of mycotoxins (summarized in Table 12.2) (Anfossi et al. 2016). These expensive and sophisticated techniques permit the detection of mycotoxins with higher sensitivity. The coupling of instruments with tandem mass spectroscopy yields various advantages such as selectivity, throughput, and accuracy (Sarkar et al. 2009). The coupling with mass spectrometry offers the advantage of various investigations of distinct mycotoxins and analogous derivatives grounded on molecular weight by providing structural data of unknown compounds. Estimation of mycotoxins via LC–MS technique is timewasting and needs extra extraction steps, pre-treatment, and cleansing (Mosiello and Lamberti 2011).

12.3.2 Immunochemical-Based Methods

The requirement of portable, prompt, easy-to-operate, and affordable systems for detecting mycotoxins has led to the introduction of immunochemical-based investigations (Tang et al. 2014). Electrochemical immunoassay and enzyme-linked immunosorbent assay (ELISA), which are commercially available, are broadly used to estimate mycotoxin due to the enhanced stability and capability of immobilizing specific antibodies and on-site testing ability (Anfossi et al. 2016). ELISA has constantly been a golden standard for the recognition of toxins. Due to

Table 12.2 Analytical techniques used for mycotoxins detection

Technique	Merits	Demerits
Chromatography		
GC	High sensitivity, confirmation via mass detection, automatic simultaneous detection of mycotoxins	Need experts for derivatization and operation, costly instrumentation, non-linear calibration curve, drifting responses, the difference in reproducibility
LC–MS	High sensitivity, no derivatization needed, identification of mycotoxins simultaneously	Costly instrumentation, quantification via matrix-assisted calibration curve, experts are needed
HPLC	High selectivity and sensitivity, less examination time	Costly instrumentation, experts are needed
TLC	Affordable, fast, and straightforward, simultaneous estimation of various mycotoxins	Only appropriate for AFs and OTs (poor precision and sensitivity), mainly useful for screening, quantification possible via densimeter
Enzymes-based assays		
ELISA (Enzyme-linked immunosorbent assay)	Simple sample preparation, inexpensive assay, screening assessed visually, lesser usage of organic solvents	Reliability issues regarding probable positive and negative outcomes, matrix interface problems that might result in cross-reactivity of associated mycotoxins
Real-time polymerase chain reaction (RT-PCR)	Great accuracy and sensitivity for high-throughput investigation	Time-consuming process and chances of errors during polymerization
Biosensors		
Electrochemical biosensors	Affordable, portable, prompt, on-spot investigation with good sensitivity and selectivity, experts not required	Choice of immobilizing matrix is significant to attain increased signal intensification

lower molecular weights, mycotoxins fail to prompt sufficient refractive index and bioluminescent/chemiluminescent signals upon successful binding to the sensor surface.

Biosensor is an immunochemical-based method involving synthetic ligands to detect mycotoxins via the binding process (Tothill 2011). A typical biosensor comprises of the following components (as depicted in Fig. 12.1):

- Ligand (enzymes, antibodies, nucleic acids, etc.) assimilated over the surface stage of the sensors

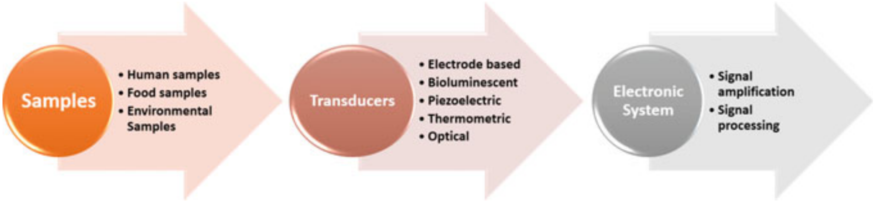

Fig. 12.1 Components of a typical biosensor

- Transducer element (electrochemical, calorimetric, mass sensitive, magnetic) that transform the binding into an electrical and optical signal
- An electronic system for convenient demonstration of the outcomes.

12.3.3 Microarrays

Microarray-dependent analytical techniques are also attractive candidates for the recognition of mycotoxin type contaminants as such approaches offer higher throughputs, increased reproducibility, higher density, higher selectivity and sensitivity, easy automation, lower sample intake, and lesser processing time. Microarrays are complex lab-on-a-chip devices that simultaneously identify many mycotoxins in one test via immobilizing two or more distinct types of ligands, which are specific and selective for respective mycotoxins. The detection of FB_1 and AFB_1 using microarray has been reported by Lamberti and co-workers (Lamberti et al. 2010).

12.4 Biosensors for the Recognition of Mycotoxins

As discussed above, the involvement of conventional techniques for detecting mycotoxins has significant drawbacks, such as the requirement of extraction of samples and tedious clean-up approaches for every assay of mycotoxin recognition. In few circumstances, post-concentration might also be compulsory. To resolve these complications, substantial consideration has been endorsed to the recognition of toxins by sensing technology, which resulted in the advancement of biosensors with the evolution of technology.

Biosensor is well-defined as the alliance of a biological factor with a transducer (physicochemical detector). This transducer further converts the received signal from the interface among the biological component and the analyte into an effortlessly quantifiable signal which the signal processor further demonstrates in a user-friendly manner. Vidal and co-workers have reviewed the usage of electrochemical affinity biosensors to identify mycotoxins (Vidal et al. 2013). A variety of biosensors have been developed, for instance, immunochemical biosensors, electrochemical biosensors, optical biosensors, electrode-based biosensors, acoustic biosensors, optical biosensors, whole cell-based biosensors, enzyme-based biosensors, whole organism-based biosensors, colorimetric biosensors, potentiometric biosensors, and amperometric biosensors (Rai et al. 2015).

Gaag and co-workers have designed a biosensor centered on the surface plasmon resonance (SPR) principle to detect four different types of mycotoxins (van der Gaag et al. 2003). Moreover, Otles and Yalcin have demonstrated carbon nanotubes (CNT) in designing a biosensor (Otles and Yalcin 2012). Chaudhary and Castle (Chaudhry and Castle 2011) have overviewed nanotechnology in foodstuffs and feeds. They described the probable usage of nanobiosensors in labeling foodstuffs with the key intention to monitor the storage and transport conditions of the packaged food with the assistant of nanosensors integrated within them. Such sensors are used for releasing food preservatives as soon as microbial activity initiates during the transportation. Campagnoli and co-workers have introduced an electronic nose as a screening tool to detect deoxynivalenol from naturally polluted wheat (Campagnoli et al. 2011).

Moreover, one of the most promising and reliable SPR principles has also been used for fabricating biosensor to detect AFs. The principle behind the development of the SPR biosensor is centered on the binding efficacy of biological molecules over the surface of a thin metal film that behaves as a sensor. This leads in mass concentration alteration that is further measured by the detector. Moreover, a chemiluminescence-based biosensor has also been developed to detect the presence of FMB1 and FMB2 (Mirasoli et al. 2012). The designed sensor was very rapid and sensitive, capable of detecting the minute concentration of FMs (up to 2.5 mg/L).

Researchers have moved closer to nanotechnology to design advanced biosensors, free from the drawbacks and with enhanced activity of existing biosensors. The inclusion of nanotechnology in biosensors has emerged as a promising technology for the fabrication of improvised biosensors.

12.5 Principle of Operational Manual of Nanomaterial in Nanobiosensors

The characteristics due to which nanobiosensors are appreciated moieties are credited to the nanomaterials employed in merging nanobiosensors. The nanomaterials support the immobilization process, amplify signals, generate signal probes, etc., which boost the sensing ability of nanobiosensors. Nanomaterials are supposed to improve the activity of biosensors at two different levels: at the bio-receptor level and transducer level. The ratio of surface area to volume in nanomaterials is very high. Applied nanomaterials provide more surface area for the attachment of biomolecules, resulting in better performance at the bio-receptor level. In a study introduced by Gan and co-workers (2013), magnetic nanoparticles have been used to immobilization over graphene oxide (GO), resulting in magnetic nanocomposites. The obtained nanocomposites were employed as bio-receptor for AFs M1 biomolecules. The outcomes have displayed the superiority of nanomaterials' at the bio-receptor level. Moreover, when nanomaterials are employed at the transducer stage, they enhance the electron transmission by acting as an active electron quencher and, therefore, improvising the action of transducers.

12.6 Versatility of Nanomaterials in Mycotoxin Detection

Numerous nanomaterials have been used for various nanobiosensing applications, such as electrode designing, immobilization of biomolecules over the surface of bio-receptors, and increasing the signal transduction toward the detector. The application of nanomaterials in the fabrication of DNA biosensors has been summarized by Khalid et al. (Abu-Salah et al. 2010). Otles et al. designed innovative nanobiosensors to detect microbial contamination present in food by using nanoparticles as an essential precursor (Yalcin and Otles 2010). Furthermore, Zhang and co-workers have reviewed the involvement of nanomaterials in designing nanobiosensors (Zhang et al. 2009). The assessment stated that CNTs, quantum dots, and nanoparticles could be considered excellent types of nanomaterials for synthesizing nanobiosensors.

12.7 Sensing Performance of Nanobiosensors for Mycotoxin Detection

Numerous nanomaterials such as distinct metal nanoparticles, nanowires, noble metal nanoparticles, quantum dots, nanoshells, nanotubes, and polymeric nanoparticles (Malhotra et al. 2014). Some of the essential characteristics that are mandatory for the usage of these nanomaterials in designing efficient nanobiosensors are as follows:

(a) High sensitivity toward the detection of mycotoxins even at minute concentrations (Yotova et al. 2013)
(b) Reproducibility of results by same nanobiosensors (Gan et al. 2013)
(c) Estimation of more than one toxins at one time (van der Gaag et al. 2003)
(d) Economical and profitable (Xu et al. 2012)
(e) Lower assay time (Xia and Ning 2011)
(f) Portability, nanobiosensors should be portable for on-the-spot recognition of mycotoxins at fields, retailers shop, or in storage (Moon et al. 2013)
(g) Specificity (Shim et al. 2009).

Due to aforementioned features, nanobiosensors are advantageous in the recognition of mycotoxins contaminants.

12.8 Nanobiosensors for the Recognition of Mycotoxins

The cutting-edge nanobiosensors attain great popularity due to their advanced parameters, viz. mobility, application time, and biocompatibility (Gan et al. 2013). The outcome of immobilization of tyrosinase on the progression of nanobiosensors was evaluated by Yotova and co-workers (2013). The immobilization step is essential as the immobilized materials enhance the contact among the sensors' signal detector elements and biometrics. Therefore, it has become very supportive in the enhancement of analytical selectivity and sensitivity of designed nanobiosensors.

Gold nanoparticles have been comprehensively studied to manufacture effective, selective, and sensitive nanobiosensors to test mycotoxins in foodstuffs. The incorporation of nanoparticles and enzyme bio-conjugates boosts the electron transferal among the electrode and the catalytic spots of the immobilized enzymes, increasing the biological stability of the sensor's surface. This leads to a rise in analytical sensitivity. Numerous sensors developed by using nanomaterials for the detection of mycotoxins are summarized in Table 12.3. Also, a typical schematic representation of nanobiosensors is illustrated in Fig. 12.2.

Apart from the nanomaterials mentioned in Table 12.3, innumerable nanomaterials and nanocomposites have been actively involved in the fabrication of nanobiosensors to detect mycotoxins in various foodstuffs. Ce-TiO_2@$MoSe_2$ nanosheets (Tang et al. 2019), CdS nanorod arrays/Ag_2S (Qileng et al. 2018b), carbon dots/MnO_2 nanosheets (Lin et al. 2017a), Mn^{2+}-doped $Zn_3(OH)_2V_2O_7$ nanobelts (Lin et al. 2017b), etc., are few of nanomaterials used for the testing of mycotoxins in foodstuffs.

Various aptasensors have been designed by using nanomaterials. Aptamers are a group of molecular recognition elements, capable of producing a special reaction with varied analytes. Due to their talent of recognition specificity for numerous analytes, aptamers have been broadly used in the designing of aptasensors acting as recognition fundamentals (Goud et al. 2018). To increase the activity of aptasensors for instance sensitivity and selectivity, numerous nanomaterials (such as covalent organic frameworks (COF), metal organic framework (MOF), noble metal nanoparticles, carbon-based nanomaterials, metal oxide NPs, and upconversion NPs) have been combined with aptasensors to design innovative nanomaterials-based aptasensors (Sharma et al. 2015). Furthermore, such nanomaterials-based aptasensors have been employed for the identification of mycotoxins. Nanomaterials perform few significant roles in nanomaterial-based aptasensors, such as signal production, immobilization support, signal amplification, replacement to the enzyme labels, and fluorescence quencher.

Recently, cutting-edge nanozymes have been used as signal amplication/generation labels for the mycotoxin aptasensing (Chatterjee et al. 2020). A colorimetric aptasensor has been used for the identification of ZEN by employing gold NPs with ZEN aptamer and peroxidase-mimicking activity (Sun et al. 2018). The designed aptasensor was capable of detecting ZEN with LR of 10–250 ng/mL and LOD of 10 ng/mL. A novel colorimetric aptasensor is dependent on aptamer-controlled $MnCo_2O_4$ nanozyme performance for OTA detection. This aptasensor was

Table 12.3 Developed nanobiosensors for the determination of mycotoxins

Mycotoxin	Nanomaterial involved	Recognition element	Detection limit	Matrix	Reference
Aflatoxin B1	CdTe quantum dots	Aptamer	0.004 μg/μL	Wheat and maize	Aswani Kumar et al. (2018)
Aflatoxin B1	Carbon nanotubes	Antibody	0.79 pg/g	Corn flour	Costa et al. (2017)
Aflatoxin B1	Gold nanoparticles (AuNPs)	Organic framework composite	2.8 pg/mL	Pistachio, wheat, rice, and peanut	Gu et al. (2019)
Aflatoxin B1	Au-PANI nanocomposites	Antibody	0.05 ng/mL	Corn	Yagati et al. (2018)
Aflatoxin B1	CdTe quantum dots	Antibody	0.001–15 ng/mL	Peanut	Lin et al. (2016)
Citrinin	Cd/Te QDs	Antibody	0.1 pM	Human serum	Shojaee Sadi et al. (2018)
Cyclopiazonic acid	Gold nanoparticles	Antibody	0.29 ng/mL	Cheese and maize	Hossain et al. (2019)
Fusarium toxins	Gold nanoparticles	Antibody	15 μg/kg (DON), 24 μg/kg (ZEA), 12 μg/kg (T-2 toxin)	Wheat	Hossain and Maragos (2018)
Fumonisin B1	Gold nanoparticles	Aptamer	2 pM	Maize	Chen et al. (2015)
OTA	2D-MoS_2	Aptamer	0.23 pg/mL	Red wine	Tang et al. (2018)
OTA	$SiO_2@Cu^{2+}$	Antibody	0.02 pg/mL	–	Qileng et al. (2018a)

capable of detecting OTA presence in corn samples with a lower LOD (0.08 ng/mL) (Huang et al. 2018). Furthermore, Fe_3O_4/GO- and Fe_3O_4@Au-based platforms have been used for simultaneous detection of AFB1 and OTA, respectively (Zhu et al. 2020).

Moreover, molecularly imprinted polymer (MIP) has also been used in biosensors, as an identification element in biosensors due to their benefits of good stability, easy preparation, low cost, high specificity, high affinity, great binding kinetics, and probable applications in severe circumstances. MIPs have emerged as a substitute to antibody for the progression of sensors and have attained great advances in using mycotoxin assays. Further, performance progression has been carried out by integrating nanomaterials such as NPs, CNTs, QDs, MOFs, and metal NPs. By employing

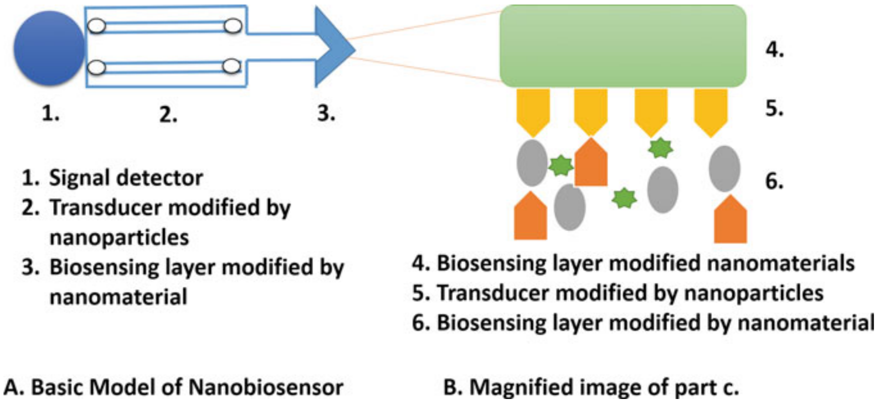

Fig. 12.2 A typical schematic representation of nanobiosensors

MIP and multi-walled CNTs to adjust a glass carbon electrode (GCE), a novel electrochemical sensor has been designed for identification of OTA. The designed MIP/MWCNT/GCE electrochemical sensor exhibited detection of OTA with LR of 0.05–1.0 μM, LOD of 0.0041 μM, and limit of quantification of 0.014 μM (Pacheco et al. 2015). An another MIP-based sensor (electrochemiluminescence) has been introduced by using $Ru(bpy)_3{}^{2+}$-doped SiO_2 NPs/chitosan composites, gold NPs, and MIP-revised GCE for the identification of FB1 (Zhang et al. 2017). $Ru@SiO_2$ NPs played the role of ECL luminophores. The AuNPs behave as a localized surface plasmon resonance source for enhancement of the intensity of ECL.

Moreover, carbon nanostructures such as graphene-based materials have arisen as a novel pervasive and promising technology because of their distinctive benefits over conventional materials (Chen et al. 2019).

12.9 Benefits and Challenges Associated with Detection of Mycotoxin by Using Nanobiosensors

The determination of mycotoxin in foodstuffs is a very effective exercise to guarantee food safety and quality and simultaneously eradicate and monitor the menace of consuming adulterated foods. Therefore, the ability to estimate mycotoxins in foodstuffs is a precedence to accomplish the legislative restrictions set by the food specialists universally. Furthermost, estimations of such toxins are still lead by employing conventional techniques, regardless of the disadvantages offered by these methods. These include lesser sensitivity at a lower concentration, time-consuming, requirement of the high workforce, highly expensive, and the necessity to send samples for analysis to the laboratory. However, the rise in mycotoxin contamination requires intense monitoring. Therefore, innovative options such as multi-array sensors built on nano- and microsystems as risk assessment tools and diagnostics have developed as an attractive candidate for the investigators (Tothill 2011).

Nanotechnology has already proven its potential in improvising food safety and quality considerably by employing innovative nanobiosensors. Moreover, nanobiosensors have displayed their efficiency in the determination of mycotoxins. They have also offered outstanding benefits over the conventional techniques and sensors, for instance, (a) ability to deliver robust, rapid, and cost-effective techniques for the on-site testing of such toxins, (b) involvement of nanobiosensors in the fabrication of ultrasensitive devices has become more accessible, (c) nanobiosensors are employed as promising diagnostic tools, (d) requires neither laboratory nor any specific instrument for estimation as on-site detection is possible, (e) provides high sensitivity toward the detection of minute concentrations of toxins in foodstuffs, (f) delivers high specificity, and (g) requirement of lesser human resources (Tothill 2009; Otles and Yalcin 2012).

Despite multiple advantages, nanobiosensors suffer from few limitations that need to be resolved to fabricate ideal commercial systems that are superior to the conventional methods employed to recognize mycotoxins. The complex chemical structure of mycotoxins polluting the food materials in distinct concentrations must be identified mandatorily. Few bio-analytical challenges faced by the applicability of nanobiosensors are needed to be resolved. Moreover, there is a requirement to enhance the stability of various nanomaterials such as quantum dots. The most significant concern related to the extensive and uncontrolled use is the toxicity risks toward the environment and humans after the disposal of these materials and devices. Conversely, such drawbacks could be resolved by safeguarding the control and limiting the usage of nanobiosensors.

12.10 Conclusion and Future Aspects

The involvement of nanomaterials in designing the biosensor is the most sophisticated route for the creation of nanobiosensors for the testing of mycotoxins in foodstuffs and feeds. The produced nanobiosensors comprise unique magnetic, mechanical, chemical, physical, and optical characteristics resulting in enhanced sensitivity and selectivity. The existing literature displays that the inclusion of nanomaterials in the construction of nanobiosensors enhances the properties of biosensors. Such designed nanobiosensors are sensitive, fast, cheap, specific, and reliable with better reproducibility. In the future, such nanobiosensors would improvise the quality of food, drinks, and feeds, resulting in minimal health menaces because of the ingestion of food adulterated by mycotoxins. Fabricating innovative sensors by involving nanomaterials is the cutting-edge area of research. Nanobiosensors, due to the involvement of nanomaterials with advanced properties, are better and different from biosensors. The inclusion of nanomaterials might enhance the optical, electrochemical, magnetic, and mechanical characteristics of biosensors. Despite the benefits offered by nanobiosensors, there is an excellent requirement of wide-ranging studies on improvising the available nanobiosensors due to certain restrictions in applicability. Additionally, extra researches are required for the designing of nanobiosensors for testing multiple toxins at a time.

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Chapter 13
Current Existing Techniques for Environmental Monitoring

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Abstract Natural and anthropogenic processes have contributed immensely to the global degradation of soil, water and atmosphere through the release of toxic gases, heavy metals (HM) and other toxic chemicals thus posing monumental problems globally. The constant increase of these pollutants has exposed the ineffectiveness of the existing conventional remediation and detection technologies thus calling for urgent effective, safe and reliable treatment methods. One of the current existing techniques for environmental monitoring is the application of nanobiosensors. Therefore, this chapter provides an insight into the carbon nanotubes (CNTs) utilization as the emerging nanobiosensors technology for environmental monitoring. Importantly, CNTs are endowed with excellent optical, chemical, physical, chemical and mechanical properties thus becoming appealing for noble applications. Their large surface area (SA), high adsorption capacity, excellent catalytic efficiency and great surface reactivity have ensured the application of CNT-based sensors in environmental detection and monitoring. This book chapter also provides advancement in CNTs-based sensor use in the discovery and tracing of heavy metal ions (HMI) and toxic gases.

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13.1 Introduction

Owing to the rapid growth of the world population, the continuing proliferation of industrial development and urbanization processes that includes construction, mining, fossil fuel, transportation, agricultural runoff and atmospheric deposition coupled with natural occurrences such as volcanic eruptions, forest fires and particle suspension in the air amongst others have hugely contributed to environmental pollution (Ukhurebor et al. 2021c; Aigbe et al. 2020). These anthropogenic and natural processes release toxic gases (asphyxiant gases, irritant gases, organic irritants and other irritant gases), heavy metal ions (HMI) [Hg(II), Cd(II), As(III), Pb(II), etc.], industrial and domestic wastewater (phenol and H_2O_2) and organophosphorus (OP) compounds (pesticides and insecticides) (Fig. 13.1) which have affected the ecosystem directly or indirectly thus posing great danger to the health of humans and ecosystem (Su et al. 2012; Ibrahim et al. 2016). For instance, toxic gases (Table 13.1) have been found to cause adverse health disorders like pulmonary disorders, headache, nausea, fatigue, coma, irritation (eyes, nose and throat) and upper respiratory tract, etc. (Lambrini et al. 2018). Also, several frequently used pesticides have been found to have endocrine-disruptive and cancer-triggering effects, high toxicity leading to loss of biodiversity (birds, plants and animals), renal and neurological disorders, reproduction and hepatic problems and high risk of psychiatric disorder in humans (Cardona and Rudel 2020; Wong et al. 2017).

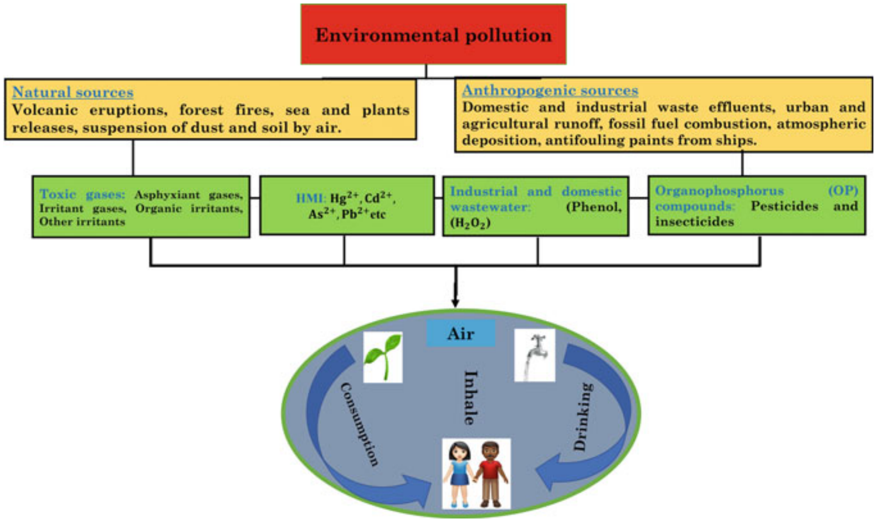


Fig. 13.1 Environmental pollutants and their sources

Table 13.1 Some basic toxic gases and their effects on the environment

Toxic gases	Effects
Asphyxiant gases such as carbon monoxide (CO), hydrogen cyanide (HCN), carbon dioxide (CO_2)	CO: can cause some medical impediments such as disorders of the cardiac and pulmonary, disturbance in the behavioural of the central nervous system, disorders in vision, pains in the head (headache), weakness, unconsciousness, shallow breathing as well as death CO_2: There are a limited reported studies about the health influences of enduring incessant CO_2 exposure on living organisms at levels less than 1.00%. According to the United States, the occupational limits of exposure to CO_2 have been set at 0.50% (5000 ppm) for 480 min. Allegedly, at this CO_2 concentration, the crews of the International Space Station often experienced pains in the head (headache), tiredness, mental sluggishness, emotional frustration and sleep interruption HCN: Some of the effects include pains in the head (headache), seasickness (nausea), giddiness, misperception, muscle feebleness, loss of harmonization, bradycardia, hyperventilation, cardiac arrhythmia, rapid loss of awareness and unconsciousness, and can likely lead to death
Irritant gases such as HCl, HBr, HF, SO_2, pentoxide (P_2O_5), oxides of nitrogen (NOx), nitric oxide (NO), nitrogen dioxide (NO_2), phosphorous	HCl: some of the effects are exasperating of the nose, eyes and throat, as well as pain, coughing, swelling and oedema of the upper tract of the respiratory system and concentrations of about 100 ppm. Inhalation (breathing in) of high concentrations of HCl has also been linked with destructive burns to the nose, eyes, throat and mouth, ulceration of the adenoidal septum, bronchoconstriction, tachypnoea and laryngeal contraction which could result in lack of oxygen (suffocation)
Organic irritants such as Acrolein, Formaldehyde	Acrolein: some of the effects are lachrimation in living organisms especially humans within about 5 s, nose exasperation, throat exasperation and lessening in the rate of respiration, the inception of pulmonary oedema, eye exasperation, etc. Formaldehyde: some of the effects are strong sensory irritation, causing slight to moderate exasperation of the upper tract of the respiratory system and eyes, bronchospasm, dyspnoea

(continued)

Table 13.1 (continued)

Toxic gases	Effects
Other irritants such as ammonia (NH_3), chlorine (Cl), phosgene ($COCl_2$)	NH_3: cruelly irritating and destructive to the respiratory tract and eyes, causes lachrimation and respiratory pain. Exposure to NH_3 could also cause exasperation to the throat, eyes and nose, inception of pulmonary oedema, fatal as a result of obstruction of the air route Cl: Restrained irritating of the upper respiratory tract and eyes, instant chest pain, queasiness and coughing, the inception of noxious pneumonitis and pulmonary oedema Phosgene ($COCl_2$)

Equally, heavy metals (HM) pose huge dangers to ecological environments and tend to accrue in the food chains. Evidently, continuous consumption of HMI-contaminated food and drinking of wastewater leads to monumental accumulation of HMI in bodies thus causing disorders and chronic diseases with others culminating in deaths. It is important to note that, in the year 2015, it is believed that 9 million deaths were ascribed to pollution globally which implies that these deaths were three times more than the deaths of tuberculosis (TB), malaria and AIDS cumulatively (Kumar and Guleria 2020). Therefore, it is fundamentally important to fabricate detection devices and techniques which are cost-effective, simple to use, field-portable that will aid in monitoring pollutants in the environment (Adetunji et al. 2021; Adetunji and Ukhurebor 2021). The monitoring will immensely assist in mitigation, reduction and even removal of these pollutants thus contributing to a sustainable and protected environment.

Notably, many traditional detection methods like inductively coupled plasma mass spectrometry (ICP-MS), high-performance liquid chromatography (HPLC), infrared (IR) spectroscopy, surface plasmon resonance (SPR), ultraviolet (UV) absorption and gas chromatography-mass spectrometry (GC–MS) has been employed to monitor the environmental pollutions (Su et al. 2012; Binions and Naik 2013). However, they have been found to be time-consuming during data acquisition, demand a high cost to assemble, high cost in maintenance requirements, they are bulk and heavy, complicated process during sample preparation and they demand trained manpower to run and conduct analysis (Binions and Naik 2013). Importantly, to remedy these problems, sensors have been developed in the recent past and successfully applied. They offer huge advantages which include easy fabrication, high selectivity and sensitivity, and they are less expensive.

A sensor consists of two main components, namely the receptor and the transducer (TSD). The high specificity property of the receptor guarantees enhanced sensing sensitivity. The TSD is typically a distinct physical or chemical component that operates with optical, piezoelectric, thermal, electrochemical and other additional detection machineries (Su et al. 2012). Specifically, in biosensors (BSs), biological recognition elements regularly are immobilized to the TSD's surface with great bioactive for targeting (Kerry et al. 2021). Here, the attachment mechanisms comprise covalent binding, encapsulation, cross-linking, adsorption and entrapment. Then, monitoring of the interaction between the target and the recognition element is done and converted into signals that are readable (Zhu 2017) as illustrated in Fig. 13.1.

Since the advent of nano (NN)-technology (technologies that work in NNmeter-scale 1–100 nm range), numerous NNmaterials (NMs) such as carbon NMs, magnetic and metallic NNparticles (NPs) have been innovatively fabricated with superior physical, excellent optical, improved electrical conductivity, mechanical and chemical reactivity properties compared to their bulk counterparts (Ukhurebor et al. 2021b). They have huge potential applications in medicine, energy, water treatment, food industry, sensing devices and pollution treatment thus presenting leapfrogging projections in the modification and improvement of already existing traditional remediation technologies (Onyancha et al. 2021; Ibrahim et al. 2016). With the exploitation of NM properties such as large SA, great surface reactivity (SR), robust adsorption capacity and superior catalytic efficiency (CE), NN-based sensors have been effectively fabricated and employed in tracing and detecting environmental pollutants (Adetunji et al. 2021; Adetunji and Ukhurebor 2021).

NM-based metals, semi-conductors and organic composites promote the improvement of electrical, chemical optical and magnetic features that are applicable in detection devices. NMs are mostly utilized for the construction of electrode and biomolecules (BioMs) tracers. When compared to enzyme labels, NPs are very stable and they offer superior sensitivity (discharge of thousands of atoms from NP). NPs are currently utilized as electrochemical labels comprising various hundreds or thousands of electroactive labels and thus driving detection limits down to some hundreds of biomolecules. Several NMs that have been employed for the immobilization of bio-elements are metallic NPs, carbon or metallic NNtubes, NNsilver-coated magnetic beads, magnetic particles, functionalized conductive polymers (PLM), etc. (Săndulescu et al. 2015).

In particular, carbon nanotube (CNT) sensors have been used to a great success in the detection of HM (Musameh et al. 2011), toxic gases (Sidek et al. 2013) and pesticides (Wong et al. 2017). Therefore, in the world of technology and specifically in applications of carbon-based NM as sensors, CNTs are popular and thus have a great role to play in environmental monitoring applications. Therefore, in this chapter, we will give an overview advancement of CNT-based sensors in detecting/tracing heavy HMI in water and toxic gases in the air. Figure 13.2 illustrates the utmost outlines of this chapter in the form of graphical abstract.

13.2 Carbon Nanotubes (CNTs)

The fact that carbon atoms can combine with each other resulting in the formation of unique and diverse structures presents the carbon element as the most intriguing element worldwide. Through hybridization of the form sp, sp^2 and sp^3, novel and specialized nanostructures like NPs, NN-diamonds, NN-onions, peapods, NNfibers,

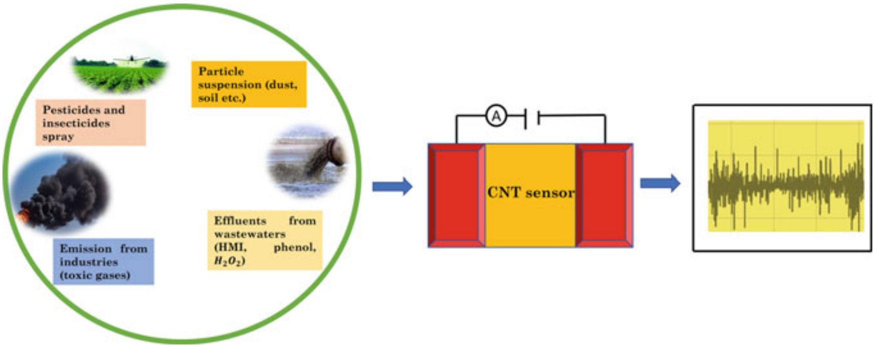


Fig. 13.2 The outlines of the chapter

NNrings, fullerenes and NNtubes have been realized. Their broad range of structures comprises 0-dimensional (D) (fullerenes and diamond clusters), 1-D (NNtubes-NTs), 2-D (graphene (Gr)) and 3-D structures (NNcrystalline diamond and fullerite) (Săndulescu et al. 2015; Onyancha et al. 2021) which have gained considerable attention. Particularly, CNT which was discovered in Iijima in 1991 (Iijima 1991) is considered a milestone in NM production as can be attested by the steady growth in volumes of existing/available articles and researches connected to the topic. The CNTs have attracted substantial attention due to the combination of excellent chemical and physical (outstanding elastic module, high electrical conductivity and great fracture strength) (Onyancha et al. 2021).

Conceivably, the CNT structure consists of one or more rolled-up sheet of graphene as shown in Fig. 13.3 (diagram schematically showing synthesis techniques and formation of SWCNT and MWCNTs from graphene layer). Here, an sp^2 hybridized meshwork of carbon atoms is arranged hexagonally to obtain cylindrical geometry of one-dimensional CNT NNstructure with a high aspect ratio. Based on the number of sheets involved, two configurations are generally used to classify CNTs. When one layer of graphene is rolled-up, a single-walled CNTs (SWCNTs) is designed with typical diameters of nearly 1 nm. The formation of multi-walled CNTs (MWCNTs) involves several layers to form concentric cylinders enclosed in each other with a spacing of layers of between 0.3 and 0.4 nm (Onyancha et al. 2021). The differences between the two classes are highlighted in Fig. 13.4, and they affect their solubility and dispersion both in surfactants and in other common solvents. Ideally, chiral vector C with indices (n, m) is employed to categorize SWCNTs into zigzag, armchair and chiral. Zigzag and armchair structures are obtained when $(n, 0)$ or $m = 0$ and $n = m$, respectively, while chiral nature exists when $n > m > 0$ (Onyancha et al. 2021). Given that SWCNTs are combined to form MWCNTs, their individual optical, chemical, mechanical and thermal properties dictate the potential applications of MWCNTs.

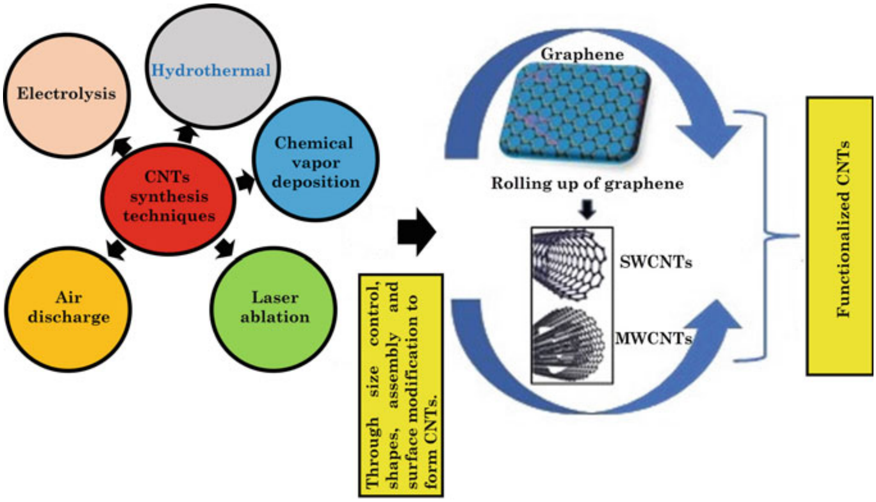
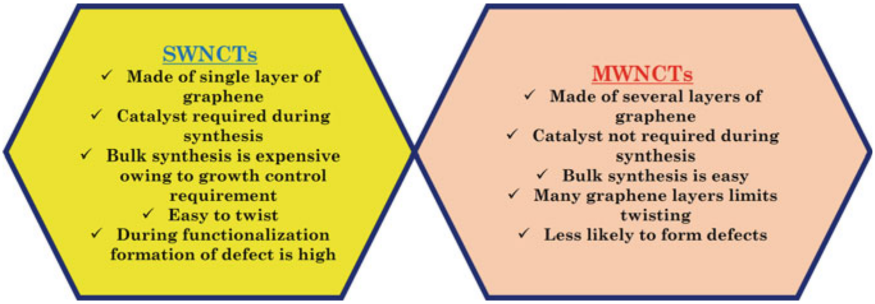


Fig. 13.3 Representation of CNT synthesis methods and formation of SWCNTs and MWCNTs from rolling up graphene sheet


SWNCTs
- ✓ Made of single layer of graphene
- ✓ Catalyst required during synthesis
- ✓ Bulk synthesis is expensive owing to growth control requirement
- ✓ Easy to twist
- ✓ During functionalization formation of defect is high

MWNCTs
- ✓ Made of several layers of graphene
- ✓ Catalyst not required during synthesis
- ✓ Bulk synthesis is easy
- ✓ Many graphene layers limits twisting
- ✓ Less likely to form defects

Fig. 13.4 Differences between SWCNTs and MWCNTs

The CNTs can be synthesized by diverse means like laser ablation, chemical vapour deposition techniques (CVD) (such as hot filament, water-aided, oxygen-aided, microwave plasma, radio-frequency, thermal and plasma-improved), electrolysis, arch discharge and sono-chemical hydrothermal methods. The pristine CNTs synthesized have remarkable properties which have catapulted them to high levels of advanced technological applications. Through reengineering, chemical modification and functionalization, the properties can even be improved. These outstanding properties have seen CNTs being used in the field of solar cells, energy storage, sensing, flexible displays, electronics (e.g. computer chips), biological field (cancer treatment, drug delivery, tissue reengineering, etc.) and water splitting (Săndulescu et al. 2015; Onyancha et al. 2021).

For instance, CNT electronic properties especially SWCNTs are distinct and show similar features to quantum dots and wires at minimal temperatures (temp) as well as Coulomb blockade and single-electron charging. Joining these properties with supplementary beneficial properties characteristic of NNstructure like the huge ratio of SA to volume, distinctive confinement effects and changing chemical and physical properties have ensued in extensive applications. The huge amount of benefits is expected to improved sensitivity, selectivity and rapid reversible reaction (rxn) by electrochemical means at normal pressure and temp. Such benefits include chemical sensors, catalyst platforms, storage and conversion of energy and electronic tools. Since they display excellent electron transfer abilities, they are employed as electrodes in an electrochemical rxn. They are also being utilized in various electrochemical sensors due to their capability to accelerate the rxn of electron transferred with electroactive varieties in the solution and interface of the electrode (Power et al. 2018).

Concerning the mechanical possessions of CNTs, these constituents have been found to be stiff and extremely strong. They are endowed with remarkable tensile strength (11–62 GPa) and Young's modulus ($E = 0.27$–3.6) (Venkataraman et al. 2019). Their density-normalized is approximately 56.0 times higher when compared to steel wire as well as around 1.70 times more when compared to that of silicon NNrods (Han et al. 2019). Now, in comparison with raw/pristine CNTs, the CNT-based composites have even higher electrical conductivity and mechanical properties which are vital ingredients for electrochemical sensing technology aimed at tracing, detecting and mitigating pollutants, pathogens and wastewater matrices (Khan et al. 2019).

13.3 Functionalization

Even though pristine CNTs exhibit numerous attractive properties and merits towards applications, they suffer dispersion activation emanating from high surface energy (Sireesha et al. 2018). As a result, they are rendered hydrophobic thus becoming unsolvable in water and other popular solvents. Consequently, CNTs ought to be functionalized to enhance solubility and evolve other vital functional possessions. Basically, for the fabrication of novel NNdevices, functionalization is deemed as a precondition. There are two possible functionalization strategies, namely covalent (C) and non-covalent (NC) surface modifications. Covalent functionalization can be classified as either sidewall or defect functionalization. Sidewall functionalization entails attaching of functional groups to the sides of CNTs while defect functionalization involves the conversion of carboxylic and other oxygen groups leading to the extended coupling of other functional groups like alcohol and amines (Onyancha et al. 2021). Sidewall oxidation is a typical path of obtaining CNTs having structural imperfections, thus producing polar solvable products appropriate for applications.

Generally, covalent functionalization relies on chemical rxn, chemical bonds existing between hydrophilic organic molecules and CNTs carbon atoms present on the tube's surface (Di Crescenzo et al. 2014). The disadvantage of this strategy is that it compromises the homogeneity and other initial properties thus altering the mechanical properties of CNTs (Sireesha et al. 2018). On the other hand, non-covalent functionalization (NCF) involves changing of CNT surface properties without altering the intrinsic structure and their novel properties thus becoming difficult to further amend. The NCF contacts comprise $\pi - \pi$ stacking, van der Waals forces, hydrophilic or hydrophobic interactions which are effective strategies of immobilizing molecules onto surfaces of CNTs (Onyancha et al. 2021).

In fact, functionalization has been used on numerous occasions to enhance CNT properties meant for environmental monitoring applications. For example, the sidewall functionalization approach was used to produce O_2-plasma-oxidized MWCNTs (poMWCNTs) meant for the electrochemical sensing of Cd(II) and Pb(II) (Wei et al. 2011). It was established that the current increased four times when compared to pristine/raw MWCNTs. This improvement was attributed to increased structural defects and oxygen-containing groups generated by plasma MWCNTs functionalization (Wei et al. 2011). Equally, poly (m-aminobenzene sulfonic acid) was employed to functionalize SWCNTs for the sensing of NO_2 and NH_3. It was realized that functionalization enhanced the sensor processing capability and sensitivity with the lowest concentration of NO_2 gas registered at 20 ppb (Zhang et al. 2007). Verma et al. functionalized MWCNTs with metallic phthalocyanines (separately using cobalt, vanadium and copper) to obtain thin film for detecting H_2O_2 vapour (Verma et al. 2011). Through ultra-sonication and suspension, MWCNT was employed as the conductive substance for the mixture of Cobalt-phthalocyanine (CoPc), Copper-phthalocyanine (CoPc) and Vanadium-phthalocyanine (VPc). Sensor fabricated by copper provided the lowest response of two seconds (2 s) and recovery time of three seconds while vanadium registered the highest values. The changes in resistance were attributed to redox rxn occurring at the surface of the thin-film composite and in presence of H_2O_2 vapours (Verma et al. 2011).

13.4 CNTs-Based Biosensors for HMI Sensing

As aforementioned, CNTs are stimulating a huge interest with various contemporary devices like TSD, for biosensing system development primarily owing to their outstanding physical, chemical and electronic features which consist of their large SA, exceptional electron transfer rxn properties and high electrical conductivity. Also, their distinctive tube-shaped structure, excellent biocompatibility (BioC) and adjustable sidewall make them a perfect contender for high-performance sensors fabrication. Electrochemical NNBSs have been utilized progressively in several forms of NMs, mainly as TSD using CNTs to enhance them from the viewpoints of automation (computerization), miniaturization (contraction) and multiplexed assessment as well as enhancing their analytic performance of such tools (Săndulescu et al. 2015; Bhalla and Singh 2016).

CNTs and, in recent times, Gr have found a huge deal of applications in biosensing (Martins et al. 2013). CNTs are employed as an electrode construction owing to their remarkable mechanical stability (firmness), large SA together with the extraordinary electrical conductivity instigated by the orbital hybridization (sp^2) amongst the neighbouring carbon atoms (Cho et al. 2020; Hwang et al. 2020).

CNTs-based BS concept was developed from the enzyme electrode description by Clark in 1962. They are composed of two parts which are the biological sensitive component and the TSD. BioMs or bioreceptors (BioRs) like protein, oligo or polynucleotides, microorganisms (microbes) or the entire tissues are used to functionalize CNTs and employed as a biological sensitive component. While the TSD is used to convert the concentration of the analytes to computable physical signals like currents (electric), absorbance (optical density), mass or acoustical (audio) variables for testing and detecting. They are categorized based on the interaction between the biological sensitive materials and the analyte into chemical and physical classes (Yang et al. 2015).

The outstanding sensitivity nature of CNTs towards their surface guarantees that they act as a perfect ingredient for NNscale BS mechanisms that are highly sensitive. But, their formidable intermolecular π–π contacts and hydrophobic (aquaphobic) nature is the main hindrance for the development of NNBS based on CNTs. To enhance their solubility and stability, their functionalizing via chemical sorption is improved by the various functionalities of CNTs. They are habitually ideal as working electrodes for devices employed for sensing due to their outstanding low detection (sensing) limit, and their hollow structure is good for enzyme sorption (Sireesha et al. 2018). They also act as stages to connect other compounds at their surface (exohedral functionalization) and can be opened and filled with losing their stability (endohedral functionalization) (Tîlmaciu and Morris 2015).

Single-walled CNTs (SWCNTs) show distinctive intrinsic characteristics like photophysical features which depend on their structure and semiconductive behaviour. Hybrid materials of SWCNTs and BioMs with some chirality, with observed bandgap fluorescence and robust resonance Raman scattering, are appropriate for obtaining excellent materials for biosensing applications, as the fluorescence bandgap of SWCNTs is greatly sensitive to the ecosystem and depicts a shift when the NTs are in interaction with other particles (Martins et al. 2013).

SWCNTs are generally employed as NNBS to improve electrical possessions. They also have decent mechanical and electronic features, and their large SA enhances the extent of the immobilized (restrained) enzymes, amplifying the rxn areas (parts) amongst the enzyme and the substrate, thereby enabling the conductivity (that is the electrical transmission) and enhancing the BS signal response (Cho et al. 2020). While the MWCNTs comprise multiple layers of concentric SWGr cylinders (tubes) structure reinforced through van der Waals forces with an interlayer spacing (layout) of 3.40 Å. They also have a sidewall composition that is comparable to the graphite (Gt) basal plane, and as a result, the speed of particle (electron) transmission could be similar to the Gt edge-plane electrode. But their outstanding conduction and electrocatalytic features are used as a customized platform (scaffold) on the electrode; see Table 13.2 as modified from (Cho et al. 2020).

Table 13.2 Outline of typical CNTs employed in electrodes and label of the electrochemical BS

Materials	Benefit	Drawbacks	Feature
SWCNTs	Huge ratio of SA to volume, a minimal charge carried density, delocalized π-orbitals and improvement in electrical conductivity	Challenging chemical functionalization and manipulation during the sensor production process, non-specific sorption of protein and reduced surface to interface with large biological components	Electrode
MWCNTs	Extraordinary conducting and electrocatalytic features	To increase BioC and irreversible aggregates in water-soluble solution, there is a need for surface functionalization	Electrode

Proper performance of the MWCNTs surface is important as the substantial structural dilapidation of MWCNTs happens all through the functionalization process ras well as decapping at the cylinders (tubes) ends and MWCNTs length dividing and breaking (Cho et al. 2020).

13.4.1 Optical Biosensors

An optical BS (OBS) is a miniature device comprising a biorecognition detection (sensing) component incorporated with an optical TSD arrangement, and the fundamental aim of the OB is to create a signal which changes in direct proportionality with the measured substance concentration (analyte) (Naresh and Lee 2021).

OBS gives excellent advances over traditional analytical approaches owing to the direct, real-time and label-free recognition of various biological and chemical substances. Their benefits consist of huge specificity, sensitivity, miniature size and cost-efficiency. In novel optical BS application, various innovative theories and exceedingly multi-disciplinary methods such as microelectronic, microelectromechanical systems (MEMSs), micro/NN-technologies, biotechnology and molecular biology are used. Exponential growth in research and technological growth of OBS has been witnessed over the last era, and this has been directed mostly to environmental applications, health care and biotechnology engineering. There are numerous prospective applications of BS in these various fields and exclusively measured are their requirements in terms of analyte concentration, their required accuracy of output, the concentration of sample required, time taken for probe completion, the required allowed time for BS reuse and the system cleaning requirements. BSs can be categorized into various classes but depend on the technique of signal transduction like optical, piezoelectric, thermometric, electrochemical and magnetic (Damborský et al. 2016).

Optical detection is accomplished by utilizing the interface of the optical region with a biorecognition component (BRC), and they are generally separated into a label-free model (LFM) and label-based model (LBM). In the LFMs, the detected (sensed) signal created is through the analysed material interface with the TSD, while in the LBM consists of the utilization of a label and the creation of an optical signal by a colorimetric, fluorescent or luminescent technique. OBS uses several biological materials such as enzymes, antibodies, antigens, receptors, nucleic acids, whole cells and tissues as BRC. The evanescent field close to the surface of the BS is used by the surface plasmon resonance (SPR), evanescent wave fluorescence and optical waveguide interferometry to identify the analyte interaction with the BRC (Martins et al. 2013; Damborský et al. 2016).

Based on various optical principles, OBs are designed, which include SPR, evanescent wave (EW) fluorescence, optical waveguide interferometry, chemiluminescence, fluorescence, refractive index and surfaced-enhanced Raman scattering. The fluorescence-based (FB), chemiluminescence-based (CB), SPR-based (SPRB) and optical fibre-based (OFB) BSs are the utmost popular OBS utilized. In the FBOB, fluorescence is the optical phenomenon, which comprises labelling for analyte or molecules detection. They are generally used for medical diagnosis, environment and food quality monitoring studies due to their elevated sensitivity, selectivity and rapid response time. Various types of fluorescent dyes used in this BS include QDs, dyes and fluorescent protein. The three methods for FB BSs are fluorescent extinguishing (turn-off), fluorescent enhancement (turn-on) and fluorescent resonance energy transfer (FRET). Lately, FRET-based OBS has been used successfully in studies owing to the intercellular process being highly sensitive, and this involves the non-radiative transmission of energy from an excited donor particle (D) to the acceptor particle (A) at the ground state through distant multi-pole interactions. They are generally used in biomedical domains like tumour therapy and aptamers assessment as they can identify alteration in angstroms to NNmeters. While in the CBOB, the phenomenon involves the chemiluminescence in which the released light energy is due to the chemical rxn. Extensive interest has been received by CB BSs lately due to their simplicity, minimal detection (sensing) limit, broad calibration limit and inexpensive instrumentation. Change in the refractive index instigated by the molecular interface at the surface of the metal via surface plasmon waves is detected using the SPRB BSs. The reduction in the intensity has a direct proportionality with the surface mass. They fall into the class of LFM biosensing technology and function on the SPR model. They are employed in the diagnosis of diseases as well as environmental and the assessment of food quality. In the OFBOB, an optical fibre developed a sensor mechanism that uses an optical field to measure biological components like proteins, aptamers and whole cells. The reliable optical fibre method is an evanescent field sensing, detected in the tapered optical fibres case (Naresh and Lee 2021). CNTs are generally employed in electrochemical BSs, and field-effect transistor (FET)-based BSs (Rao et al. 2011) are discussed in the subsequent section.

13.4.2 Field-Effect Transistor (FET) Biosensors

An increased interest has been received by FET sensor over the last two eras, which is inspired by the requirement for inexpensive BSs that are capable of the precise and quick recognition of the analyte particles deprived of the requirement for cost-effective and time-consuming labelling stages (Lowe et al. 2017).

In the label-free type of sensing model, the field-effect transistor (FET)-based BSs are suitable ingredients for an array of TSD. Owing to their important benefits like scalability, ultra-sensitivity, quick real-time recognition, intrinsic amplification, the requirement of minimal power, mass fabrication at economical rates and direct electrical readout in contrast to SPR, microcantilever sensors, fluorescent devices and other techniques, they have received much attention in current years. However, the accessibility of mature production methods like the complementary metal–oxide–semiconductor (CMOS) procedure help offer the benefit of scale down, parallel sensing (detection) and permitting the incorporating with other system and circuits. This is vital for the real development of biosensing tools and generally preferred in the situation that the target BioMs contain electrostatic charges or bioactivities which modify the device electrostatic potential (Wadhera et al. 2019).

A characteristic FET device contains a drain and a source, with current passing from the source to the drain. Also, the FET holds a gate that is utilized for controlling the current (electric) passing between the source and the drain. An electrical region and the current (electric) flow are generated and controlled by the gate material (Rao et al. 2011).

ISFET is electrochemical impedance spectroscopy (EIS) that is similar to the MOSFET, which was first invented in the early 1970s to identify solution pH values. Biosensing applies to the state of the art of ISFET with corresponding bio-probes attached on the ISFET surface. Innovative ISFET machineries are established on the CMOS design, and the consistent design platform has resulted in considerable development in ISFET study since the 2000s (Syu et al. 2018; Wadhera et al. 2019).

They have performed as the best-established alternatives between numerous forms of BS owing to the various benefits they offer. The various types of FET-based sensing platform employed for biological domains are the ion-sensitive field-effect transistors (ISFETs) and metal oxide (MO) semiconductor field-effect transistors (MOSFETs) which are grounded on the applied gate voltage method, design and gate substance and the channel region. The ISFETs are the most widespread forms of integrated tools for micro electrochemical LOC compositions and their utilization as a TSD indicates a favourable device for biological purposes. Their arrangement is comparable to the conventional MOSFETs, but the only distinction is in their sensitive region or the transistor gate that transduces ion concentration to a quantifiable voltage. The metal gate and gate oxide of a MOSFET is represented by a reference electrode immersed in a sample solution and the insulating layer that are sensitive to ions. ISFET sensor sensitivity and capability are very reliable on the insulating layer nature. The FET channel is secluded from the liquid by the insulating layer, and the surface layer charge is coupled into the channel electrostatically, with the dielectric layer made of various constituents like Si_2N_4, Al_2O_3 or Ta_2O_5 on SiO_2, act selectively for H^+ ions due to the oxide surface-active sets. Ionophores are employed to improve the device specificity and permit other analytes detection, sensing of ion or ion-blocking membranes. To make them more penetrating to BioMs, a biorecognition layer is attached to their surface and the typical ISFET is known as a biological sensitive FET (BioFET). The coating provides for the selective interaction with the analyte have a reduced non-specific binding and create a charge transfer, bonds to the TSD surface by either electrostatic or chemical contacts (Lowe et al. 2017).

The ISFET also uses a MO semiconductor (MOS) with a natural variation in the metal gate electrode replaced by a series grouping of a dielectric layer, electrolyte and reference electrode. It comprises a silicon substrate (p-type) with dual n-doped areas, making up the source and the drain and which are divided by a small channel protected by the gate dielectric layer working as a sensing (detection) membrane (Wadhera et al. 2019).

MOSFET is a metal–insulator–semiconductor composition with a metal gate electrode positioned on the upper part of a protecting coating of oxide (Park et al. 2014). The MOSFETs are the very common type of insulated gate FET (IGFET) exploited in several forms of switching in electronic circuits and electronic signal amplification. They are the foundation of integrated circuits (IC), and because of their small sizes, they can effectively be employed in a single chip. The key features of the MOSFETs are their MO gate electrode and SiO_2 is usually used as an ultra-thin layer of protecting materials, to isolate the directing gate from the core channel situated in the centre of the drain and source and hence making the MOSFET feedback resistance remarkably high. They can easily be damaged owing to the huge amount of static charge build-up stemming from huge feedback resistance (Sadighbayan et al. 2020).

The MOSFET operation is based on the use of an electric field controlled by the shape and size of the source–drain outlet, which is known as the outlet length control and outlet outline control. In rxn to a target analyte, the carrier flow is controlled by the gate electrode control (electrons and holes) via an outlet created between the source and the drain, thus resulting in an alteration in the drain electric (current) (Park et al. 2014).

13.4.3 Electrochemical Biosensors

An electrochemical BS is a diagnostic tool that converts biochemical actions like enzyme–substrate rxn and antigen–antibody interface to electrical signals like current, voltage and impedance (Kerry et al. 2021). The first form of an electrochemical BS was developed for blood glucose by Clark, with several forms of this BS been successively created and marketed for different applications. In this BS, the main part is the electrode, which is used as reliable backing for BioMs immobilization (enzyme, nucleic acid and antibody) and movement of electron. Depending on the chemical sets on the electrode presence or the supporting materials nonappearance, several chemical variation techniques are utilized for this reason through amine and carboxyl (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide: EDC), aldehyde (hydrazide) and thiol (maleimide). The inapt immobilization may lead to less specificity, loss of activity and minimal BioC. Hence, it is important that the positioning and the biological activity of the BioMs upon immobilization should be maintained. Also, a significant procedure for the superior routine of BSs utilizing proper functional constituent for the electrode (Cho et al. 2020).

Electrochemical BSs are divided into amperometric, potentiometric, impedimetric, voltammetric and conductive (conductometric) sensors and are based on the transduction principle (Naresh and Lee 2021; Yang et al. 2015). Electrochemical BSs are inexpensive and need uncomplicated instrumentation. In amperometric BSs, it consists of a triple-electrode system which includes the sensing (detection), reference (position) and auxiliary electrodes, which are submerged in an appropriate electrolyte. A continuous potential is applied on the sensing (detection) electrode, and the ensuing current (electric) related to the analyte concentration is measured (Ukhurebor 2020; Ukhurebor and Adetunji 2021). Also, most electrochemical BSs use voltammetric methods like differential pulse or square wave, and cyclic voltammetry. In potentiometric researches involves the development of potential amongst dual electrodes measured at a large impedance voltmeter. The sensing electrodes hold the biological element and while the other electrodes serve as the reference (position) electrode. The triple-electrode system is employed in the impedimetric sensor, and the plot of impendence is made in redox compound such as ferrocyanide presence. CNTs are mostly employed in amperometric and impedimetric sensors and voltammetry-based BSs (Rao et al. 2011).

They show elevated sensitivity, elevated selectivity and elevated capability of sensing (detection). The electrochemical rxn happens on the TSD surface amongst the BioRs and the analyte creating measurable electrochemical indicators as regard the current, voltage, impedance and capacitance. In the potentiometric BSs, the charge amassed owing to the interaction between the analyte and BioRs at the working electrode comparative to the reference (position) electrode under no electric (current). Ion-selective electrode and ion-sensitive field-effect transistors are employed when converting a biochemical rxn into a possible signals. While in the amperometric BSs measures the current created owing to the electrochemical oxidation or reduction (redox rxn) of the electroactive classes at the working electrode when the working electrode is supplied with a constant potential with regard to the reference (position) electrode. The electric (current) created on the working electrode surface is comparative to the analyte present in the solution concentration. This technique allows for sensitivity, quick, accurate and rectilinear response which makes it further apposite for mass fabrication when compared to the potentiometric BSs. But, the interference from other electroactive substance and poor selectivity are these sensor drawbacks. The modification in the conductance amongst the electrodes pair is quantified by the conductometric BSs owing to the electrochemical rxn (alteration in the analyte conductivity properties). While in the impedimetric BSs, the electrical impedance created is measured at the interface of the electrode/electrolyte with the application of a small sinusoidal excitation signal. This consists of the low-slung amplitude AC voltage application at the electrode device, and the quantified in/out phase electric (current) response as a function of the frequency is achieved applying the impedance analyser. Ultimately, the voltammetric BSs identify analyte by electric (current) measurement all through the regulated distinction of the employed potential. These BSs have elevated sensitivity measurements and instantaneous multiple analyte sensing (detection) (Naresh and Lee 2021).

CNT based-stripping voltammetry as a detection technique in the electrochemical field has been applied for the detection of HMI (Wang and Yue 2017). In the study done by (Yue et al. 2012), anodic stripping voltammetry (ASV) was used to detect Pb(II), Cd(II) and ZN(II) by using catalyst-free CNT electrodes synthesized through carbothermal carbide conversion (CTCC) process which ensures metal-free residual transmission in the structure of CNT. The material was able to show good detection limits of 13, 32 and 50 nM corresponding to Pb(II), Cd(II) and ZN(II). Also, CNT threads-based electrodes were used for the sensing (detection) of Cu(II), Pb(II), Cd(II) and Zn(II) through ASV. It is imperative to note that the CNT thread electrodes provide sharp stripping, very reproducible and well-defined signals during HMI detection. The detection limits were established to be 0.27 nM, 1.5 nM, 1.9 nM and 1.4 nM meant for Cu(II), Pb(II), Cd(II) and Zn(II), respectively (Zhao et al. 2014).

13.5 Applications of CNT-Based Sensors in Gas Sensing

One of the major problems confronting the natural environment is the release of HM and basic toxic gasses into the environment (Ukhurebor et al. 2021a). These substances (HM and toxic gasses) have caused serious adverse effects to man, other living organisms and the entire ecosystem. Therefore, there is a need to continually explore contemporary means in mitigating the adverse effects. Hence, this section will consist of some of the work on CNTs utilized for sensing applications basically for environmental monitoring vis-à-vis CNT-based sensors in gas sensing. Table 13.1 comprises a summary of some basic toxic gases and their effects on the environment as reported by (Wakefield 2010; Lambrini et al. 2018).

13.5.1 CNT-Based Gas Sensors

As a result of the interface with particles, a transfer of charge ensues between CNTs and particles that can considerably modify the electrical conductivity (EC) of CNTs. Gas sensors (GS) operating with CNTs being in interaction with dual metallic electrodes depend exactly on this condition. As reported by (Kong et al. 2000), where they established that a sole semiconducting (SCT) SWCNT reduces or rises its conductance when uncovered to NH_3 or NO_2 gas correspondingly. This designed

GS displays a very quick response of down to below 60 s, and this could be ascribed to the exceptional SA of the CNT. Nevertheless, the utmost outstanding possession is perhaps the fact that it functions at room *t* temp. MO has frequently been utilized for sensing of NH_3 or NO_2, but they need to function at temp greater than 200.0 °C to attain adequate sensitivity (Shimizu and Egashira 1999).

The sensor designed by (Kong et al. 2000) as an alternative works at room temp with a sensitivity of about a thousand, despite the fact it can recuperate in an hour upon strengthening at 200.0 °C or in half a day (12 h) if left at normal temp underflow of unadulterated argon (Ar). The enhanced SA, which gives CNT-based GS a quick response, is perhaps similar to the reason for their sluggish recovery. Additionally, to strengthening the sensor at advanced temp, another approach to advance its recovery duration as reported by (Li et al. 2003) is to illuminate the CNTs with infrared radiation.

It has been reported that some SCT CNTs turn out to be metallic during exposure to oxygen (Camilli and Passacantando 2018); however, not all the CNTs are sensitive to oxygen, and gasses such as nitrogen helium (He) or Ar have no obvious doping effects on them (Collins et al. 2000).

In term of some inert gases, such as hydrogen, it has been found that palladium-modified CNTs can sense (detect) it down to about 100.0 ppm concentrations (Ding et al. 2007). Generally, sensors built on metal-adorned CNTs have more sensitivity than those built on pristine CNTs for a larger number of vapours and gases (Penza et al. 2010; Kauffman et al. 2010; Leghrib et al. 2010; Abdelhalim et al. 2015).

The combination of CNTs with other gas constituents in a multifaceted mechanism GS that could possibly be valuable not only for the improvement of the intrinsic sensitivity of the CNTs but also for the improvement of the other characteristics. One of the utmost issues with CNT-based GS is the low-slung selectivity to precise vapours or gases. In a situation where a CNT GS is exposed to both water and NO_2, the signal proceeding for the sensing (detection) of the former can mask the sensing (detection) of the latter; this is as a result of the fact that although the adsorption of NO_2 onto the walls of the CNTs inclines to rise the EC, water tempts a reverse response (implying a decrease in EC). To alleviate this constraint (Evans et al. 2016) in their study, joint CNTs with zeolites. Although plain CNTs are incapable of detecting NO_2 in humid settings, hence, CNTs joined with exceedingly hydrophilic zeolites effectively sense (detect) it; the aim is that the zeolites trap water particles before these could essentially get to the CNTs, which consequently only detect (sense) NO_2.

However, studies carried out in the past few years have revealed that GS that combine MO with CNTs can certainly work better even at room temp (Wei et al. 2004; Espinosa et al. 2007; Nguyet et al. 2018).

13.5.2 Photosensors for Gas Sensing

The idea behind a modest single-CNT photosensor (PTS) lies in the photon hitting a SCT CNT, if its energy is greater than the bandgap of the CNT, at that moment an electron duo is produced within the NNstructure. Hence, an incorporated potential can then discrete the dual charge carriers to measure the photocurrent (Camilli and Passacantando 2018). To produce the incorporated potential, it is essential for a junction (either Schottky type or p–n type) to be formed; in the first instance, a p–n junction can be formed within a NNtube in an electrostatic setting (Lee et al. 2004), or by doping chemically (Zhou et al. 2000).

However, it has been reported that both methods are somehow technically stimulating and have allegedly caused some limitations. In reducing some of these limitations (Liu et al. 2016) invented (ultraviolet) PTS where only highly disinfected SCT CNTs were reportedly utilized. CNTs are auspicious for the sensing (detection) of ultraviolet since their absorption coefficient is an order of scale greater than that of the traditional ultraviolet huge constituents (Itkis et al. 2006). In the devices designed by (Liu et al. 2016), photovoltage was employed as a signal in place of the photocurrent, as frequently described. This permits significant improvement of the ratio of the signal-to-noise due to the following; report noise and 1/*f* noise are repressed, and the signal could be increased via the introduction of virtual interactions. With the mechanism that works at room temp and non-bias setting, they have shown the detectivity of more than 1.0×10^{11} Jones, broadband response range of 7.85×10^2–2.100×10^3 nm, and excellent temp and sequential stability; these together with the utilization of a walkable solution-grounded production technique make CNT-based ultraviolet PTS very competitive as compared to the traditional (conventional) huge ultraviolet detectors, such as Si, HgCdTe, Ge and InGaAs (Rogalski 2003; Xu et al. 1998; Jiang et al. 2004).

Another popular conformation for CNT-based PTS is found when a CNT film is positioned on a doped Si substrate to produce a hetero-junction. The benefit of this hybrid PTS as compared to that of a Si PTS is that the CNTs outspread the range of the device (sensing) detection into both the near-IR and near-ultraviolet areas of the electromagnetic (EMT) range (Aramo et al. 2017; Afrin et al. 2012).

However, several researchers have revealed that the photo-response in CNT-built photo-mechanisms could be enhanced with functionalization of CNTs with other NNstructures; particularly, as they are adorned with either SCT (Li et al. 2010; Kongkanand et al. 2007) or metallic NMs (Scarselli et al. 2012; Zhou et al. 2013).

13.5.3 FET Sensors for Gas Sensing

Plain CNTs hardly display captivating affinity towards biological particles, so before utilizing CNTs for biosensing purposes (BSs), so it is necessary to be functionalized such CNTs with BioRs, such as microorganisms, proteins and polynucleotides (Yang et al. 2015); these BioRs have a noticeable affinity with biotic particles, so they function as the sensitive parameters, while the CNTs function as the TSD, by amassing and translating the signals, as a result of the interface between the sensitive parameters and the biological particles to be sensed, in a further measurable physical signal. Depending on the possessions (such as the acoustic signal, electric, heat and optical absorbance) of the physical signal as well as the technique of measurement, diverse CNT-based BSs have been reportedly designed (Yang et al. 2015). However, this subsection emphasis will be on CNT-based BSs that are functioning with a FET arrangement (CNT-FET).

Supposedly, the operational principle of CNT-FETs is comparable to that of the CNT GS; particularly, when the target biotic particle interrelates with the functionalized CNT; consequently, the electrical conductance of the CNT vicissitudes and the target particle is sensed (detected). However, due to the functionalization, CNT-based BSs could be exceedingly precise towards a precise target particle, and this is generally different from that of CNT-based GS (Patolsky et al. 2004).

It has been shown that only CNTs functionalized with antibody receptors that are precise to the target organic (biotic) particle (virus) can effectively interact and subsequently detect such target particle (Patolsky et al. 2004). It follows that the BioRs employed to functionalize the CNTs would as well dictate the magnitude of the sensed (detected) biotic samples, ranging from minor particles such as proteins, sugars or DNA remains to more multifaceted systems such as bacteria and virus as well as cells and minor remains of tissues (Camilli and Passacantando 2018).

Nevertheless, these theoretically simple sensors could still grieve from unspecific binding, particularly in the case of the detection of protein bonding. However, as reported already about GS, also the specificity of CNT-BS can be enhanced if the CNTs are joined with another substance to form a complex arrangement. Supposedly, CNTs are not the sensing (detecting) parameter, but due to their high characteristic ratio, high electric conduction and elasticity of the mechanical system, they aid in the understanding of ultra-thin sensing mechanisms with reasonable specific SA and quick response time. To elude the problem with unspecific binding (Star et al. 2003), employed coated CNT-based GS using a combination of hydrophilic PLM; the PLM attached to biotin particles that in turn bond explicitly to the target streptavidin particles. The PLM coating was also employed to connect the molecular receptor (biotin) to the sidewalls of the CNTs shorn of functionalization, which would cause hindrance of damaging the physical possessions of the CNTs. Similarly, Villamizar et al. (2008) adopted the same stratagem to intensify the specificity by coating the CNT-FETs with Tween 20. They performed the sensing studies with three diverse and hypothetically competing microorganisms, viz *Shigella sonnei*, *Streptococcus pyogenes* and *Salmonella infantis*. The results revealed that neither *Shigella* nor *Streptococcus* inhibited the detection process of the target microorganisms. Hence, CNT-based BSs with this conformation have similarly been utilized in monitoring rxn of biological samples (Camilli and Passacantando 2018).

The foremost reported restrictions of CNT-FETs are possibly the occurrence of background noise (BGN) originating from the electrostatic interactions (Camilli and Passacantando 2018). In CNT-FETs, the detected signals measured in the range of millivolt-scale, but this BGN ensues fluctuation of the electrostatic interactions in the same range scale, eventually restraining the routine of the sensing mechanism. Not until recently the source of such noise was not that know, until when (Sharf et al. 2012.) executed an orderly experimental and theoretical study based on the charge noise model (CNM). They observed that the noise is produced primarily by the contacts of the substrate and the surface adsorbates, and if they are effectively detached the power spectral density of circumstantial voltage oscillations could be possibly condensed by 19-fold.

13.5.4 Pressure Sensors for Gas Sensing

In the past few years, there has been a rising interest in the elastic and stretchy electronics domain amongst researchers. Remarkably, wearable and skin-mountable straining sensor mechanisms are predominantly captivating as a result of their potential, and they are modified for health monitoring, the detection of human motion, environmental monitoring, soft automation as well as prosthetic solutions (Camilli and Passacantando 2018). Due to their outstanding mechanical possessions (Salvetat et al. 1999), enhanced mobility and current density (CD) (Ebbesen et al. 1996), CNTs have been considered as one of the utmost superlative building slabs for such mechanisms. The foremost advantages of utilizing CNTs for these applications instead of contending constituents (e.g. semiconductor NNwires and biological PLM) are enhanced mobility and CD, environmental steadiness and better mechanical possessions.

Instead of utilizing plane films of CNTs for manufacturing pressure sensors (PS), an alternate design entails the use of a huge, 3-D assembly of CNTs. An illustration of this composition is characterized by the supposed CNT scrubbers, which are formed from lengthy and intertwined CNTs creating a haphazard skeleton with open holes (Gui et al. 2010). Consequently, during the application of pressure to the CNT scrubber, the inter-tube holes are hugged, this makes the CNT scrubber denser and ultimately allows additional CNTs to touch each other. As soon as the compressive force is free, the scrubber elastically recuperates its normal outline and subsequently the original amount of tube interactions, so that the EC recuperates its initial rate as well.

13.6 Conclusion

This chapter showcased the significant research work and studies done on CNT-based sensors in HMI and toxic gas detection as one of the contemporary techniques for environmental monitoring. It initially provided insights into CNTs, their functionalization and then their use as CNT-based sensors to detect HMI and toxic gases. Based on the articles reviewed here, it is quite evident that CNTs NMs are emerging as the effective, efficient, reliable and cost-effective technology in the environmental monitoring field. They are very stable, possessing both high SA and high adsorption capacity, excellent catalytic efficiency, superior selectivity and sensitivity when compared to conventional sensing and detection techniques. Equally, based on their superior electrical and mechanical attributes, the use of CNT-based sensors in environmental monitoring application will drastically change tech-based industries in regard to the cost of fabrication, energy consumed and size of sensors.

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Chapter 14
Molecularly Imprinted Polymers-Based Nanobiosensors for Environmental Monitoring and Analysis

Ayushi Singhal, Pushpesh Ranjan, Mohd Abubakar Sadique, Neeraj Kumar, Shalu Yadav, Arpana Parihar, and Raju Khan

Abstract The increasing concentration of environmental pollutants is an alarming issue all over the world. These pollutants not only affecting environmental conditions but also show harmful effects on human as well as animal health and therefore monitoring and analysis of the environmental pollutants are essential, for maintaining the quality of the environment and living beings. Fast, errorless, and inexpensive identification and quantification can functionally control the risks raised from environmental contaminants. Pharmaceuticals, pesticides, and heavy metals are some of the major pollutants found in the environment. There are many existing techniques based on chromatographic and spectrometric detection, however, molecular imprinting technology surpasses them and has emerged as an important sensing technique and is nowadays used widely for sensing applications. Molecular imprinted polymers (MIPs) combined with other detection techniques are used for selective detection of various pollutants in different sources. The MIPs possess numerous advantages over the classical natural biorecognition element/bioreceptor-based techniques as they are more physically and chemically stable, cheaper, highly selective, and sensitive. MIPs are often modified with nanomaterial for enhancing their performance, various nanomaterial modified MIPs have been reported for sensing application. The presented chapter will provide an insight into molecular imprinted polymers-based nanosensors and their application for the detection of various environmental pollutants.

Keywords Pollutant · Pharmaceuticals · Pesticides · Heavy metals · Molecular imprinting technology · Nanomaterial

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14.1 Introduction

The contamination of environmental pollutants on public health has plucked worldwide attention and research. It is believed that many health issues or diseases can be prevented by maintaining healthy environments (Prüss-Üstün et al. 2016). Environmental risks to health were defined, in the study by the WHO, as "all the physical, chemical and biological factors external to a person, and all related behaviors, but excluding those natural environments that cannot reasonably be modified." The modifiable factors include pollution of air, noise, occupational risks, build environments, agricultural practices, man-made climate, electromagnetic fields, etc. Contaminants are chemical elements or compounds or groups of materials that are poisonous, persevering, and can bioaccumulate in the biotic ecosystem and are not generally present in the environment and if present they are found in higher concentrations than natural background (Tornero and d'Alcalà 2014; Chapman 2007).

The environmental contaminants include pharmaceutical products, pesticides, heavy metals, and various components used in industrial synthesis. To reduce the disease threat to human health, the monitoring and analysis of environmental contaminants are crucial. Various techniques have been used to identify and quantify various environmental pollutants, some of the techniques used are tandem and hyphenated chromatographic and spectroscopic techniques, biological techniques, and immunoassays. The chromatographic and spectrometric techniques are very costly, required well-sophisticated instrumentation, and well-skilled manpower, that limit the use of these techniques. However, the immunoassays which include enzyme-linked immunoassays, radioimmunoassay, fluorescent immunoassays, etc., are far better than the chromatographic and spectroscopic techniques as they are cost-effective, fast, and reliable. The immunoassays have been precisely used for various environmental contaminant identification and quantification. The various immunoassay techniques required biological antibodies for the sensing application, which affect the sensitivity and stability. Although antibodies reflect high selectivity, the sensitivity is always a major concern. In addition to the high cost of antibodies, low stability, and their storage at specific conditions, antibody-based techniques are costly and time-consuming which limits their use in sensing applications. To overcome the limitations of the above-mentioned techniques, a novel material with ultrasensitivity, high stability, less expensive, thermally, and chemically stable which can mimic the properties of biological antibodies is required.

Molecularly imprinted polymers (MIPs) are proved to be a stepping stone in the fabrication of nanosensors for various food and environmental monitoring and analysis applications. In comparison to biological antibodies, MIPs are economical, more stable, withstand high temperatures, and can also sustain basic and acidic mediums of solvents. Moreover, they have a low degree of degradation, and can also prepare in large batches unlike natural antibodies, which cannot be prepared in large batches. The MIPs work on the principle of lock and key mechanism; they bind specifically to the target molecules similarly to bioreceptors such as aptamers and antibodies. A detailed stance on molecularly imprinting technology, fabrications of sensor, design, and the basic principles for the detection of various contaminants are presented in this chapter. Moreover, the types, concept, and relevance of biosensors and nanobiosensors along with their working principle has been discussed in detail. Furthermore, their applicability in the detection of several environmental contaminants like pesticides, pharmaceuticals, heavy metals, etc., areas reviewed extensively.

14.2 Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are artificial materials with imprinted recognition sites, able to bind specifically and selectively to an analyte. These MIPs are obtained by the polymerization of the various monomeric unit in the presence of crosslinkers. The monomers are selected based on the capability to interact with functional groups present on the surface of the template (Turiel and Esteban 2020). There are many advantages of using MIPs, as they are highly selective, stable against extreme conditions by pH, heat and mechanical stress, economically beneficial, long storage life, are functional several times, and are chemically inert in acidic, basic, and other organic solvents (Singhal et al. 2022a; b). However, imprinting of long-chain peptides, proteins, etc., molecules of high molecular weight is still a major concern. The diverse diagnostic applications of MIPs are represented in Fig. 14.1. A basic comparison between natural molecules and MIPs is explained in Table 14.1 (Whitcombe et al. 2011; Tarannum et al. 2020a, b).

MIPs can be used in different fields, such as environmental, clinical, and food. MIPs with different analytical tools and techniques such as gas and liquid chromatography, mass-spectrometry, and tandem mass techniques can be used for the detection of various analytes such as drugs and their metabolites, chemical components, and pollutants. MIP-SPE (Screen Printed Electrode) is vastly utilized for the detection of analytes in different matrices. It is also used for the pretreatment (clean up and preconcentrate) of samples, which can be used directly as a column in HPLC or can be used with SPE, in both cases, it reduces the time of pretreatment and the degree of contamination. The MIPs act as selective sorbent and are used for cleaning, extraction, and detection of the target from different matrices. Nowadays, the most highlighted usage of the MIPs in biosensing applications and many researchers are working in this field to produce sensors with the help of MIPs so that they can detect various analytes selectively with high sensitivity. Catalysis is another important application of MIPs, due to high selectivity and stereospecificity MIPs proved to be an important tool (Tarannum et al. 2020a, b). The biosensing application of MIPs will be discussed in more detail under heading 4. Owing to their properties such as biocompatibility, low toxicity, and biodegradability, MIPs are effectively used in drug delivery systems and treatments of several diseases such as cardiovascular, cerebrovascular diseases, and cancer. It can be administered through different routes such as intravenous, transdermal, ocular, as well as oral (He et al. 2020).

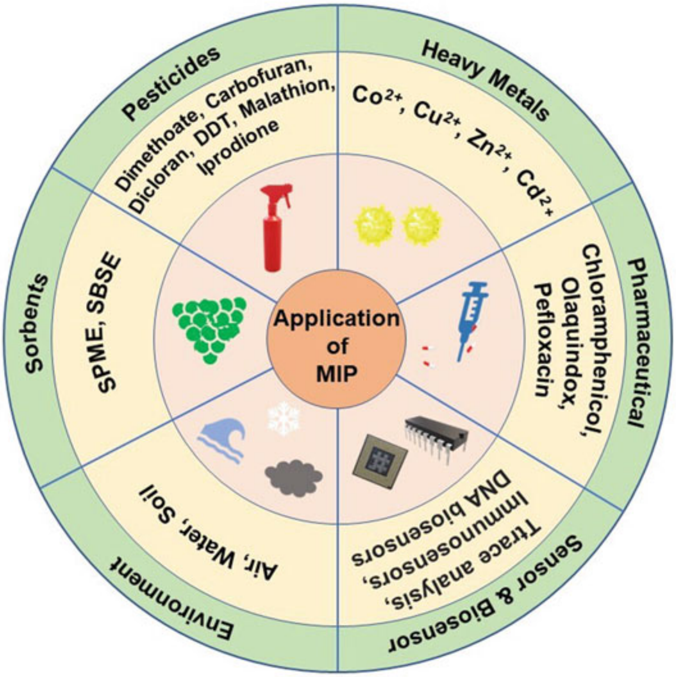

Fig. 14.1 Versatile diagnostic applications of MIPs

Table 14.1 Comparison between MIPs and antibodies

MIPs (artificial)	Antibodies (natural)
Chemically, physically, thermally stable	Relatively unstable
Comparatively inexpensive	Expensive
Can be reused up to 100 times	Non-reusable
High sensitivity	Low sensitivity
Enhanced cross-reactivity	Low cross-reactivity
Long storage time	May denature on long-term storage
Rapid preparation (days)	Delayed preparation (months)
Resistance to bio elements	Non-resistance to bio elements
Insoluble copolymers	Soluble
Can perform in organic media	Poor performance in organic media
Can be used for a wide range of analytes	Used only for a limited number of analytes

14.3 Application of MIP-Based Nanobiosensors for Environmental Monitoring and Analysis

14.3.1 Pesticide Detection

Pesticides are agrochemicals that show a hazardous effect on the ecosystem. They affect the biotic as well as the abiotic factors, in various ways such as deteriorates the soil as well as water quality which affects the living system. Excessive and repetitive use of pesticides affects the flora as well as the fauna present in the biosphere. Pesticides can cause harm to public health and are the reason for many chronic diseases like Parkinson's, Sclerosis, Cancer, and Alzheimer's diseases in humans. Long-term exposure to pesticides can affect the functionality of various organs of the human body and they are even included in the category of mutagens, which can cause mutation, DNA degradation, and chromosomal alterations within the body. They are also considered as endocrine disruptive agents as they can cause the endocrine toxicity in males and females and are the reason behind various types of cancer. Pesticides are even identified as immune system suppressors, which affect the normal responses shown by the immune system within the healthy body. In short, we can conclude that pesticides are major harm to lives, and their monitoring and analysis are much needed (Parween and Jan 2019). For the monitoring and analysis of pesticides, many researchers came forward and proposed sensors. The contribution of many MIPs-based nano-biosensors for the detection of pesticides is described in Table 14.2.

Dimethoate, an organophosphorus pesticide when encounters living organisms, can reach vital organs such as the kidney and brain through systemic circulation. Carbofuran is a widely used pesticide that belongs to the carbamate class of pesticides. It can cause diabetes and cancer when entering the human body through various sources in the food chain or water. Çakır and Baysal prepared a MIP-based SPR sensor chip nanofilms by process of photopolymerization using MATrp, ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN) as a monomer, cross-linker, and initiator, respectively, for dimethoate and carbofuran detection. The prepared sensors for dimethoate and carbofuran are reusable and can use up to six times. Moreover, they showed high sensitivity, high accuracy, precision, and short response time. The LOD was found to be 8.37 ng L^{-1} and 7.11 ng L^{-1} with a linear range of concentration ranging from 0.04–4.36 nM and 0.05–4.50 nM for dimethoate and carbofuran, respectively, and was successfully used for the pesticide's detection in an environmental water sample (Çakır and Baysal 2019).

In another work, Shahtaheri et al. have discussed a MIP-dependent electrochemical sensor for dicloran (2,6-dichloro-4-nitroaniline), which can cause phototoxicity in human skin and cause severe damage to the liver, kidney, spleen, and hematopoietic system (Xu et al. 2018). The linear range was found to be 1×10^{-6} to 1×10^{-9} mol L^{-1} with LOD 4.8×10^{-10} mol L^{-1}. The fabricated sensor was effectively applied for detecting dicloran in different real water samples and urine samples (Shahtaheri et al. 2017). Lin et al. described selective and sensitive sensors for the

Table 14.2 Applications of MIPs based sensors in pesticides analysis

Template	Sensor configuration	Monomer	Matrix	Technique	LOD	Linear range	Refs.
Dimethoate	MIP-SPR sensor	MATrp	Environmental water	SPR	8.37 ng L^{-1}	0.04–4.36 nM	Çakır and Baysal (2019)
Carbofuran					7.11 ng L^{-1}	0.05–4.50 nM	
Dicloran	MIP-electrochemical sensor	–	Environmental, biological samples	SWV	4.8×10^{-10} mol L^{-1}	1×10^{-6} to 1×10^{-9} mol L^{-1}	Shahtaheri et al. (2017)
Methyl parathion	MIP-QCM sensor	Poly (vinylidene fluoride)	Vegetables	QCM	68 nM	–	Lin et al. (2018)
Carbofuran	PFF-MIP/Au-QCM sensor	MAA	Standards	QCM	0.21 µM	0.5–1000	Sroysee et al. (2019)
Profenofos		PVP & DPDI			0.38 µM	5–1000 µM	
Carbofuran	MIP/Au NPs/GCE-electrochemical sensor	*p*-Aminobenzoic acid	Vegetables	DPV	2.4×10^{-8} mol L^{-1}	5.0×10^{-8} t 0.0×10–4 mol L^{-1}	Qi et al. (2018)
Malathion	Au-SPE/MIP-electrochemical sensor	Acrylamide	Olive fruits and oils	DPV	0.06 pg mL^{-1}	0.1–1000 pg mL^{-1}	Aghoutane et al. (2020)
Iprodione	MIP-QCM Sensor	MAA	Aqueous solution	QCM	17.954 nM^{-1}	–	Yang et al. (2020)
Cyhalothrin	SiO_2/ZnO/MIPs-fluorescence sensor	Acrylamide	River water	Fluorescence	0.13 µmol L^{-1}	1.0–80 µmol L^{-1}	Li et al. (2017)
DDT	PDA@Fe_3O_4-MIP electrochemical impedance sensor	Dopamine hydrochloride	Radish	EIS	6×10^{-12} mol L^{-1}	1×10^{-11}– 1×10^{-3} Mol L^{-1}	Miao et al. (2020)

detection of pesticide simulants of chemical threat agents principally for Methyl parathion. In this study, a polymer was polymerized before rather than generally used post-polymerized polymers, which makes the process swift. The sensor has a detection range ranging from 1.0 to 87.0 μM with a LOD of 68.0 nM. The sensor displayed good selectivity even in presence of other similar competitive analyte molecules. The sensor also showed good sensitivity, repeatability, reproducibility, and stability and was successfully applied for the real sample (vegetables) analysis (Lin et al. 2018). In another study, for the detection of insecticides carbofuran and profenofos, MIPs were designed. Carbofuran-MIP is based on methacrylic acid (MAA) as a monomer, EGDMA as a crosslinker, and AIBN as an initiator. The profenofos-MIP comprised of polyurethane-based on poly (4-vinyl phenol) (PVP), and diphenylmethane-4,4′-diisocyanate as functional monomers, phloroglucinol as the cross-linker, and diphenylmethane as the porogen. The sensors showed a linear detection range of 0.5–1000 μM for carbofuran and 10–1000 μM for profenofos with LOD of 0.21 μM and 0.38 μM for carbofuran and Profenofos, respectively, which was lesser than the other previous methods (Sroysee et al. 2019). For the same above-mentioned pesticide carbofuran, another sensor was constructed using AuNPs, which amplified the signals produced and hence show high sensitivity. Aminobenzoic acid (ABA) was used as a monomer and was deposited on the AuNPs/GCE through the electro-polymerization process. The constructed sensor showed good reproducibility and stability with high selectivity and wide linear response of 5.0×10^{-8} to 4.0×10^{-4} mol L^{-1} with a LOD of 2.4×10^{-8} mol L^{-1} and was successfully applied for the real vegetable sensors (Qi et al. 2018). A detailed procedure of detection of carbofuran with the help of MIPs is shown in Fig. 14.2.

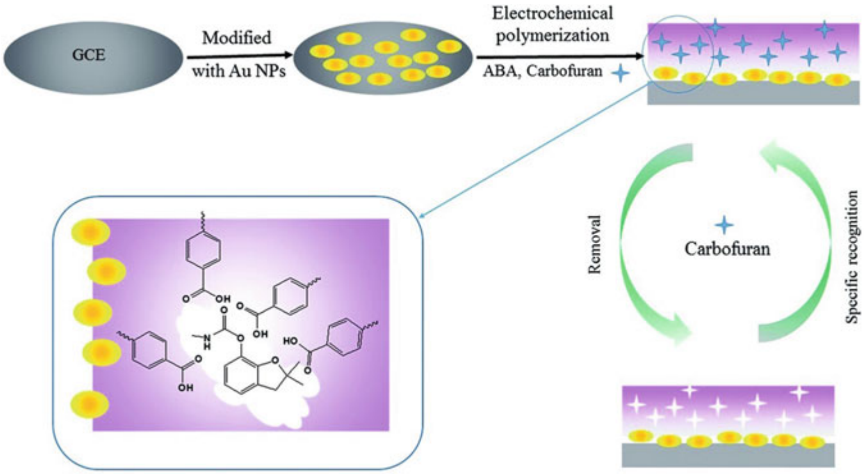

Fig. 14.2 A detailed procedure of detection of carbofuran with the help of MIPs. Reprinted with permission from Qi et al. (2018). Copyright 2018 @ Creative Commons Attribution-Noncommercial 3.0 Unported Licence

For the determination of Malathion, an organophosphorus pesticide in olive oils and fruits. Aghoutane et al. fabricated a MIP-based screen-printed gold electrode. The preparing procedure is found quite simple and completed in few steps. The sensor satisfactory work with good repeatability, selectivity, recovery results, and showed a linear concentration range of 0.1–1000 pg mL^{-1} and a LOD of 0.06 pg mL^{-1} (Aghoutane et al. 2020). A fungicide, iprodione is a harmful pollutant to public health and the atmosphere. Many researchers worked to detect iprodione from the different matrices, in the same run, a molecularly imprinted quartz crystal microbalance (MIP-based QCM) sensor was fabricated. The micro-contact printing technique (μ-CP), via ultraviolet (UV)-assisted photopolymerization was employed to synthesize MIP-based QCM sensor. The patterned MIP-QCM sensors showed better results than planar MIP-QCM sensors owing to their larger surface area. The study concluded that heterogeneous surface MIP films influence the binding affinity and number of binding sites. The sensor showed LOD of 17.954 nM^{-1} with good recovery and rapid detection (Yang et al. 2020) Cyhalothrin is a broad spectrum, widely used pyrethroid pesticide, used against insects and pests, but its long-term use causes it remains in the environment which can pollute the environmental sources and can be detrimental to public health. For the detection of such pyrethroid, a thin shell and sunny shape molecular imprinted fluorescence sensor was constructed. The sensor showed high sensitivity because of the QDs and high selectivity with competing analytes due to the MIPs. The SiO_2 acts as substrate and ZnO QDs act as the fluorescence material. The sensor presented a linear range of 1.0–80 μmol L^{-1} and the LOD was 0.13 μmol L^{-1} and was efficiently used for the analysis of river water samples (Li et al. 2017).

Dichlorodiphenyltrichloroethane (DDT) is a widely used insecticide and due to its toxicity, it is also considered a persistent organic pollutant. The application of DDT has become a threat to the ecosystem, and therefore effective detection of this pesticide is needed. For instance, an electrochemical impedance sensor was constructed for the differentiation and detection of DDT. The sensor was based on molecularly imprinted polymer magnetic nanoparticles. The sensor showed a linear range of concentration ranges from 1×10^{-11} to 1×10^{-3} mol L^{-1} with a LOD of 6×10^{-12} mol L^{-1}. The sensor was able to detect DDT in food with good sensitivity, selectivity, and high reproducibility (Miao et al. 2020).

14.3.2 Pharmaceutical Product Detection

In our daily life, we come across many pharmaceuticals, daily drug consumption, and they are manufactured on a large scale. The effluents from the manufacturing units and other sources generate chemical toxicity in the environment. Chloramphenicol (CAP) is a broad-spectrum antibiotic effective against a wide variety of bacteria and generally used for treating various infectious diseases and it is also used as a growth promoter in food-producing animals. However, CAP has also toxic side effects such as bone marrow suppression, aplastic anemia, gray baby syndrome, and leukemia. It

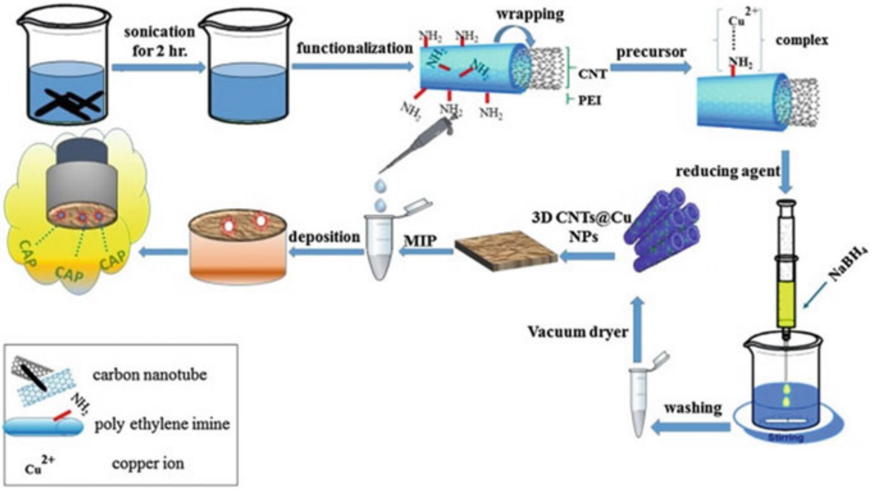

Fig. 14.3 Schematic illustration of strategy to show 3D imprinted nanohybrid for the detection of chloramphenicol. Reprint with permission from Munawar et al. (2018) @ 2017 Elsevier

has been banned in several parts of the world, but still, it is easily available. Munawar et al. deposited copper nanoparticles on carbon nanotubes and then a hybrid structure is formed by coating molecularly imprinted polymer on 3D CNTs@Cu NPs and is used to fabricate a sensor for CAP determination in the milk sample. The developed sensor possesses good sensitivity and selectivity and LOD of 10.0 μM (Munawar et al. 2018). The strategy to fabricate 3D imprinted nanohybrid for the determination of chloramphenicol is illustrated in Fig. 14.3.

Olaquindox (OLA) is a growth promoting and anti-infection agent used to treat dysentery and bacterial entities in animals. OLA possesses carcinogenic, mutagenic, genotoxic, and photoallergic effects and due to this developing an effective method to detect OLA attracted widespread attention of researchers. Wang et al. developed a sensor named MIP/AuNPs/cMWCNTs/GCE for determining the amount of OLA in pork and fish. Under the optimized conditions, this developed sensor showed a high percentage of recovery, high sensitivity, and selectivity with LOD of 2.7 nM and linear range of the sensor ranges between 10.0 and 200.0 nM (Wang et al. 2017). Pefloxacin (PFX) is a widely used fluoroquinolones antibiotic. Due to the misuse of PFX, a high amount of PFX residues is found in animal products and environmental samples, which has plucked the attention of researchers. Shi et al. fabricated a sensor for the detection of PFX in milk samples. The nanocomposite of gold nanoparticles/reduced graphene oxide/single-walled carbon nanotubes were used to fabricate a MIP/AuNPs/RGO/SWCNTs/GCE sensor; the fabricated sensor showed a linear range from 5.0×10^{-7} to 2.0×10^{-5} mol L^{-1}, and the detection limit was 1.6×10^{-8} mol L^{-1}. The sensor exhibited high stability, high reusability, and was a low-cost process (Shi et al. 2020). Clenbuterol was used as a drug to stimulate β-receptors, it is also used to increase muscle mass and reduce body fat as it can enhance protein deposition and lipid degradation; therefore, it is also misused by bodybuilders. The residues of clenbuterol once get deposited in the body, can cause adverse effects like increased heart rate, muscle tremors, or vomiting, etc. Due to the safety of food and public health, the WHO has imposed restrictions on the use of clenbuterol, and therefore its detection is needed (Ma et al. 2020). Liu et al. fabricated an electrochemical sensor employing tetraethyl orthosilicate as functional monomers and (3-aminopropyl) triethoxysilane (APTES) as cross-linking agents. Under the optimized conditions, this developed sensor showed excellent stability, extensive sensitivity, and selectivity with LOD of 31.0 nM and linear range of the sensor ranges between 2.0 μM and 0.1 mM and was effectively applied for the determination of clenbuterol in food samples (Liu et al. 2021).

In another work, a magnetically assisted imprinted sensor was fabricated to determine antibiotics selectively. The developed sensor was based on surface-enhanced Raman scattering, Ag@Fe_3O_4 composites were used as SERS substrate, and dopamine was used as monomer. The prepared sensor displayed good magnetic separation, reproducibility, selectivity, recovery, and was applied to detect enrofloxacin hydrochloride in a water sample. The sensor was found to have impressive sensitivity with a LOD of 0.012 nmol L^{-1} (Li et al. 2020). The contribution of mentioned MIPs-based nanobiosensors for the detection of pharmaceuticals is described in Table 14.3.

14.3.3 Heavy Metals Detection

Heavy metals are elements that possess a higher density than water and are toxic even at a low concentration. Heavy metals occurred in the environment from both natural well as anthropogenic sources. Weathering and volcanic eruption are considered as major natural sources of heavy metal contamination, whereas anthropogenic sources include metal processing, fuel combustion, power stations, various processing units, etc. Heavy metal can disrupt various cellular organelles, even hormones, and enzymes and can cause cancer and kidney damage. They are also recognized as known or probable human carcinogens by US Environmental Protection Agency. Hence analysis and monitoring of heavy metals are crucial to conserve the environment and human health (Madkour 2020; Tchounwou et al. 2012). The contribution of many MIPs-based nano-biosensors for the analysis and monitoring metals of heavy metals is described in Table 14.4.

Contamination due to Cobalt (II) (Co^{2+}) generally occurs from burning fuels, mining activities, and industries effluent, and they are considered a threat even at trace levels. For the sensitive determination of Co^{2+}, Li et al. designed a molecularly imprinted electroluminescence switch sensor. The sensor was based on the interaction of bovine serum albumin with the metal Co^{2+} and the MIPs recognition offering a dual recognition effect. The sensor possessed high detection limits of 3.07×10^{-10} mol L^{-1} and was successfully used in the identification and quantification of ultra-trace Co^{2+} levels in different real samples (Li et al. 2019).

Table 14.3 Lists of MIPs based sensors in pharmaceutical product analysis

Template	Sensor configuration	Monomer	Matrix	Technique	LOD	Linear range	Refs.
Chloramphenicol	3D CNTs@Cu NPs@MIP	MAA	Milk	CV	10.0 µM	–	Munawar et al. (2018)
Olaquindox	MIP/AuNPs/cMWCNTs/GCE	o–PD	Pork and fish	DPV	2.7 nM	10.0–200.0 nM	Wang et al. (2017)
Pefloxacin	MIP/AuNPs/RGO/SWCNTs/GCE	o–PD	Milk	DPV	1.6×10^{-8} mol L^{-1}	5.0×10^{-7}–2.0×10^{-9} mol L^{-1}	Shi et al. (2020)
Clenbuterol	Cl/MIP/Au-electrochemical sensor	APTES	Spiked milk	DPV	0.031 µM	2–100 µM	Liu et al. (2021)
Enrofloxacin hydrochloride	Fe_3O_4@Ag@MIPs-SERS imprinted sensor	Dopamine	River water	SERS	0.012 Nmol L^{-1}	–	Li et al. (2020)

Table 14.4 List of MIPs based sensors in heavy metal analysis

Template	Sensor configuration	Monomer	Matrix	Technique	LOD	Linear range	Refs.
Co^{2+}	MWCNT/Cu/C-dots-ECL switch sensor	o-aminophenol	Spiked water, soil and agricultural product	ECL	3.07×10^{-10} mol L^{-1}	$10–1000 \times 10^{-10}$ mol L^{-1}	Li et al. (2019)
Cu^{2+}	Alga-OMNiIIP-electrochemical sensor	Acryloylated-algae	Lake water and soil	DPASV	0.0018 ng mL^{-1}	0.008–7.807 ng mL^{-1}	Prasad and Fatma (2016)
Cu^{2+}	Alga-MIP modified PGE-electrochemical sensor	NMGA	Lake water, soil	DPASV	0.0038 ng mL^{-1}	0.01–6.24 ng mL^{-1}	Prasad and Singh (2016)
Zn^{2+}	GNS@Ag NPs/Zn^{2+}-IIP NPs /RTIL)-CPE-potentiometric sensor	MAA	River water and industrial wastewater	Potentiometry	1.93×10^{-1} μg L^{-1}	$2.62 \times 10^{-1}–6.54 \times 10^{5}$ μg L^{-1}	Shirzadmehr et al. (2016)
Cd^{2+}	IIP/ERGO/GCE-electrochemical sensor	oPD	Lake and river water	SWASV	0.13 ng mL^{-1}	1–50 ng mL^{-1}	Wang et al. (2020)

Copper and zinc ions are an essential nutrients, their deficiency, and their toxicity both lead to the defects, therefore, ultra-trace levels of these ions need to be identified. For the selective determination of copper ions in lake water and soil. Prasad and Fatma developed an alga-OMNiIIP (One MoNomer Ion Imprinted Polymer) modified pencil graphite electrode. The sensor concentration ranges from 0.008 to 7.807 ng mL^{-1} with a detection limit of 0.0018 ng mL^{-1} (Prasad and Fatma 2016). In another study, an electrochemical imprinted sensor was constructed using algae which substituted the MWCNTs. The main advantage is that the algae are way much cheaper than MWCNTs and it also enhances the electroconductivity of the film (Prasad and Singh 2016). On the other hand, for the determination of zinc ions in environmental and bio samples, Shirzadmehr et al. constructed a potentiometric sensor based on nanographene/ion-imprinted polymer composite. MAA was used as a monomer and ionic liquid 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl) imide as a conductive binder, which improved the sensor performance. The sensor showed a good linear range ranging from 2.62×10^{-1} to 6.54×105 μg L^{-1} with LOD of 1.93×10^{-1} μg L^{-1} (Shirzadmehr et al. 2016). For the determination of Cd^{2+}, a heavy water pollutant, et al. fabricated an electropolymerized ion imprinted poly (o-phenylenediamine) PoPD/electrochemically reduced graphene (ERGO) composite. The sensor has been displayed to have good properties in terms of selectivity, repeatability, and stability (Wang et al. 2020). Detailed procedure diagrams for fabrication of the IIP/ERGO/GCE for the determination of Cd^{2+} are illustrated in Fig. 14.4.

14.4 Conclusion and Future Perspective

Environmental monitoring and analysis to examine the various pollutants such as gases, heavy metals, pharmaceuticals waste, adulteration chemicals, pesticides, and herbicides is the prime requirement of sustainable development to make the world a healthier place. To minimize the adverse effect and management of these pollutants, various sensors were developed. Moreover, MIP-based sensors gain much attention due to their several advantages like ultra-sensitivity, specificity, robustness, prolonged stability, and low detection ability. Since, they are made up of synthetic chemicals, where they work as artificial bioreceptor like natural bioreceptor. In addition, they are easily synthesizable as well as they are chemically and thermally stable which results in long-term stability. Further, the sensitivity and low detection ability can be improved by the utilization of hybrid nanomaterials and synthesis strategy which increase their electrical conductivity. In addition, these sensors are fabricated by the polymerization of monomer, which is available at a low cost, so it makes them cost-effective, as well as the receptor could be designed according to the target candidate which enhances their selectivity. On the other hand, they are reusable and sustainable in a wide pH range, and solvents, as a result, have wide applicability. Overall, the advantages of the MIPs-based sensor make them a suitable candidate for not only analysis of the environmental pollutants but also the detection of biological species as diagnosis of numerous disease biomarkers such as

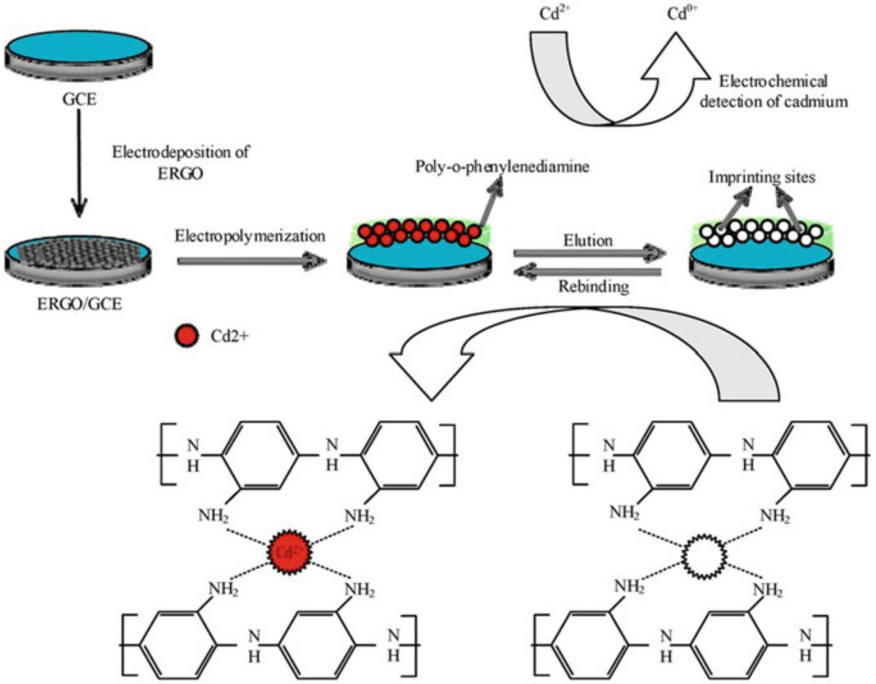

Fig. 14.4 Detail procedure diagrams for fabrication of the IIP/ERGO/GCE for the determination of Cd^{2+}. Reprint with permission from Wang et al. (2020). Copyright 2020 @MDPI Creative Commons Attribution License 4.0

cancer, cardiac, viral, and bacterial. In near future, they could be miniaturized into a portable device and commercially available which extends their future applicability in sensing applications.

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Chapter 15
Plasmonic Nanoparticles for Naked-Eye Detection of Environmental Pollutants

Semra Akgönüllü, Monireh Bakhshpour, Nilay Bereli, and Adil Denizli

Abstract In recent years, the spread of pollutants including toxins, pesticides, or heavy metal ions in soil, water, and air environments has been increasing. Environmental contamination issues are very important for human health. For this reason, it is necessary to fabricate a quick detection and real-time monitoring system for hazardous compounds via high correctness and sensitivity in the environmental sample. Methods that provide the detection of hazardous compounds in real time by using a colorimetric sensor that is also visual by the naked-eye are extremely required for applications of environmental control. Plasmonic nanoparticles can be used to detect these hazardous compounds. Nanomaterials display unique features. In recent years, noble metal nanoparticles have attracted attention due to their physicochemical features and applications in areas including biotechnology, electronics, catalysis, and plasmonic. The surface plasmon resonance features of silver and gold nanomaterials have been helpful for a kind of application. Plasmonic nanoparticles have recently been utilized as favorable materials in colorimetric detection methods. As a result, rapid and sensitive detection of hazardous compounds and small contaminants without the requirement of anyone device (i.e., naked-eye watching change of colors) can be achieved using a UV–visible spectrophotometer. Herein, this chapter summarizes the importance of plasmonic nanoparticles and the use of these materials in environmental monitoring and analysis is extensively discussed.

Keywords Environmental analysis · Environmental pollution · Hazardous compounds · Plasmonic nanoparticles · Naked-eye detection

15.1 Introduction

Most environmental pollutants including pesticides, toxins, heavy metals, and polycyclic aromatic hydrocarbons could be hazardous for humans and other living organisms. For instance, pesticides, mercury batteries, and paper industries could

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reason damage to the nervous system, tremors, gingivitis, psychological changes, and even more negative influences on human health. Furthermore, heavy metal ions are bioaccumulator. Hence, the long-term continuous consumption of low heavy metal amounts could be harmful. In addition, the overproduction of greenhouse gases, which are air and environmental pollutants, causes serious global climate changes, and air pollution is thought to be responsible for global warming potential, melting glaciers, and sea-level rise (Jacobson 2009; Singh et al. 2011; Kołątaj et al. 2020).

A variety of methods such as electrochemical, spectroscopy, chromatography, chemiluminescence, flow injection analysis, and capillary electrophoresis have been applied to the hazardous compound’s detection. These time-consuming techniques have high precision and selectivity but require expensive tools and well-trained professionals (Sepahvand et al. 2021). As shown above, environmentally hazardous substances are very harmful to human health. There is therefore a big requirement for good analytical devices for the detection of diverse harmful contaminants with perfect sensitivity and specificity. Plasmonic nanoparticles are used as one of the ways to detect these dangerous compounds.

Nanomaterials are utilized to fabricate accurate, miniaturized, and sensitive sensor systems for environmental pollutant detection. These smart nanomaterials, particularly plasmonic nanomaterials, are good sensitive and selective toward environmental hazardous pollutants (Ding et al. 2016). Therefore, the improvement of a highly selective colorimetric sensing technology based on silver and gold nanomaterials is of big attention owing to their unique electronic and optical features (Faghiri and Ghorbani 2019). The noble metal nanoparticles based on surface plasmon resonance (SPR) have been a major research topic in the past half-century. SPR is the interaction between free electrons in metals and electromagnetic fields (Liu et al. 2018). Plasmonic nanoparticles have unique optical properties for naked-eye detection assays. Recent advances in the development and utilization of plasmonic nanoparticles as colorimetric substrates have led to a broad range of applications (Mohamad et al. 2019). The development of plasmonic nanoparticles is being followed more seriously in the environmental fields. The plasmonic nanoparticles have important advantages including on-site, optical, fast, and cost-effective detection. The advantage of defined plasmonic nanoparticles is that they provide real-time monitoring with naked-eye detection, without sample pre-treatment. Herein this chapter, we investigated the effectiveness of the plasmonic nanoparticles as high selectivity and sensitivity detection methods for environmental pollutants and applications. Detection of hazardous contaminants including toxic heavy metals, organo-phosphate pesticides, aromatic compounds, and other contaminating compounds as model compounds with materials based on plasmonic nanoparticles has been reviewed.

15.2 Colorimetry-Based Plasmonic Nanoparticles

The plasmonic nanoparticles-based colorimetric strategy provides on-site, visual, quick, and cost-effective detection. Plasmonic nanoparticles (usually silver and gold nanoparticles) have taken enormous attention in the design of effective colorimetric sensors owing to their SPR and great extinction coefficients in the visible light range. Nanoparticles-based colorimetric techniques base indirectly or directly on different interactions between the plasmonic nanoparticles and target compounds. These specific interactions reason for changes in the optical features and color of plasmonic nanoparticles and thus allow visual detection of the target compound (Saha et al. 2012; Sepahvand et al. 2021). Colorimetric biosensors for quick and sensitive detection of target molecules with the naked-eye are needed in point-of-care diagnostics, environmental monitoring, and resources constrained analysis, where analytical devices may not be available (O'Connor et al. 2016).

15.3 Plasmonic Nanoparticles

Nanoparticles are described as particles measuring sizes between 1 and 100 nm (Mody et al. 2010; Boholm and Arvidsson 2016; Nowak et al. 2020). Nanoparticles have been used and developed for diverse applications such as in environmental monitoring (Chakraborty and Pal 2018), drug delivery systems (Sur et al. 2019), medical therapy (Aghebati-Maleki et al. 2020), catalysis (Zhang et al. 2018), electroanalysis, and photoelectrochemical (Shu et al. 2018) analysis to name a few (Sharifi et al. 2019). The surface modification process can be easily adapted for the corresponding applications. Moreover, nanoparticles have unique optical features for naked-eye detection tests (Kim et al. 2017) or in microscopy (Hemelaar et al. 2017; Brown et al. 2018). Such advantages have allowed a wide range of applications in nanoparticle-based sensing with enhanced sensitivity (Mohamad et al. 2019). Nanomaterials, especially plasmonic-based materials, are utilized to generate accurate, miniature, and sensitive sensing systems for hazardous pollutant detection (Faghiri and Ghorbani 2019).

Plasmonic nanoparticles are based on the interaction between material and light through the surface plasmon resonance characteristics. In general, surface plasmon resonance mentions the whole electromagnetic oscillations of electrons between the metal and dielectric (Scholl et al. 2012; Koyun et al. 2019; Akgönüllü et al. 2020). Such an electronic oscillation can be spread during a straight interface (surface plasmon polariton: SPP) or can be limited by a subwavelength structure (localized SPR: LSPR). The SPR forms can restrict the influence of the electromagnetic area at a deep subwavelength scale, which can essentially rise the local area to ensure light manipulation below the light dispersion (Lance Kelly et al. 2003; Liu et al. 2018; Sharifi et al. 2019).

Plasmonic-based nanoparticles have recently been the topic of research owing to their unique chemical, optical, mechanical, and electronic features (Guzman et al. 2008). The most prominent distinction in features of nano-sized and bulk noble metallic nanoparticles is their optical features. Silver and gold nanomaterials can change from red to violet, which is based on their shape and size (Fig. 15.1). The bulk silver nanoparticles are a gray color. The bulk gold nanoparticles are yellow color

(Liz-Marzán 2004; Kołątaj et al. 2020). Electron microscope images of gold-silver nanoparticles in different shapes were shown in Fig. 15.2.

Matchless optical features of gold and silver plasmonic nanoparticles are the result of the stimulation of LSPR. It is occurring when the tiny plasmonic nanoparticle is irradiated by light with a suitable wavelength. SPR is understandable as a collective oscillation of conduction electrons near surface of nanoparticles (Fig. 15.3). Under the excitation of an external electric area, the motion of free electrons in metal nanoparticles can be defined by the following Eq (15.1):

$$E_{\text{out}} = E_0\vec{x} - \alpha E_0\left[\frac{\vec{x}}{r^3} - \frac{3x}{r^5}(x\vec{x} + y\vec{y} + z\vec{z})\right] \tag{15.1}$$

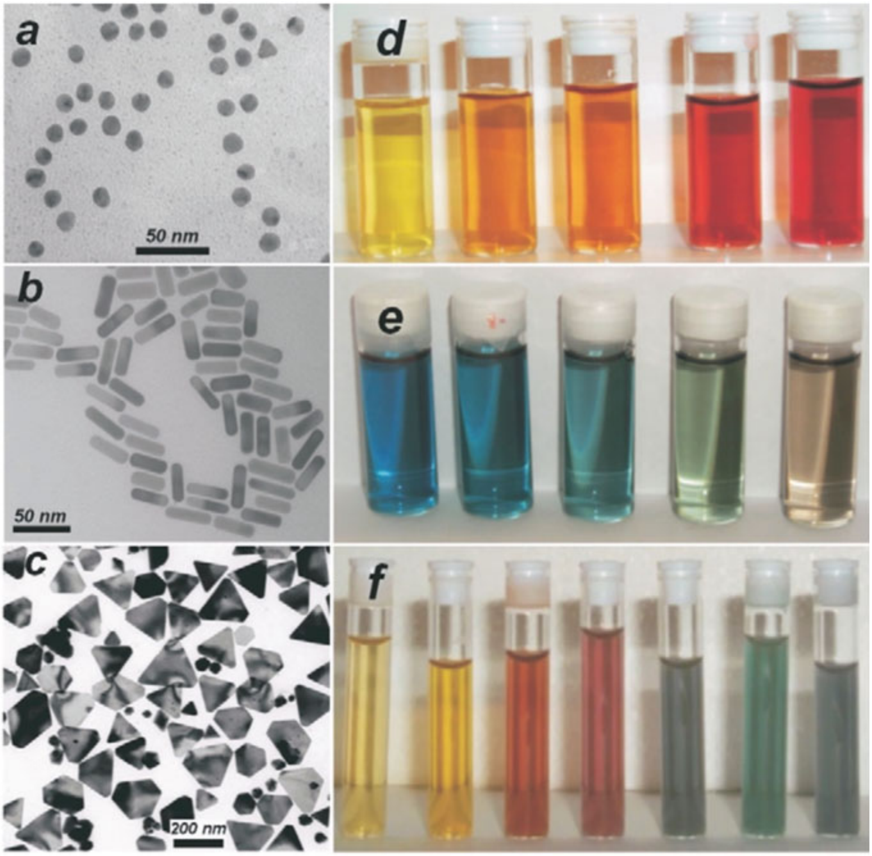

Fig. 15.1 Transmission electron micrograph (TEM) images and photographs of colloidal dispersions of Au nanoparticles, nanorods **a**, **b**, and Ag nanoprisms **c**; photographs of colloidal dispersions of **d**–**e** Au nanorods; and **f** Ag nanoprisms (*Source* Reprinted with permission from Liz-Marzán 2004)

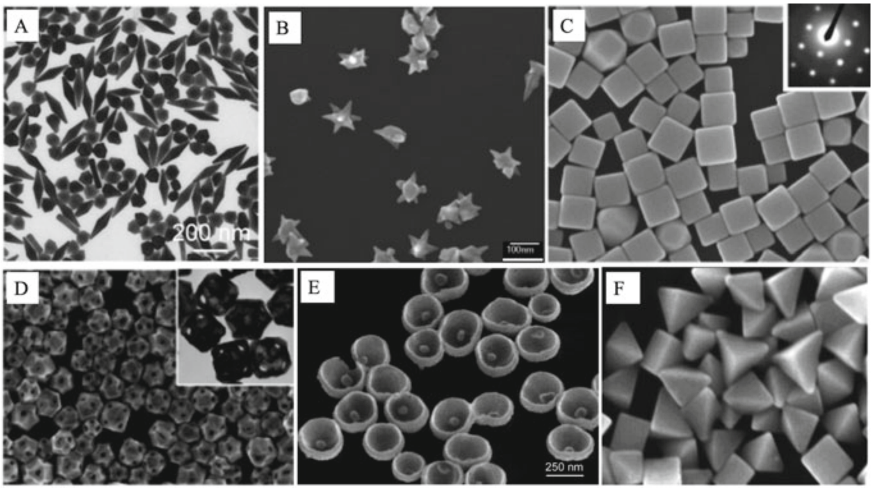

Fig. 15.2 Gold and silver nanoparticles with different size and shapes; **a** gold nanobipyramid (*Source* Reprinted with permission from Chow et al. 2019); **b** gold nanostar (*Source* Reprinted with permission from Nehl et al. 2006); **c** silver nanoboxes (*Source* Reprinted with permission from Sun and Xia 2004); **d** silver nanocubes (*Source* Reprinted with permission from Chen et al. 2006); **e** gold nanobowls (*Source* Reprinted with permission from Ye et al. 2009); and **f** silver bipyramids (*Source* Reprinted with permission from Wiley et al. 2006)

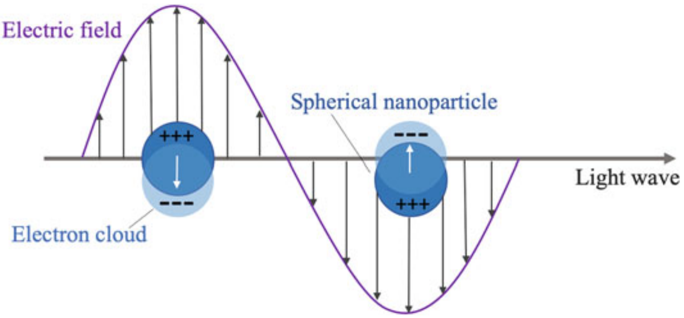

Fig. 15.3 Schematic diagram of plasmon oscillation on a metal nanoparticle, display charge distribution relative to the nuclei

where E_0 is the magnitude of the external electric field, a is the metal polarizability, x, y, z are Cartesian coordinates, r is the radial distance, and $\vec{x}$, $\vec{y}$, $\vec{z}$ are unit vectors (Krajczewski et al. 2017).

Nanomaterials play a significant role in current biosensor technology. For instance, nanoparticles like gold, silver, and copper have been broadly utilized in visual detection thanks to their optical feature known as SPR. Plasmon resonance scattering of gold and silver nanoparticles has been utilized for bio-affinity sensing (Schuster et al. 1993; Jain et al. 2006; Piriya et al. 2017). In general, molecular interaction on the surface which is modified or functionalized with certain functional groups or nanoparticles has been used in the plasmonic detection technique. The role of plasmonic nanoparticles in colorimetric detection is reported, and their results have displayed that label-free analysis is also possible with nanoparticles (Piriya et al. 2017).

15.4 Noble Metal Nanomaterials

Nanoscience is nanomaterial discovery by manufacturing nanometer-sized materials with new and developed features that can influence all fields of chemical and physical nanoscience. Nanomaterials and nanoparticles display unique features. The noble metal nanoparticles are important nanomaterials (Asefa et al. 2009). They are of attention for their physicochemical features and various applications in areas including plasmonic, biotechnology, electronics, and catalysis. As the sizes of nanoparticles dispersed in the liquid medium decrease to nanometer size, a robust UV–visible band seems that is not present in the bulk metal spectrum (Hatamie et al. 2014). Noble metal nanoparticles have a strongly enhanced SPR, and this makes them perfect scatterers and absorbers of visible light at optical frequencies (Jain et al. 2006). The plasmonic nanoparticles-based whole analytical methods have high specific optical features. There are a lot of highly sensitive analytical devices using the simulation of surface plasmons and their aggregates. Plasmonic sensor technology is very selective and sensitive. It is possible to observe the dependable signal response even in the detection of a sole molecule of an analyte (Kołątaj et al. 2020).

15.5 Plasmonic Nanoparticle-Based Naked-Eye Colorimetry Method

Signal production techniques for the detection of compounds with the naked-eye colorimetry method are becoming progressively popular in biosensor technology (Paterson and De La Rica 2015). These approaches are especially advantageous in those cases in which tools are not available, for instance, in-field works (Lee et al. 2007; Bui and Abbas 2015), analyses in resource-constrained settings (De La Rica and Stevens 2012), and point-of-care diagnostics (Parolo and Merkoçi 2013). Among the various methods for naked-eye detection, colorimetric sensor mechanisms are frequently preferred owing to the simple detection that just needs comparing the color of control and assay samples. Moreover, colorimetric signals can be facilely recorded by taking a picture with a smartphone, which is a common and portable smart technology that can be found anywhere (Vashist et al. 2015; O'Connor et al. 2016).

Plasmonic nanoparticles ensure the quantitative determination and detection of many analytes. Plasmonic nanoparticles based on the aggregation method are the most commonly used in various colorimetric systems. It is based on the aggregation or agglomeration of plasmonic nanoparticles. Plasmonic nanoparticles that have been used in the colorimetric analysis technique are dramatically cheap than the equipment needed in other analytical devices (Kołątaj et al. 2020). Various hazardous compound applications have been reported based on the unique features of plasmonic nanoparticles, that show the importance of plasmonic nanoparticles in environmental activities. Colorimetry is an alternative sensing system that has been found helpful in sensitive and low-cost sensor systems for hazardous compound analysis (Faghiri and Ghorbani 2019).

15.6 The Applications of the Plasmonic Nanoparticles in the Environmental Pollutant's Detection

The potential application of plasmonic nanoparticles is used in the analysis of the environment such as imaging and chemical detection applications. Environmental pollutions pose several risks to the environment and also to human health (Li et al. 2014a). Due to the serious effects of the pollutants, interest in the detection and imaging the environmental pollution such as toxic heavy metal ions, chemical compounds, and organo-phosphate pesticides in aqueous eco-systems is continued. Plasmonic nanoparticles-based sensing is used with the aggregation of nanoparticles or changes in the local refractive indexes; therefore, the detection or imaging of the toxic heavy metal ions, chemical compounds, and other environmental pollutants is obtained by changing the color of the sample such as soil and water in the presence of any pollutants (Li et al. 2008).

Environmental pollutions are one of the serious environmental problems in the world and inhibit the problem development of the economy and society (Che Sulaiman et al. 2020). The presence of heavy metal ions, chemical toxins, and organic and inorganic pollutants need to be detected frequently to safeguard the environment (Xue and Mirkin 2007; Lou et al. 2011). Recently, the combination of chemistry, biology, physics, and also nanotechnology is present as sensitive imaging and sensing methods for application in many fields (Tang et al. 2017; Zhang et al. 2019; Zhou et al. 2020; Cassano et al. 2021; Koh et al. 2021). In the past decade, the surface of nanoparticles (silver/gold/magnetic) and quantum dots is modified to sensitive binding of the target (Wang et al. 2008, 2019; Lin et al. 2010; Liu et al. 2010; Diao et al. 2020).

15.6.1 Toxic Heavy Metal

Cr(II), Cu(II), Hg(II), Cd(II), and Pb(II) as heavy metal ions are the most environmental pollutants and pose substantial public health hazards. Gold nanoparticles are commonly utilized for sensitive heavy metal ion detection as colorimetric sensors. Many techniques are used commonly for the selective and sensitive detection of heavy metals (Bakhshpour and Denizli 2020). However, they do not need expensive equipment is the major advantage of plasmonic sensor methods that cause to use of the metal nanoparticle-based colorimetric sensor. In the study, the opposite effects of single-stranded DNA area in DNAzyme on the Au nanoparticles were used for the detection of Pb(II). High ionic strength can be spontaneously assembled DNAzyme-based Au nanoparticles to their substrate sequence, due to the stacking interaction of the DNA base. The DNAzyme-functioned Au nanoparticles, in presence of Pb(II), cleave the substrate; therefore, an ssDNA area occurs in the middle of the rigid loop. Here, this procedure can show an effective plasmon resonance increase of the gold nanoparticles for sensitive detection of Pb(II) ions. The limit of detection value was reported as 8.0 nM concentration of Pb(II) in this study.

Figure 15.4a depicts the schematic of Pb(II) detection with this method. The cleavage of the DNA and/or RNA chimera substrate sequence and the principle of this study are summarized. Figure 15.4b shows the quantitative analysis of Pb(II), (a) extinction spectra, and (b) photographs of different Pb(II) concentrations. In addition, *c* is the variation of the LSPR intensity of the DNAzyme-functionalized gold nanoparticles with $R^2 = 0.99$, and (d) linearly relationship between the relative change and Pb(II) in the different concentration range (0–100 nM) with ($R^2 = 0.97$) (Diao et al. 2020).

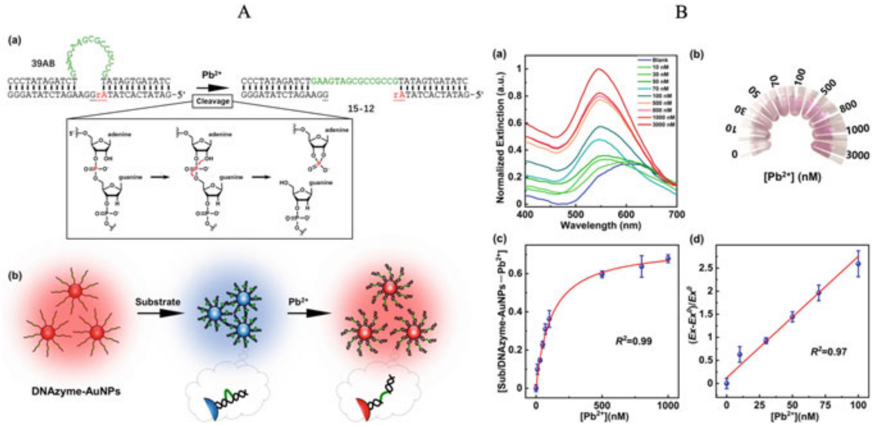

Fig. 15.4 **a** Schematic representation of Pb(II) detection using DNAzyme-functioned Au nanoparticles; **b** quantitative analysis of Pb(II) (*Source* Reprinted with permission from Diao et al. 2020)

In another study, a non-aggregation-based silver-coated Au nanoparticle probe was prepared for the real-time and sensitive detection of Cu(II). Using silver-coated Au nanoparticles on the surface plasmon resonance can be dramatically leaching, therefore, decreasing the amount of the nanoparticles. Colorimetric sensors circumventing this disadvantage provided selective and sensitive imaging with a 1.0 nM Cu(II) detection value. The rapid, selective, and highly sensitive detection of Cu(II) ions was done using a cost-effective probe. This probe can be utilized in the real-time detection of Cu(II) in water. Figure 15.5a shows the schematic illustration of the sensing mechanism of silver-coated gold nanoparticles for the colorimetric detection of Cu(II). Photographs and the absorption responses of nanoparticle solution with Cu(II) are shown in (A, B), respectively. The plot of ($A_{\text{blank}} - A_{\text{Cu}}$)/$A_{\text{blank}}$ values against Cu(II) concentration is shown at 405 nm (Lou et al. 2011).

Some heavy metal ions such as mercury, lead, and cadmium cause serious health and environmental impacts, so monitoring toxic metal ions is essential (Şener and

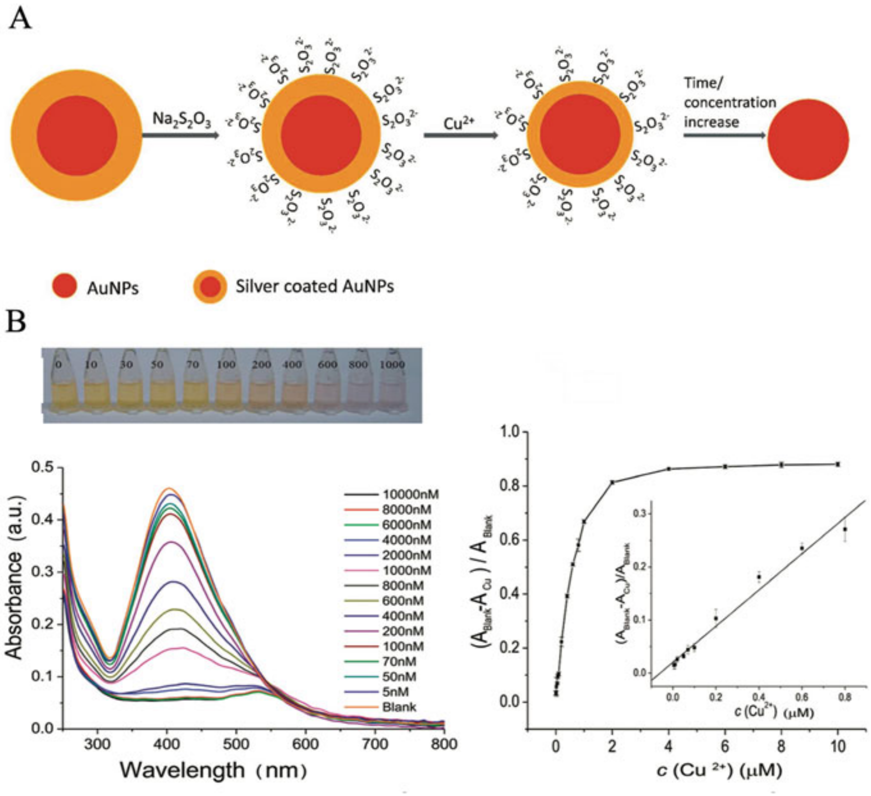

Fig. 15.5 **a** Schematic illustration of the sensing mechanism of silver-coated gold nanoparticles for the colorimetric detection of Cu(II) ions; **b** photographs and the absorption responses of nanoparticles solution with Cu(II) and plot of ($A_{\text{blank}} - A_{\text{Cu}}$)/$A_{\text{blank}}$ values against Cu(II) concentration (*Source* Reprinted with permission from Lou et al. 2011)

Denizli 2019b, 2019a). Denizli groups developed a rapid, sensitive, inexpensive, and simple sensor for the detection of Hg(II) in water samples. Firstly, they synthesized gold nanoparticles that were capped with citrate. Then, they allowed to aggregation of the prepared nanoparticles with the Hg(II) ions in the presence of the lysine as a positive charge amino acid. They reported a very fast, selective, and sensitive detection of Hg(II) with 2.9 nM of the detection value via this colorimetric assay. Figure 15.6a illustrates the schematic representation of Hg(II) detection mechanisms. As seen in Fig. 15.6b, in the nanoparticle solutions, with the 0.4 mM concentration of lysine and 10 μM concentration of Hg(II) the color change has occurred. Figure 15.6c demonstrates TEM images of gold nanoparticle solutions: (a) Bare AuNPs, (b) with lysine, (c) with Hg(II) ions, and (d) with lysine and Hg(II) ions. The aggregation of nanoparticles was obtained by adding lysine and Hg(II) ions. They also tested other amino acids by adding 10 μM Hg(II) to the gold nanoparticle solutions. Then, they added 0.4 mM amino acid solutions to the nanoparticle solutions. The successful results were obtained only with lysine and arginine among the 14 amino acids (Sener et al. 2014a).

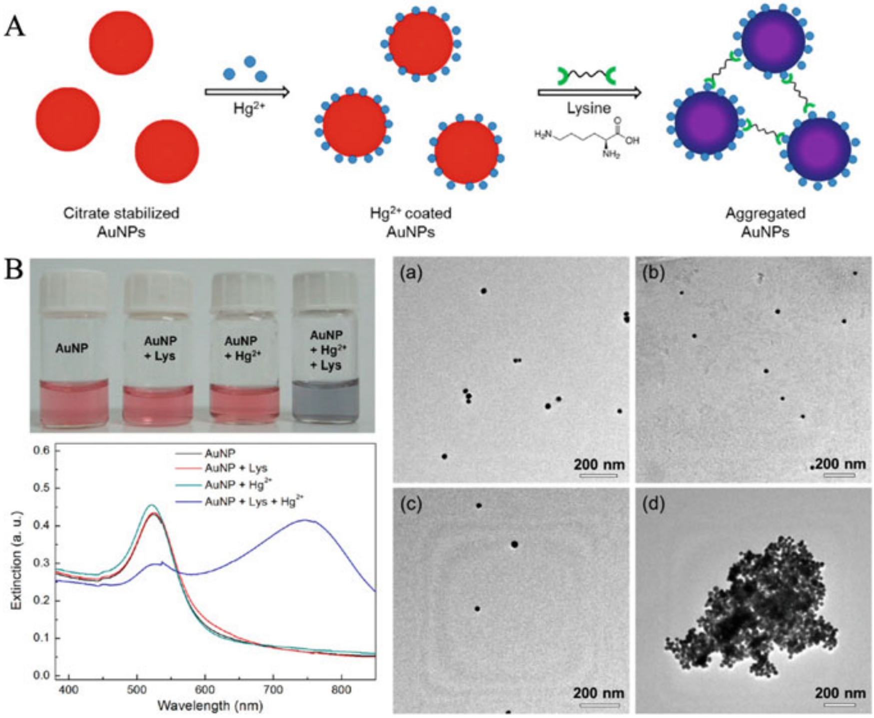

Fig. 15.6 **A** Schematic illustration of Hg(II) detection mechanisms; **B** photographs and extinction spectra; and TEM images of gold nanoparticle solutions: **a** Bare AuNPs, **b** with lysine, **c** with Hg(II) ions, and **d** with lysine and Hg(II) ions (*Source* Reprinted with permission from Sener et al. 2014a)

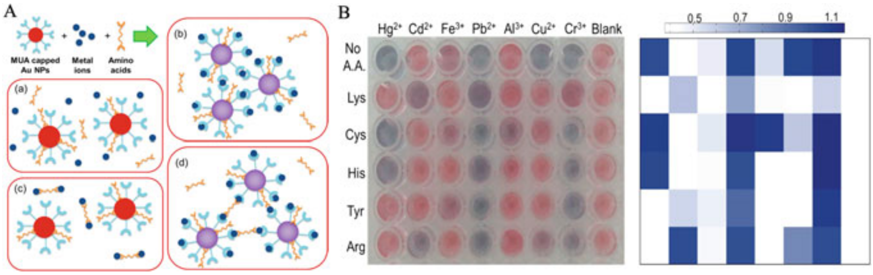

Fig. 15.7 **a** Schematic diagram of metal ions, gold nanoparticles, amino acids, and the proposed metal ion interactions; **b** The colorimetric sensor array response against 20 μM of metal ions, and blue-scale representation of the colorimetric sensor array response. White demonstrates no-aggregation, and blue demonstrates the aggregation of gold nanoparticles (*Source* Reprinted with permission from Sener et al. 2014b)

In another study, they reported a sensitive colorimetric sensor for multiple detections of heavy metal ions using gold nanoparticles capped with 11-mercaptoundecanoic acid. They tested cysteine, lysine, histidine, arginine, and tyrosine in this study. Figure 15.7a represents the schematic of gold nanoparticles, amino acids, and the proposed metal ion interactions. Also, Fig. 15.7b shows the image of metal ion detection with the colorimetric sensor (Sener et al. 2014b).

15.6.2 Organo-Phosphate Pesticides

The other toxic materials are organo-phosphorus pesticides that are usually employed in agriculture. Organo-phosphorus pesticides are amides, thiol, or ester derivatives of either thiophosphoric or phosphoric acid (Bai et al. 2015; Chiou et al. 2015). Organo-phosphorus pesticide, among the other pesticides, is the most commonly used, that inhibits acetylcholinesterase (AChE) (Kim et al. 2011). The use of pesticides poses health risks to non-target species and the environment (Saylan et al. 2017). Many techniques are used commonly for the sensitive detection of these compounds such as gas chromatography and liquid chromatography. However, the expensive equipment and time-consuming of these methods cause to use of the metal nanoparticle-based colorimetric sensor. The perfect properties of the nanoparticles such as high target specificity, droplet size, and surface-to-volume ratio allow them to utilize for the specific and sensitive detection of pesticides. This part ensures a wide summary of the new studies of the recent development in the usage of the nanoparticle-based colorimetric assay for naked-eye detection of organo-phosphate pesticides (Oujji 2014).

Dissanayake et al. developed plasmonic nanoparticles for organo-phosphorus pesticide detection. Plasmonic silver, gold, and also bimetallic silver-gold nanoparticles have been used for sensitive detection and separation of organo-phosphorus pesticides. They used nanoparticles for real-time detection of the thion-pesticides. The three plasmonic nanoparticles demonstrated real-time detection with a ppm limit of detection values of pesticides. In addition, they tested the capability of the plasmonic nanoparticles for selective detection of pesticides in the environmental sample (Dissanayake et al. 2019).

In the other study, sensitive detection of paraoxon, parathion, diazinon, and fenitrothion was demonstrated using Au nanoparticles from the lake, tap, and mineral water via inhibition of AchE. Firstly, TCh as a positive charge was absorbed on the negative charge Au nanoparticles that were capped with citrate. As seen in Fig. 15.8a, the color of the solution was changed after the aggregation of the Au nanoparticles. By binding organo-phosphate compound to the active serine site of AchE that inhibits the activity of AchE, the positive charge TCh molecule reduces. Thus, pushing force among the negative charge of Au nanoparticles can protect the aggregation of nanoparticles (Satnami et al. 2018). In a similar work, cysteine was used as a capping onto Au nanoparticle surface for ethyl parathion compound detection (Fig. 15.8b). The authors reported a 0.081 ng/mL detection value for the spike samples (Usman Ali et al. 2010). In another similar study, AchE-enzymatic inhibition using paraoxon was shown. The production of TCh is greatly increased by increasing the enzymes; therefore, aggregation of nanoparticles is obtained (Pavlov et al. 2005).

Li et al. developed an uncomplicated, real-time, rapid, and novel method for detection and naked-eye imaging of the organo-phosphorus pesticides. They used the citrate-capped method via silver nanoparticles and AchE. The aggregation of

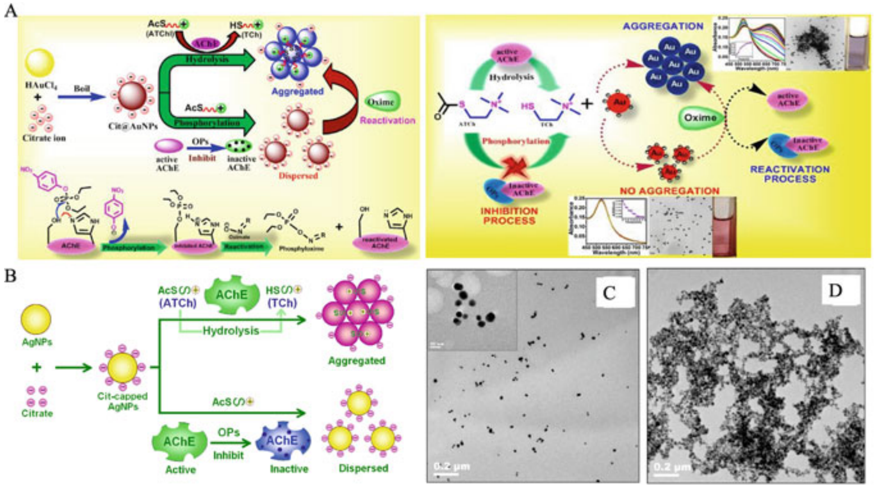

Fig. 15.8 **a** Schematic illustration of the probing inhibition and reactivation of AchE; **b** Schematic representation of enzyme inhibition of AchE with silver nanoparticles (*Source* Reprinted with permission from Satnami et al. 2018); and **c**–**d** TEM images of citrate-capped silver nanoparticles before and after the addition of 2.25 micromol/L ATCh and 400 mU/mL AChE, respectively (*Source* Reprinted with permission from Li et al. 2014b)

nanoparticles was caused to change the color of nanoparticles from bright yellow to pink in the solution. The limit of the detection was found to be 0.18 ng/mL (Li et al. 2014b). TEM images of citrate-capped silver nanoparticles before and after the addition of different concentrations of AchE are shown in Fig. 15.8c–d.

15.6.3 Aromatic Compounds

Aromatic compounds are broadly utilized in the synthesis of dyes, and also are compounds of antiseptics and disinfectants. Aromatic compounds are known as hazardous environmental pollutants. The high level of anilines and phenols created concern about fish, mammals, and others. Chen et al. developed a highly sensitive, quantitative colorimetric sensor for naked-eye detection of aromatic compounds. They synthesized silver nanoparticles and functionalized them with beta-cyclodextrin. The silver nanoparticles can be aggregated in the presence of aromatic compounds; then, the color of the solution was changed. They reported a 5 × 10^5 M limit of the detection value for aromatic compounds (Chen et al. 2010). Schematic diagram of modified-silver nanoparticles with aromatic compounds and photographs of nanoparticle solution is demonstrated in Fig. 15.9.

In another study, Champaiboon et al. designed a polydiacetylene-based multilayer film for sensitive and real-time naked-eye determination and imaging of aromatic compounds. They prepared a blue-colored polyelectrolyte-based multilayer film for the detection of aromatic compounds from water. They showed the changes in the color of the multilayer film from blue to red within 5 min by adding 10 mM-cyclodextrin solutions (Champaiboon et al. 2009).

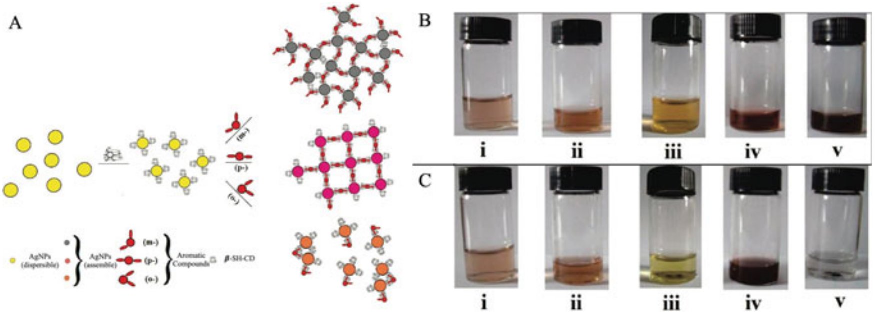

Fig. 15.9 **a** Schematic illustration of modified-silver nanoparticles with aromatic compounds and photographs of nanoparticle solution before (b, i–v and c, i–v) and after adding with aromatic compounds (10^4 M) for 2 h (*Source* Reprinted with permission from Chen et al. 2010)

15.6.4 Other Pollutant Compounds

Sulfide ions, as known very toxicity liberated hydrogen sulfide form, are usually found in water and wastewater samples. It is a major contamination index. The sulfide detection is necessary for biological research and environmental protection. Hatamie et al. synthesized a Cu nanoparticle-based novel technology for the detection of sulfide ions. This simple and sensitive method was developed for rapid and real-time colorimetric imaging sulfide ion detection in water. The color of Cu nanoparticle-based colorimetric sensor was changed in the presence of micromolar levels of sulfide ions. This color-changing can be observed with naked-eye, also the results showed with UV–vis spectrophotometer. They reported 12.5×10^{-6}–50.0×10^{-6} M concentration range of sulfide ions in this study. The river and tap water samples were successfully analyzed. They showed excellent value for the deception of sulfide ions in the water without using any readout equipment. They present a new method with a high potential for sensitive and real-time detection of environmental monitoring of sulfide ions (Hatamie et al. 2014).

15.7 Conclusions and Future Outlooks

A securely sensing or detection of hazardous compounds is one among various environmental contamination problems worldwide. The uncontrolled or controlled release of hazardous compounds from different ever-growing industrial applications is the main source of environmental issues. Important investigation works have been made to handle this problematical matter to meet the developing requirements of the modern world. In this context, the rise in the concentration of these hazardous and toxic compounds in the environment is of critical worry; therefore, real-time monitoring is promptly needed. In this chapter, we investigated plasmonic-based technology and its potential to address the detection destiny of toxic and hazardous compounds such as toxic heavy metals, organo-phosphate pesticides, aromatic compounds, and other pollutant compounds as model elements.

Among the detection strategies utilized so far, sensors have received special attention owing to their remarkable potential, compared to conventional chromatography-based techniques. Among current sensors, the optical-based sensor class of broad and particularly fluorescent and plasmonic materials has several advantages over other types of electrochemical sensors, including real-time tracking, miniaturization, and increased selectivity and sensitivity. As a result, the risk associated with environmental pollutants can be carefully identified, measured, and controlled using a sound strategy. Smart systems that provide the real-time detection of environmental pollutants using plasmonic nanoparticles-based colorimetric technology that is visible by the naked-eye are highly required for environmental control.

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Chapter 16
Utility of Nano Biosensors for Heavy Metal Contamination Detection in the Environment

Chansi, Ruchika Chauhan, Punya, and Tinku Basu

Abstract Heavy metal ions act as major pollutants due to their noxious effect on human health. Contamination of surface and groundwater has become one of the major environmental problems. Thus, it is imperative to detect their trace levels present to prevent their deadly impact. Conventional methods include spectroscopic and chromatographic based approaches that provide precise detection. However, there is a grave need for highly sensitive, selective, reliable, simple, cost-effective, and real-time response techniques. In this chapter, the statistical availability of various heavy metal ions across different continents along with their epidemiology is discussed. Among, heavy metal arsenic (As) has a wide prevalence and is most toxic. Consumption of contaminated water and food pose a threat to public health, demands for several techniques for its detection. Hence traditional methods for As monitoring are discussed. Further wastewater monitoring and treatment-related nano biosensors, majorly applied for the recognition of As are reviewed. These biosensors are categorised into two types, micro fuel cell (MFC) based biosensors and nanomaterial-based biosensors. At last online chip-based commercially available biosensors and their challenges have been discussed.

Keywords Nano biosensor · Arsenic · Electrochemical · Optical · Sensing

16.1 Introduction

Metal ions form essential part of human body and are required to maintain various biological functions and their absence or scarcity led to disease. Even heavy metal ions like Zn, Fe, Mn, Cu play a vital role in growth as well as required to support biological activities. Metal ions are also important in controlling enzyme-catalyzed

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processes for altering electron transport in substrates or enzymes. The irony of the fact is that the excess of these (metal ions) beyond the desired amount is toxic to humans (USEPA 2015). However, few heavy metal ions including Pb, Hg, As, Cd have been deemed as contaminants and poisonous substances even at minimum level concentrations, which can be regarded as a serious risk to the humans and environment. Unlike organic pollutants, heavy metals are non-biodegradable, tend to accrue within living beings, and can behave as a potential carcinogen. The natural sources account weathering of metal-bearing rocks and volcanic eruptions. The worldwide developments of industrialization and urbanization comprising mining, industrial and agricultural activities have headed toward rise in the anthropogenic apportion of heavy metals in the environment (Verma and Kaur 2016). Application of chemical fertilizers, especially, phosphate fertilizers, and incineration of fuels also contribute to the existence of heavy metals in the environment. Since heavy metals are persistent in the environment, they either pile up in biota or percolate down into ground waters setting off serious implications for human health. All these forces for precise quantification of heavy metals in the environment. The sensing of heavy metal ions at low concentrations entails the use of conventional analytical approaches including spectroscopic methods such as atomic absorption spectroscopy (AAS), inductively coupled plasma/atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectroscopy (ICP-MS), ultraviolet-visible spectroscopy (UV-vis) and Chromatographic methods. Even Though these analytical procedures are highly sensitive and selective, they endure constraints such as tedious sample preparation, expensive tools, preconcentration methods, and qualified personnel. However, the present trends especially the nanomaterials-based sensors/biosensors have displayed great potential in the recognition of heavy metals owing their substantial surface area, high catalytic efficacy, high surface responsiveness, and effective adsorption capacity. Biosensors can be summarized based on signal transduction mechanisms as electrochemical, piezoelectric, and optical (Fluorescent, colorimetric, surface plasmon resonance (SPR), Luminescence) sensors. Besides these, several portable currently available commercial devices (optical and Electrochemical) are accessible for on-site/in-site quantification.

Hence, the present chapter describes statistical prevalence of different heavy metal ions and their consequences on human health. Further, the traditional approaches for the detection of arsenic are discussed due to its toxic effects and wide prevalence. A detailed overview of various nanomaterial-based approaches for quantitative identification of arsenic at the trace level is provided. Finally, several commercially available techniques and kits are also discussed.

16.2 Statistical Prevalence and Epidemiology of Heavy Metal Ion Contamination

Heavy metals are present organically in earth's crust, but the anthropogenic activities related to technology or research have created drastic variations in their geochemical cycles. Concentrations of different heavy metal ions differ in water bodies across the five continents as reported in Table 16.1. Contamination of Heavy metal ions is higher in Africa, Asia, and South America, and it is lowest in North America and Europe.

The primary sources of heavy metal ions include mining, smelting, foundries, and other industries, leaching of metals from special sources consisting of landfills, waste dumps, excretion, cattle manure, runoffs, vehicles, and roadworks. Use of heavy metals in the field is secondary source together with the usage of insecticides, pesticides, and fertilizers. Natural causes include volcanic eruptions, metallic corrosion, metal evaporation from soil and water and sediment re-suspension, soil erosion and geological weathering, etc. also increase contamination. Out of Thirty-five metals cause a hazard to human health on suburban /professional exposure, are heavy metals (Mosby et al. 1996). Studies have stated various consequences of presence of heavy metals in drinking water (ATSDR 2015; USEPA 2015) and classified them as carcinogenic or not. Among different heavy metal ions-As, Cd, Pb, Cr, Cu, Hg, and Ni are of key concern, primarily due to their existence at moderately elevated concentrations in drinking water and their impacts on human health (ATSDR 2015).

Arsenic (As) a natural element of the earth's crust is highly poisonous in its inorganic form and widely scattered throughout the environment. It is recognized as the "king of poison" due to its toxicity and is ranked number one amongst unsafe

Table 16.1 Presence of heavy metal ions (mg L^{-1}) in water bodies across five continents along with permissible concentration as per WHO

Metals	Africa	Asia	Europe	North America	South America	WHO
$Cd2^+$	45.04	17.75	5.69	1.12	63.54	3
$Pb2^+$	83.82	92.70	14.31	163.28	332.93	10
Cr^{3+}	388.77	383.93	13.61	5.42	903.78	50
Hg^{2+}	528.50	4.17	0.15	1	40	1
Zn^{2+}	1169.00	889.57	1338.99	86.94	680.49	1000
Cu^{2+}	190.79	345.85	14.63	15.90	142.64	2000
Ni^{2+}	131.69	54.84	137.47	10.93	33.55	20
Al^{3+}	945.48	3130.88	569.75	223.38	–	200
Mn^{2+}	483.54	967.77	257.89	57.26	89.36	100
Fe^{3+}	2012.82	3152.78	243.75	274.06	1203.89	300
As^{3+}	33.46	178.30	18.54	0.33	–	10
Co^{2+}	12.60	28.83	0.36	0.17	6.78	–

substances. The International Agency for Research on Cancer (IARC) has categorized them into different groups (Table 16.2) and As is considered as class I carcinogen, mutagens, and teratogen. The ultimate hazard to public health from arsenic occurs from polluted groundwater. The worldwide data reveals that 107 countries in different continents have been affected by As contamination and the percentage composition is as depicted in Fig. 16.1. Countries with a presence of inorganic As is naturally present at high levels include Argentina, Bangladesh, Chile, China, India, Mexico, and the United States of America.

Smith et al. (1992) have stated that intake of 1 L/day water with As of 50 μg/L over entire lifetime can cause cancer of liver, lung, kidney, or bladder. The record exists for enhanced manifestation of skin lesions that arise owing to As dosage of 0.0012 mg/kg/day ingested by drinking contaminated water (Ahsan et al. 2006). Arain et al. (2009) have reported an association between As in drinking water and other respiratory disorders. As has been reported to be part of central nervous system

Table 16.2 Classification of heavy metal carcinogenicity

Category	Carcinogenicity level in humans	Evidence	Heavy metal classification
Category 1	Carcinogenic	Observed in humans	• Al^{3+} Compounds
			• As compounds
			• Cd Compounds
			• Ni^{2+}
			• Nickel refining
Category 2(a)	Probably carcinogenic	Few evidence in humans, confirmed evidence in other animals	• Pb compounds
Category 2(b)	Possibly carcinogenic	No evidence in animals	• V_2O_5
			• Mb_2O_3
			• Methylmercury
			• Co^{2+}
			• Ni^{2+}
Category 3	Carcinogenicity not segregated	Insufficient usage in humans	• Cr^{3+}
			• Chromium metallic compounds
			• Cu^{2+}
			• Hg and Hg^{2+} compounds
			• As compounds not metabolized
Category 4	Not carcinogenic	No carcinogenic properties	• Mn
			• Ag
			• Zn

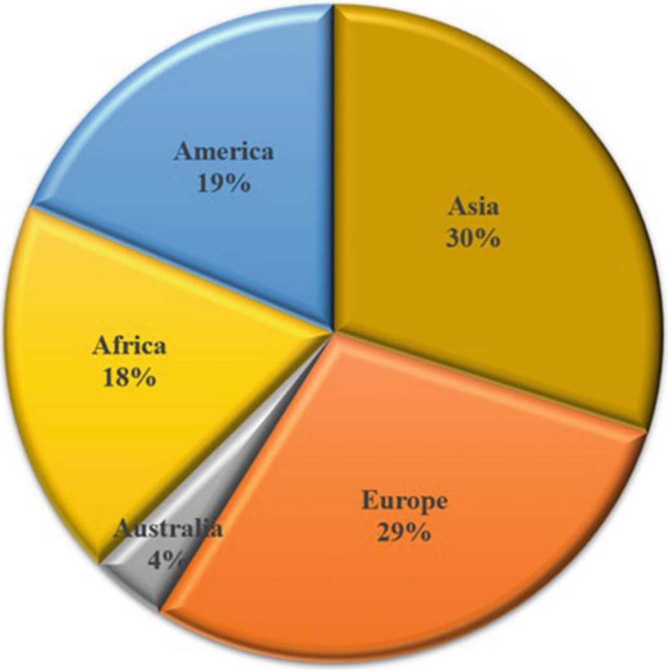

Fig. 16.1 Pie-chart showing percentage of total contamination of As in different continents

and cognitive development in children (Rosado et al. 2007). It is found that accumulation in fingernails and hair (Choong et al. 2007). Sub-chronic consequences, majorly the peril of stillbirths, is soared by six-fold in women who devour As $\geq$ 200 μg/L throughout their pregnancy (von-Ehrenstein et al. 2006).

It is startling that at least 140 million people in 50 countries have been drinking water containing As above the limit provided in the WHO provisional guideline value (10 μg/L). The accounts from US national research council have indicated that as many as 1 in 100 added cancer deaths may be due to life-long exposure to drinking water with As levels above 50 μg/L As. Long-term exposure to As has a major impact on human health as summarized in Fig. 16.2.

As is one of the top 10 chemicals accounted by WHO as a major public health concern. Considering the high prevalence and magnitude of the epidemiological effects of As contamination it has become obligatory to detect even its traces beyond the threshold limit. Hence, the subsequent section provides an overview of various approaches followed (both traditional and current) for detection of As content in water bodies. Subsequently, the commercially available As detection kits are also discussed.

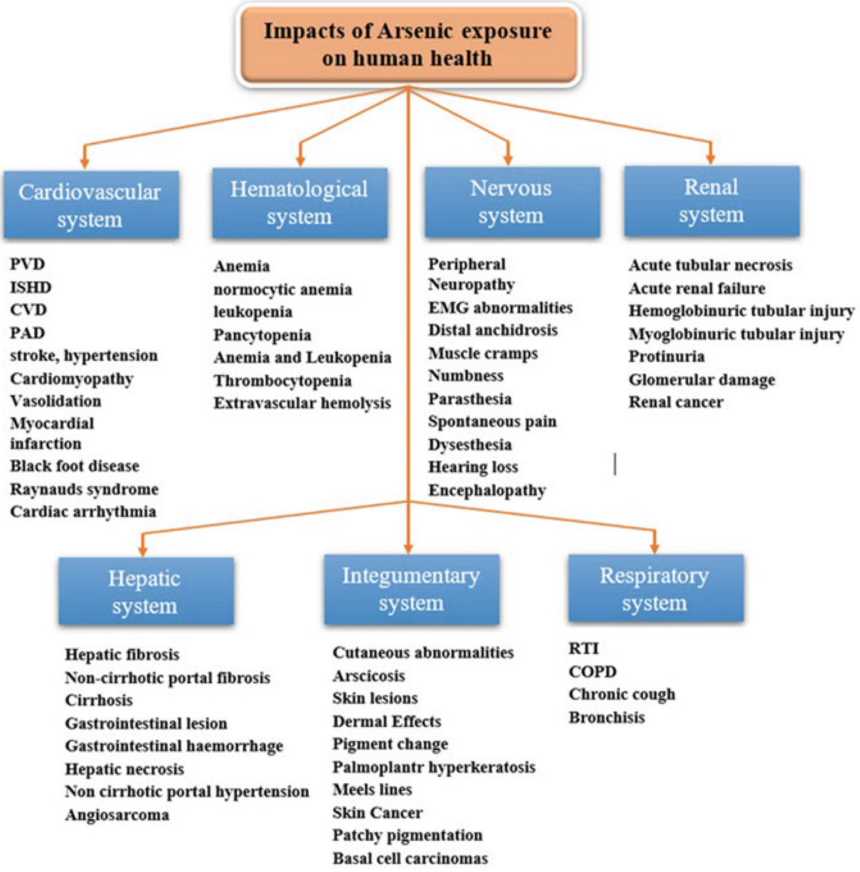

Fig. 16.2 Major impact on human health due to arsenic contamination

16.3 Traditional Approaches for Arsenic (As) Monitoring in Water

As the prevalence of As contamination is rising a range of approaches, i.e., classical to contemporary analytical procedures have been used for quantification of total As content and individual As species in water. Figure 16.3 provide an overview of various traditional methods used for As detection.

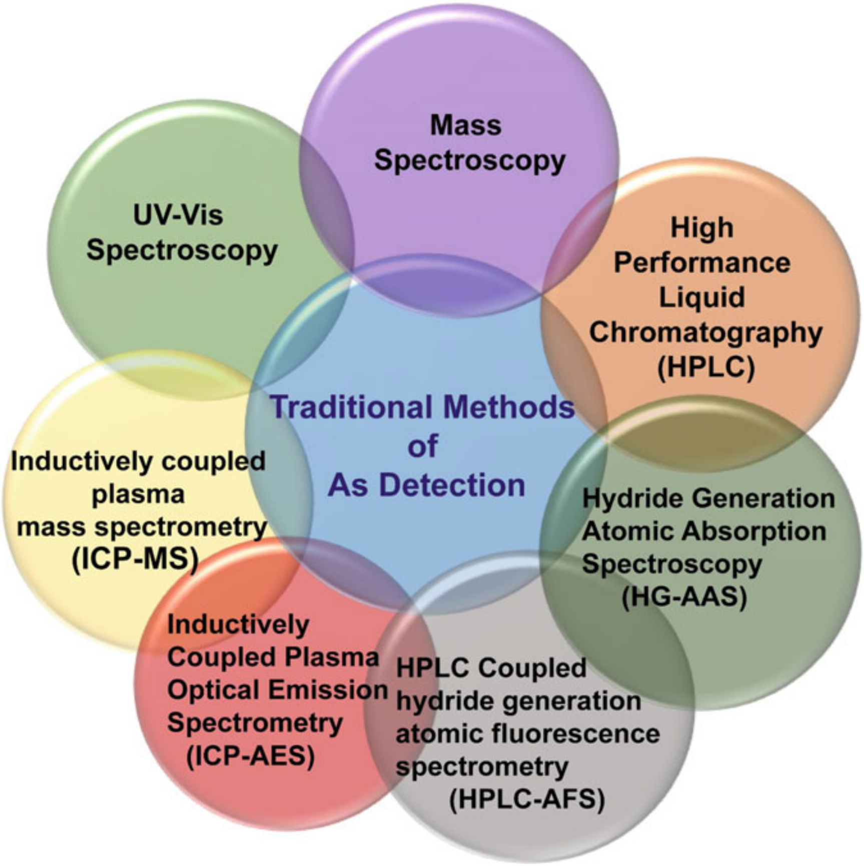

Fig. 16.3 Overview of various traditional methods available for As detection

16.3.1 Standard UV-Visible Technique

The advantages of high precision, detection efficacy, nondestructive sample, ecofriendly protection, low cost have made UV-visible (UV-vis) spectroscopy in combination with a variety of advanced tools as an excellent and effective method for rapid pollutant detection in aqueous settings. Based on the Lambert–Beer Law, UV-visible spectroscopy is applied for quantitative analysis of the quality of water and the same is effectively employed for quantification of arsenic in water. Colorimetry is a method to identify the amount of materials by coordinating the variations in color or measuring the color intensity of solution. Table 16.3 provides an overview of work done on detection of As by colorimetric technique. By selecting the appropriate reagent (usually a colored dye), the change in wavelength or intensity of color of the solution due to the interaction of the inorganic [As(III)] if present is monitored with respect to the standard solution of dye for quantitative and qualitative estimation of arsenic in water.

Vanillin-2-amino nicotinic acid (VANA) which can form a colored complex visible at 350 nm in acidic medium (pH 5.0) has been used to detect [As(III)] within the range 0.128–2.66 μg/ml. The method has observed negligible interference against an array of reagents (Citrate, W(v), Tartarate, Mn(II), Urea, Pb(II), Iodate, Cr(VI), Bicarbonate, Zn(II), Thiocyanate, Cd(II), Sulfate, Co(II), Oxalate, Ni(II), Thiourea, Fe(II), Nitrate, Au(III), Acetate, Pt(IV), Phosphate, Ti(III), Bromide, Ag(I), Chloride, V(V), Fluoride and Cu(II)) when present along with As. Similarly, 2,4-Dihydroxy benzophenone-2-amino thiophenol (BPBT) has been explored for sensing As(III) within detection range 0.125–2.637 μg/ml. Thiophene-2-carboxaldehyde thiosemicarbazone has been utilized for spectrophotometric detection of As(III) at acidic pH showing maximum absorbance at 580 nm within detection range of 0.1–1.0 mg/L (Rahman et al. 2015).

Another proposed methodology demonstrates the reaction of As(III) with KIO_3 under acidic conditions to liberate iodine. The liberated iodine would, in turn, blanches the violet color of azure B (chromogenic dye) at an absorbance of 644 nm for As(III) detection within a range of 0.2–10 mg/mL and limit of detection (LOD) of 0.002 μg/mL has been reported (Cherian and Narayana 2005). Colorimetric detection of both As(III) and As(V) is reported due to color change of Benzothiazole Schiff base with limit of detection of 7.2 ppb for As(III) and 6.7 ppb for As(V) (Chauhan et al. 2017). A novel colorimetric detection for arsenic has been employed using $KMnO_4$ and CH_4N_2S reducing and oxidizing agents for As(V) and As(III) respectively within linear range 100–800 μg/L and LOD as low as 8 μg/L through the formation of molybdenum blue complexes with minimum interference from non-analyte (Hu et al. 2012).

Nanomaterials hold unique physical and chemical properties owing to their high surface area and nanoscale size. The aggregation of nanomaterials with As(III) and As(V) have been tracked by using UV-vis and colorimetric methods for sensing of As. Mostly, gold nanoparticles (AuNP) are employed due to their high molar absorptivity and size-dependent optical properties. Hence, AuNPs have been functionalized with GSH or glutathione and act as an excellent colorimetric sensor for the detection of As(III) due to the ability of binding of As(III) ions with GSH ligands inducing the aggregation of AuNPs, resulting in a rapid color change to attain LOD of 0.12 ppb in the range of 0.1–1 ppb (Zheng et al. 2021). GSH-DTT-CYs-PDCA functionalized AuNPs showed a linear range from 2 to 20 mg/L. The average value of the analytical recovery has been noticed to be 99.7% with a LOD of 2.5 mg /L and interference due to other metal ions is negligible (Domínguez-González et al. 2014). In-situ formation of AuNPs by introducing gaseous hydrides into the $HAuCl_4$ solution containing PVA-ethanol and KI results in color change has been utilized for detection of As(III) for a wide range of 10–200 μg/L and LOD of 10 μg/L. On exploitation of $NaBH_4$ both the range (1–25 μg/mL) and LOD (0.392 μg/mL) have improved drastically (Damirchi and Heidari 2018).

Table 16.3 Comparative evaluation of various studies for detection of As using UV-visible spectroscopy

S. No.	Methodology	Electrode/reference material	Limit of detection	Range	References
1	UV-vis spectroscopy	Vanillin-2-amino nicotinic acid (VANA)	0.06 µg/ml	0.128–2.66 µg/ml	Deepa et al. (2015)
2	UV-vis spectroscopy	AuNPs functionalized with GSH-DTT-CYs-PDCA	2.5 µg/L	2–20 µg/L	Domínguez-González et al. (2014)
3	UV-vis spectroscopy	Acridine red	0.25 µg/L	0.8–280 µg/L	Saleh et al. (2022)
4	UV-vis spectroscopy	BPBT		0.125–2.637 µg/ml	Deepa and Lingappa (2014)
5	UV-vis spectroscopy and colorimetric detection	Probe L	7.2 ppb-As(III) 6.7 ppb-As (V)	5–100 ppb	Chauhan et al. (2017)
6	UV-vis spectroscopy, visual sensing	BF2-curcumin solution	19.8 µg/L-UV-vis 1. 87 mg/L-Naked-Eye Detection 2.25 mg/L-colorimetric	0–100 µM—UV-vis 25–100 µM—naked-eye detection 10–70 µM—colorimetric	Sirawatcharin et al. (2014)
7	UV-vis spectroscopy and colorimetric detection	Aptamer-CTAB-AuNPs	16.9 ppb	1–100 ppb	Thao Nguyen et al. (2018)
8	UV-vis spectroscopy and colorimetric detection	TMT-Au NPs and NCDs	0.87 ppb	50–100 ppb	Li et al. (2020)
9	UV-vis spectroscopy and colorimetric detection	$Fe_3O_4/MB/H_2O_2$	0.358 nM	0–1 µM	Babu Christus et al. (2018)

(continued)

Table 16.3 (continued)

S. No.	Methodology	Electrode/reference material	Limit of detection	Range	References
10	UV-vis spectroscopy, colorimetric detection and mass spectroscopy (ICP-MS)	DTT-Fe_3O_4@Au	0.86 ppb	0–20 ppb	Banerjee et al. (2019)
11	Colorimetric detection and UV-vis spectroscopy	Glucose/AuNPs	0.53 ppb	1–14 ppb	Mohan et al. (2019)
12	UV-vis spectroscopy	Iodine	0.02 µg/mL	0.2–10 µg/mL	Cherian and Narayana (2005)
13	UV-vis spectroscopy	PbS NPs	4.5 µg/L	10–100 ppb	
14	UV-vis spectroscopy	Thiophene-2-carboxaldehyde Thiosemicarbazone impregnated with alumina		0.1–1.0 mg/L	
15	UV-vis spectroscopy and colorimetric detection	LS modified AuNPs	2 µg/L	5–500 µg/L	Shrivas et al. (2015)
16	UV-vis spectroscopy	AuNPs functionalized with GSH-DDT-CYs-PDCA	2.5 µg/L	2–20 µg/L	Domínguez-González et al. (2014)
17	Colorimetric detection and UV-vis spectroscopy	AuNPs	10 µg/L	10–200 µg/L	Sharma et al. (2019)
18	Colorimetric detection and UV-vis spectroscopy	PEG functionalized AuNPs	5 ppb	5–20 ppb	Mohan et al. (2019)

(continued)

Table 16.3 (continued)

S. No.	Methodology	Electrode/reference material	Limit of detection	Range	References
19	UV-vis spectroscopy and colorimetric detection	PHTH	0.35×10^{-6} M	0–240 mM	Deepa and Padmini (2019)
20	Colorimetric detection and UV-vis spectroscopy	DTT-AuNRs	38 nM	0.13–10.01 μM	Ge et al. (2018)
21	Colorimetric detection and UV-vis spectroscopy	Rano-Cu NPs	1.6×10^{-8} M	3.0×10^{-7} to 8.3×10^{-6} M	Laghari et al. (2019)
22	Colorimetric detection and UV-vis spectroscopic	GNP-MMT@Eu	10 ppb	1−1000 ppb	Nath et al. (2018)

Conjugation of AuNP with sugar is also employed for detection of As(III) due to their excellent chemical stability, extinction coefficient, and Surface Plasmon Resonance with sucrose (mostly by capping). AuNPs/sucrose can detect As(III) within the linear range 10–800 μg/L with LOD of 4 μg/l (Shrivas et al. 2020) and glucose offers a linear range of 1–14 ppb with a remarkable LOD of 0.53 ppb via rapid color change from red to bluish possessing (Boruah et al. 2019). Polyethylene gylocol (PEG) functionalized AuNPs has been found to detect As(III) within 5–20 ppb and LOD upto 5 ppb (Mohan et al. 2019).

Protein or peptide-bound AuNP is also exploited to quantify As by the visible color change. Lakatos et al. have functionalized AuNP by a protein oligomer of *Lysinibacillus sphaericus* for colorimetric sensing of As(V) linearly in the range of 0.17 μM–170 mM, sensitivity up to 24 ppb and LOD of 50 nM (Lakatos et al. 2015). Similarly, naked-eye detection of As(III) through AuNP functionalized with phytochelatin-like peptide (g-Glu-Cys)3-Gly-Arg has been observed. As(III) prevents the peptide from affixing to the surface of AuNPs by coordinating to all the three Cys and has triggered color change due to AuNPs aggregation resulting in ultralow detection range (0.04–1.2 μM) and LOD (2 nM) (Xia et al. 2012).

Colorimetric sensing system based on Fe_3O_4 NP/MB/H_2O_2 can detect As(V) in the range of 0–1 μM with LOD (0.358 nM) monitored at 664 nm due to Fenton-like catalytic activity of Fe_3O_4 NP, oxidizing methylene blue to bring color change at low pH (Babu Christus et al. 2018).

Another nanoparticle that has been utilized for the detection of As sensing is lead (Pb) due to its high optical sensing properties showing LOD close to 4.5 ppb and detection within linear range 10–100 ppb (Priyanka et al. 2017).

16.3.2 *Mass Spectroscopic Techniques*

Mass spectrometric analysis is a technique for measuring the mass/charge ratio (m/z) of different molecules present in a test sample and is often employed to determine the exact molecular weight of each component in the sample. Inductively coupled plasma (ICP) technique is most widely applied method integrated and automated for speciation analysis of As species at a higher level in diverse matrices (Liévremont et al. 2009; Tomlinson et al. 1995). ICP tool utilizes the plasma to ionize segments, whereby the sample is treated with acid and splashed into the plasma. High temperature of the plasma atomizes and ionizes all types of arsenic so the reaction doesn't fluctuate with species as in the more conventional often, ICP has been used by coupling with different analytical techniques, including MS (Gao et al. 2015; Johannesson et al. 1996) and atomic emission spectroscopy (AES) (Ryssel et al. 1993), with inclusion of ICP exterminates the preparation time of samples.

ICP-AES is a rarely utilized method and is employed for highly precise comparison of a multi-component sample. In contrast, the ICP-MS technique is one of the most widely employed analytical procedures for arsenic detection. Several reports on the analysis of arsenic in water have appeared (Townsend et al. 2001; Goossens et al.

1993; Stroh and Völlkopf 1993; Bolea-Fernandez et al. 2015; Huang et al. 2011; Liu et al. 2013). The main benefit of ICP-MS is that the analysis of the isotopes is possible with very high precision. The only limitation of this method is presence of a direct nebulizer causing the interference due to chloride ions formed by argon chloride in plasma having a similar mass like As (Sheppard et al. 1992). To overcome this issue sample introduction by electrothermal vaporization (ETV) have been achieved (Gao et al. 2015; Liu et al. 2015b).

Though this method is highly efficient, however in real sample analysis where the concentration of arsenic is low, the sensitivity is not up to mark due to poor ionization of ICP. The above issue is circumvented using ICP-MS coupled with hydride generation (Klaue and Blum 1999). Matousek et al. have registered an ultrasensitive technique for arsenic (As) species chemical analysis (both trivalent and pentavalent species) based on selective hydride generation (HG) (Matoušek et al. 2013) with preconcentration by cryo-trapping (CT) and ICP-MS with LOD of 3.4, 0.06, 0.14 and 0.10 pg mL^{-1}, respectively. Table 16.4 provides a list of available literature on detection of As using mass spectroscopic technique.

Matoušek et al. (2013) have utilized a continuous-flow HG system devoid of the conventional gas–liquid phase barrier as a model induction device for flow injection ICP-MS analysis to quantify trace amounts of As(III) concurrently with Bi, Sb, and Hg with high resolution. ICP-MS can effectively separate peaks of nearby metals such As and Ar. For the first time, Domínguez-Álvarez (2020) have reported the method of capillary electrophoresis paired with electron spray mass spectrometry for detection of As with a limit of 0.02 $\mu g\ L^{-1}$.

Peng et al. have reported the application of 3-(2-aminoethylamino) propyltriethoxysilane (AAPTS) functionalized with MWCNTs as an adsorbent for detection of As(V) for solid-phase extraction (SPE)-ICP-MS. Further, the ICP-MS coupled with chromatographic technique has been investigated to enhance the analysis power with respect to sensitivity and LOD.

16.3.3 Chromatographic Techniques

Although mass spectrometry has emerged to be an important technique for species analysis of a single element, however, the speciation of multi-element through HPLC or HPLC coupled with ICP/MS is currently the most popular trend.

Based on the physicochemical properties and differences in the anionic character of different arsenic species anion exchange chromatography has been utilized to analyze several As compounds including As(III) and As(V) (eg., thioarsenosugar, (As III), phenylarsenals. As(V) with low pKa values (2.19 and 6.98) is the last compound to be eluted while As(III) being a neutral compound is first to be drained out from the elution column (Liu et al. 2018). To enhance the separation of various As species the process parameters like pH of the mobile phase, partitioning of mobile and stationary phase have been optimized. Due to short retention of As(III) in chromatographic column, it is not identified in presence of other non-retained neutral and the cationic

Table 16.4 List of available literature on detection of As using mass spectroscopic technique

S. No.	Methodology	Matrix	Limit of detection	Range	References
1	Mass spectrometry (ICP-MS)	Gold microwire	0.1 μg/L	0.08–2.0 μg/L	Alves et al. (2011)
2	Mass spectrometry (ICP-MS/MS)	Methyl fluoride as a reaction gas	0.2 ng/L	Not studied	Bolea-Fernandez et al. (2015)
4	Mass spectrometry (ICP-MS)	Buffer/electrolyte	As(III): 0.12 μg/L	0–100 μg/L	Bradley et al. (2011)
5	Mass spectrometry (ICP-MS)	Fe_3O_4-RTIL/SPCE	102.6 ppb	1–10 ppb	Gao et al. (2015)
6	Mass spectrometry (ICP-MS)	Thin film PVG coupled to collision cell ICPMS	3 pg/g	0.01–200 ng/g	Gao et al. (2015)
7	Mass spectroscopy (ICP-MS)	Amino-modified silica coated magnetic nanoparticles (MNPs)	0.21 ng/L	Not mentioned	Huang et al. (2011)
8	Mass spectroscopy (ICP-MS)	Resins-HY-AgCl resin, HY-Fe resin and SBAE resin	0.2 μg/L	0.030–20 μg/L	Issa et al. (2011)
9	Mass spectroscopy (CE-ICP-MS)	Buffer/electrolyte (NaH_2 PO_4 and HBO_3)	279.9 and 22.7 ng/g	20–250 ng/g	Klaue and Blum (1999)
10	Mass spectroscopy (DLLME) combined with ETV-ICP-MS)	DTC chelating agent	2.5 ng/L	0.01–10 μg/L	Liu et al. (2015b)
11	Colorimetric detection	ZIF-8	5 mg/L	0.01–10 μg/L	Parajuli et al. (2018)
12	Mass spectroscopy (liquid chromatography coupled to ICP-MS)	Buffer/electrolyte	0.02 mg/g	20–248 mg As/kg	Pell et al. (2013)
13	Mass spectroscopy (SPE-ICP-MS)	(AAPTS) functionalized multi-wall carbon nanotubes (MWCNTs)	15 ng/L	0.05–100 ng/mL	Peng et al. (2015)

(continued)

Table 16.4 (continued)

S. No.	Methodology	Matrix	Limit of detection	Range	References
14	Mass spectroscopy (ICP-MS, ICP-OES)	Buffer/electrolyte	2.5 μg L^{-1}	0.7–190 μg L^{-1}	Pfeiffer et al. (2015)
15	Mass spectroscopy (ICP-MS)	Field kit (buffer/electrolyte)	4.4 μg/L	0–100 μg/L	Safarzadeh-Amiri et al. (2011)

species. Generally, a higher pH mobile phase is included (Liu et al. 2015a; Peng et al. 2014).

With enhanced analytical power, HPLC coupled with the ICP-MS technique (Saverwyns et al. 1997; Lindemann et al. 2000) can critically separate and quantify individual arsenic species through matching with the available standards. It is observed that the ion-exchange column provides better results (selectivity and separation) than the reverse column analysis. Cation Exchange Chromatography is utilized for the assessment of positively charged As compounds (Falk and Emons 2000; Zhou et al. 2013). Due to direct interaction between analyte and stationary phase, it is less prone to the interference that arises from the co-chromatographed components of the matrix. The retention of the arsenic compounds is reliant on the strength of the cationic charge, for example, a cationic exchange column (PRP-X200) has been used for detection eight different compounds in 15 min (Wolle et al. 2014).

Ion-pair chromatography is selectively used for the separation of both neutral and ionic species. This method utilizes a standard reversed-phase column for the reagents in the mobile phase. Tetra alkyl ammonium (especially tetra alkyl ammonium) is the most frequently used chemicals for the separation of ionic species. For ion-pair separation of cationic and neutral species hexane sulfonic acid and 1-pentene sulfonic acid have been harnessed. Modifiers such as methanol and acetonitrile are also put into the mobile phase for reducing the time of retention. A combination of cationic and anionic methods has been used for the separation of mixed charged As species in 12 min (Klaue and Blum 1999; Liu et al. 2013). Though ion-pair chromatography can detect different types of arsenic compounds, however, presence of ion-pair reagents leads to ion-suppression.

For hydrophilic interaction, LC utilizes aqueous mobile phase and the hydrophilic stationary phase is coated by mobile phase, detection species are retained either through the partitioning from the water layer or on the surface of polar stationary phase. Due to polar stationary phase, this method is suitable for arsenic speciation analysis. Wennrich and coworkers effectively have detected nine different organoarsenic compounds using a zwitterionic column tracked by ICP-MS based detector (Xie et al. 2008).

Apart from the standard materials used as the chromatographic column, several new materials have been used for the detection of arsenic compounds. Graphene oxide (GO) as it contains several available functionalities (carboxylic, hydroxyl, epoxides) along with π-electron backbone serves as an efficient and highly adsorbent material. Nanomaterials have unique assets relative to their bulk counterpart which reveal their beneficial characteristics as a stationary phase. Cheng et al. have utilized graphene and graphene derivative as a stationary phase in an HPLC-ICP-MS technique to speciate As(III), As(V), MMA, and DMA along with several other compounds (Xie et al. 2008). The ion-pair reagent forms complex with As species and gets adsorbed on the surface of GO with the attached aromatic species (Sherlala et al. 2018; Xu et al. 2017; Gong et al. 2016). Though GO has proven to be an effective material as a stationary phase, however, the retention mechanism is still not understood clearly. Recently, Fluorinated alkyl columns have been employed for analysis of As compound (Baba et al. 2014; Suda et al. 2016). Boba et al. have demonstrated the separation of five As compounds in five min (Ishikawa et al. 2016). Owing high-level electronegativity of fluorine, a negative charge is attached to the stationary phase. Hence anionic species As(V) move out of the column whereas As(III) is retained due to availability of hydrogen bonds amid hydroxyl group and fluorine. By means of a mobile phase with pH < 3, the fluoride-based column allows the segregation of both cation and anionic species.

16.4 Current Approaches for Monitoring of Arsenic

Though the contemporary analysis techniques (e.g., HPLC, GC-MS, UV-vis) as discussed in earlier section are highly sensitive and precise they often require sample pre-treatment, skilled personnel, and are expensive too. Furthermore, these techniques are not appropriate for urgent action monitoring applications where quick results are required in the field or on-the-spot analysis. Simple and portable biosensors are the best alternative for this and are potentially applicable for their online practice (Justino et al. 2017). Biosensor is an analytical device developed by the integration of bio-element and a transducer. The transducer reads the signal, as current or voltage is the gradient of electrochemical transducers, while intensity, absorbance, or reflectance of fluorescence are a gradient of optical transducers, and change in frequency is the gradient of acoustic transducers (Justino et al. 2017). The advanced biosensors are compact, cost-effective, and disposable. There have been majorly two types of biosensor microbial fuel cell (MFC) biosensor and nanomaterial-based biosensor developed for the measurement and treatment of heavy metals in water (Arya et al. 2011; Koedrith et al. 2015).

16.4.1 Microbial Fuel Cell (MFC) Based Biosensor

A new category of biosensor relied on the microbial fuel cell (MFC) is in trend for the detection as well as treatment of poisonous materials in water (Patil et al.

2010; Kumlanghan et al. 2007; Kim et al. 2003). MFC biosensor is a device where a biological organism (bacteria) converts chemical energy into electrical energy. Figure 16.4a shows the MFC biosensor, it encompasses two chambers divided by an ion-exchange membrane. Bacteria grow on the anode in one chamber, it oxidizes the organic material and produces electrons. While in other chambers, electrodes act as a cathode and accept the electron. The potential difference between electron donor and electron acceptor provides energy to the microorganisms. The MFC biosensors are cost-effective, easy to operate, sensitive, and reproducible can be monitored continuously. The advantage of the MFC biosensor over other biosensors is, it consists one-step process to generate the signal and read it (Fig. 16.4b). Moreover, no genetically modified microorganism is required, only natural microorganisms are used (Logan et al. 2006). The main emphasis of MFC research is focused on chemical production, energy, and nutrient recovery in waste water. Still more specific research is required to design and control their operation, also the effect of these issues (Hasan et al. 2005) on sensitivity, robustness, and reproducibility of the sensor. Research on microbial fuel cell-based biosensors describes mostly the use of batch tests (Kim et al. 2003, 2007). A microbial fuel cell has been fabricated with biofilms of Enterobacter cloacae grown over anode for sensing of As due to reduction in current with a detection range (46 μM for arsenate and 4.4 μM for arsenite lead to decrease in power output as sensor is self-powered (Rasmussen and Minteer 2015). A bioelectrochemical system (BESs) designed over genetically engineered strain *Shewanella oneidensis* has exhibited an increased current response in presence of As with a detection range of ~ 40–100 μM (Webster et al. 2014).

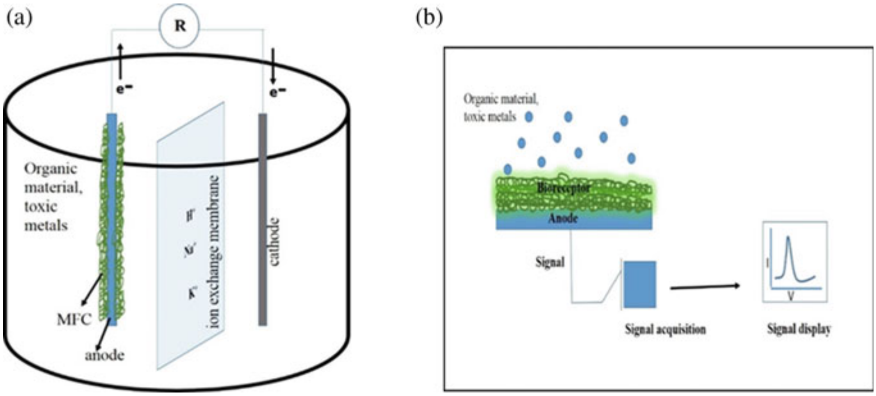

Fig. 16.4 **a** Dual chamber MFC biosensor **b** scheme of MFC biosensor

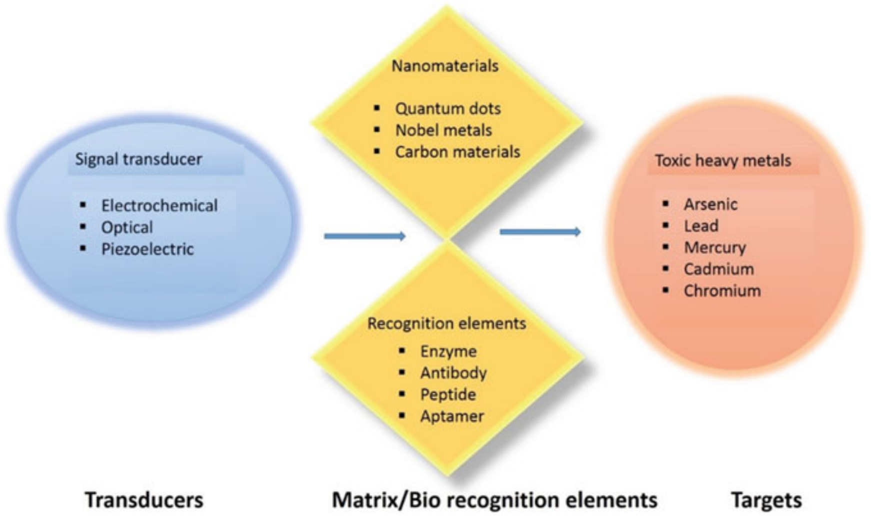

Fig. 16.5 Nano biosensor consists of transducer, nanomaterial, and biorecognition element for their targets (toxic heavy metals)

16.4.2 Transducer Based Biosensors for Heavy Metal Detection

Integration of nanoparticles into biosensors provides a platform for rapid selective and sensitive detection. The nanoparticles consist of high surface-to-volume ratios and confinement of electrons at the nanoscale. The biosensor is a device with two main parts a biorecognition element and a signal transducer (Geppert 1988).

Based on the transducer, biosensors are divided into three main types: electrochemical, optical, and piezoelectric. A nano biosensor consists of a transducer, nanomaterial, and biorecognition element for their targets (toxic heavy metals) as shown in Fig. 16.5.

16.4.2.1 Electrochemical Biosensors

Electrochemical biosensors have attracted more attention due to their low cost, sensitivity, and portable behavior. It works on the concept of electron transfer between electrode surface and analyte that causes the change in voltage or is currently recorded as voltammogram. The sensitivity of the electrochemical sensor majorly is affected by the selection of nanomaterial as well as biorecognition elements (enzyme, antibody, peptide, etc.) (Vo-Dinh 2008). Metal nanoparticles (Au, Fe) (Hossain et al. 2008) and carbon nanoparticles (Duoc et al. 2020) (carbon nanotubes, graphene, etc.) have the intrinsic property of high conductivity and electrochemical stability therefore often used for the detection of hazardous materials (Pb, As, Hg, etc.) in wastewater (Hara and Singh 2021; Martínez-Huitle and Ferro 2006). The enzyme-based electrochemical sensors are the most common, generally, peroxidase, glucose oxidase, tyrosinase, and urease have been used for the detection of hazardous materials (Bachan Upadhyay and Verma 2012; Maleki et al. 2017; Moyo et al. 2014; Rao et al. 2014). Recently researchers are more inclined toward aptasensors, because of strong reliable interaction with hazardous metals or metal ions (Zhang et al. 2018; Hayat and Marty 2014).

Integration of nanomaterials in biosensors becomes an advantage to improve its sensitivity (Lan et al. 2012; Chen and Huang 2014; Jimana et al. 2018; Song et al. 2018; Nguyen et al. 2018; Chen et al. 2020). Table 16.5 provides a list of various electrochemical sensors fabricated for arsenic detection.

Nanostructured gold (AuNP) has been extensively used for low-level sensing of As (Wu et al. 2014; Chen and Huang 2014; Yang et al. 2016a). Babar et al. have developed electro-generated nanotextured gold assemblage-based electrodes using Au foil via electro for ultralow detection of 0.08 ppb of As(III) in water (Babar et al. 2019). Rehman et al. have introduced polycrystalline multi-walled carbon nanotube (MWCNT) over Au electrode to detect the heavy metals in industrial waste with detection limit of 0.28 ppb.

A functionalized reduced graphene oxide (rGO) based nano biosensor has been fabricated for multi-detection of metal ions in industrial wastewater using L-cysteine as a biorecognition element (Ganjali et al. 2011). A LOD of 5 ppt has been reported by Mardegan et al. using gold nanoelectrode ensembles (GNEEs). Chen et al. have demonstrated a sensing mechanism using the synergy effect of various sulfhydryl compounds and attained a LOD of 0.5 ppb. Voltammetric co-detection of As and Cu has been reported for alkaline Britton-Robinson buffer system using carbon SPE modified with gold nanostars validated with 87.2% recovery (Sullivan et al. 2020). A mercaptoethylamine modified Au electrode for LOD of 0.02 μgL^{-1} with a preconcentration time as 100 s under optimized conditions has been provided (Li et al. 2012).

Electrochemical assays relied on self-assembled monolayers (SAMs) (Chen et al. 2011a; Long and Nagaosa 2008) have been developed for the highly sensitive determination of As(III) in water samples. The combination of glutathione (GSH), dithiothreitol (DTT), and N-acetyl-L-cysteine (NAC) mixed SAMs improved the detection specificity and sensitivity due to immobilization of a huge number of arsenic moieties over the surface of gold electrode coupling with As–O and As–S linkages. Fuletra et al. have reported the fabrication of an electrochemical sensor based on template-free growth of gold nanoisland (AuNis) over ITO surface integrated with 1, 4-dithiothreitol (DTT) as illustrated in Fig. 16.6. A bilayer assembly of Au seed (Auseed) was obtained by insertion of a di-thiol (1,12-dodecanedithiol (DTH)) in between two nano gold seed layers resulting in the formation of nano-islands (AuNis) on the surface. As compared to colloidal monolayers, metal AuNis are directly grafted on ITO substrate for trace level detection of As(III) in water within detection range 0.1–100 ppb with ultralow LOD of 0.085 ppb with minimum interference from other heavy metal ions.

Table 16.5 List of various sensors fabricated for detection of As

S. No.	Working electrode	Linear range (ppb)	LOD (ppb)	References
1	GSH/DTT/NAC Au	3–100	0.5	Chen et al. (2011a)
2	ChOx/DWCNTs-Gr/SPE	1–10	0.287	Duoc et al. (2020)
3	NPG/ITO	0.1–50	0.054	Chen et al. (2020)
4	IIP/NPG/GE	2.0×10^{-11} to 9.0×10^{-9} M	7.1×10^{-12} M	Ma et al. (2020)
5	Fe-Chitosan	0–7.5	1.12	Hwang et al. (2019)
6	Bismuth-film graphite	0.0200–18.0	0.012	Long and Nagaosa (2008)
7	Bismuth-modified EG electrodes	20–100	5	Ndlovu et al. (2014)
8	PANI/GC	–	0.4	Yang et al. (2010)
9	nano-Au/GC		1.8	Hossain et al. (2008)
10	Mn_2O_3/CeO_2 nanocube modified gold		3.35	Ren et al. (2018)
11	Eu-MGO/Au@MWCNT	0.9–100	0.27	Roy et al. (2016)
12	Gold nanostar modified CSPE	0–100	2.9	Sullivan et al. (2020)
13	Mercaptoethylamine modified Au electrode (MEA/Au)	0.2–300	0.2–300	Li et al. (2012)
14	Au–Pd NPs/GCE	1–25	0.25	Lan et al. (2012)
15	np–Au modified GCE	0.5–15	0.137	Yang et al. (2016a)
16	MnOx/AuNPs	0.5–18 18–80	0.057	Wu et al. (2014)
17	Au-nanoparticle-embedded Nafion (NF(Au nano)) composite	0.1–12.0	0.047	Huang and Chen (2013)
18	Aunano@GCE	0.1–15.0	0.0025	Chen and Huang (2014)
19	Au-IrM	$10–50 \times 10^8$ $1–10 \times 10^8$	5×10^8	Touilloux et al. (2015)

(continued)

Table 16.5 (continued)

S. No.	Working electrode	Linear range (ppb)	LOD (ppb)	References
20	AuNPs/α-MnO_2 nanocomposite	0.5–50 ppb	0.019	Yang et al. (2016a)
21 ara>	Nanoelectrode ensembles (NEEs)		0.005	Mardegan et al. (2012)
22	Au/Fe_3O_4 SPCE	0.1–100	0.0215	Li et al. (2018)
23	AuNPs/CeO_2eZrO_2	0.5–15	0.137	Yang et al. (2018)
24	Graphene oxide decorated gold microelectrode	1–10	0.162	Yang et al. (2017)
25	Au–Cu bimetallic nanoparticles	10–100	2.09	Yang et al. (2016a)
26	Hanging mercury drop electrode	10–100	0.2	De Carvalho et al. (2006)
27	Cobalt nanoparticle/reduced graphene oxide modified exfoliated graphite	1–50	0.31	Jimana et al. (2018)
28	DTT/AuNis/DTH/Auseed/APTMS/ITO	0.1–100	0.085	Fuletra et al. (2021)
31	Ruthenium(II)-tris(bipyridyl), graphene oxide	0.05–0.8 μM	0.03 μM	Gumpu et al. (2018)
32	Ruthenium(II) bipyridine-graphene oxide (GO) complex	0.1–1.2 μM	2.3 nM	Gumpu et al. (2017)
33	Ag	Upto 30 ppb	1 ppb	Kim et al. (2013)
34	Au	0.94–3.75 ppb	0.8 ppb	Liang et al. (2013)

Uniform assembly of AuNP dispersed over the porous cobaltosic oxide (Co_3O_4) microsheets (AuNPs/Co_3O_4 nanocomposites) over the carbon-based SPE has been used for detection of As(III) using square wave anodic stripping voltammetry (SWASV) with an ultra-high sensitivity of 12.1 ± 0.2 μA ppb^{-1} and a LOD of 0.09 ppb (Li et al. 2021).

A dumbbell-like Au/Fe_3O_4 nanoparticles-based screen-printed carbon electrode demonstrating excellent sensitivity of 9.43 μA ppb^{-1} and LOD of 0.0215 ppb has been reported (Li et al. 2018). A novel As(III) sensor has been fabricated by co-electrodepositing a Fe-Chitosan composite over carbon SPE with the LOD of 1.12 ppb for mining wastewater (Hwang et al. 2019).

De Carvalho et al. have reported adsorptive stripping voltammetric method of As(III) detection using hanging mercury drop electrode (HMDE) in the presence of sodium diethyl dithiocarbamate (SDDC) by adsorptive deposition of an As(III) complex with SDDC with LOD of 0.3 $\mu g\ L^{-1}$ in water (de Carvalho et al. 2006).

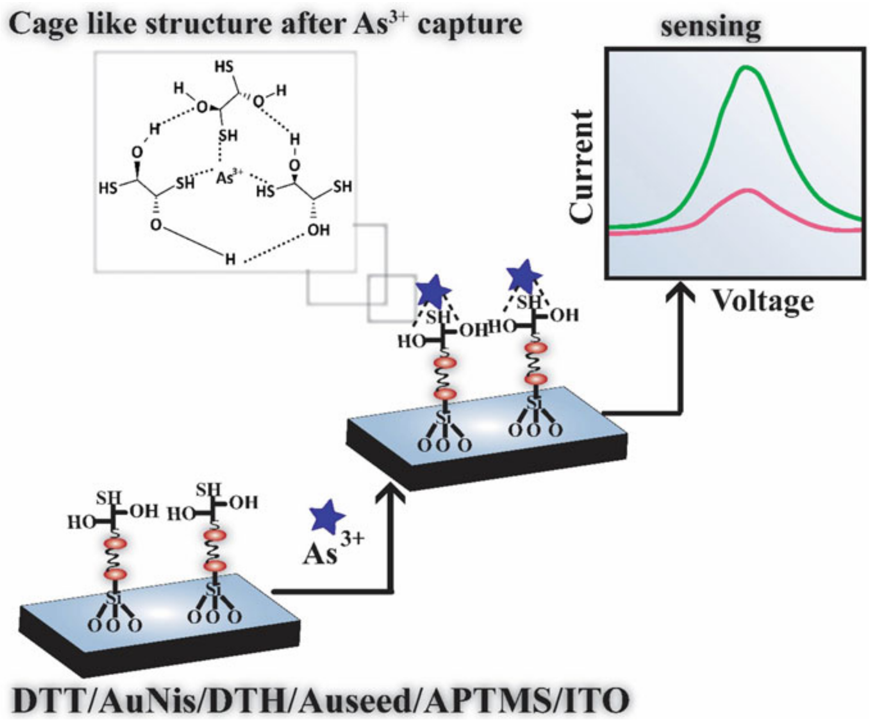

Fig. 16.6 Detection of As ions by DTT/gold nanoisland(AuNis) based novel platform (Reprinted from Fuletra et al. 2021)

Hetro-nanostructures of Ceria cubes (Ren et al. 2018) decorated with manganese oxide nanoparticles (Mn_2O_3/CeO_2 nanocubes) have been made to alter Au electrode for detection of As with LOD of 3.35 ppb.

16.4.2.2 Piezoelectric Biosensor

Piezoelectric biosensors are mass-dependent and work on changes in frequency due to changes in mass over the piezoelectric material. There have been several natural and synthetic materials that exhibit piezoelectric properties among these, quartz crystal (Monošík et al. 2012; Pohanka 2017) is used frequently. Piezoelectric biosensors are very simple, cost-effective, and sensitive techniques to detect the waste material at ppb level in polluted water. Chen et al. have fabricated an AuNPs functionalized DNA-relied piezoelectric biosensor for sensing of Hg^{2+}. On increasing its concentration, a specific T–Hg^{2+}–T hairpin complex was formed with subsequent elimination of AuNPs resulting in a decrease in frequency (Chen et al. 2011b). Similarly, Teh et al. have detected Pb^{2+} ions by using DNAzyme-based QCM-D biosensor (Teh et al. 2014). Asadnia et al. have modified AlGaN/GaN transistor-based sensor with poly(vinyl chloride) (PVC). PVC acts as an ionophore and is able to detect metal ions in polluted water (Asadnia et al. 2016). Piezoelectric devices are sensitive to surface charge and reference electrodes are not required like other ion sensors.

16.4.2.3 Optical Biosensor

Optical biosensors are orchestrated on the alternation in light intensity as well as wavelength due to the interaction of the biorecognition element and the target analyte, which relates with the amount of analyte present. Based on the function of light with analyte concentration optical biosensors are categorized. Table 16.6 provides an overview of various optical sensors available for detection of As.

Absorption-based biosensors working on absorption property of a particular analyte at a certain wavelength, these are simple, portable, and cost-effective devices (Chen et al. 2011b; Shahat et al. 2018; Siangproh et al. 2016). AuNP conjugated with 2-mercapto-4-methyl-5-thiazoleacetic acid (MMT) coupled with europium chloride ($EuCl_3$) based sensor shows a visible color change to detect both As(III) and As(V) ions in an aqueous medium, with a detection limit of $\leq$ 10.0 ppb (Nath et al. 2018). A colorimetric dip strip-based sensor designed by encapsulation of ammonium molybdate in a PAM–PVA hydrogel can detect As(V) with LOD (10 μg L^{-1}) in water (Das and Sarkar 2016). Paper-based analytic devices relied on silver nitrate can sense total As content in water within a threshold limit of 7 ng mL^{-1} (Pena-Pereira et al. 2018). Dimercapto-succinic acid-functionalized Aunanorod entrusted paper strip-based sensor can detect both As(III) and As(V) using spectroscopic analysis within a detection limit of 10 ppb in groundwater (Priyadarshni et al. 2018).

Surface plasmon resonance (SPR) biosensors are based on electromagnetic waves (plasmons), which are generated due to the interaction of metal surface and amplified incident light. The results measured by the variation in the refractive index of the material on the metal surface, due to specific binding of the analyte and biomolecule (Teh et al. 2014; Asadnia et al. 2016). Synergistic molecular assembly of aptamer and CTAB over AuNPs based platform can detect As(III) in water by visual colorimetric recognition within broad linear range (1.0–100 ppb) and LOD (16.9 ppb) within 30 min (Thao Nguyen et al. 2018). Ranolazine-derived copper NP (Rano-Cu NPS) can detect As(III) in the linear range of 3.0×10^{-7} to 8.3×10^{-6} M and LDL of 1.6×10^{-8} (Laghari et al. 2019). A smartphone-based microfluidic device harnessing the plasmonic resonance property of AuNP conjugated over a dual microchannel PDMS kit can simultaneously trace As(III) (710–1278 mg L^{-1}) and mercury ions (10.77–53.86 mg L^{-1}), respectively.

Fluorescence-based optical biosensors can capture frequency change of electromagnetic radiation emitted by the analyte. The detection is mostly carried out with fluorescence labels or fluorescence energy transfer (FRET). Wu et al. have designed a quantum dots (QDs) labeled DNAzymes nano biosensor for multiple detections of metal ions in wastewater, using two quenchers. The nano biosensor designed on fluorescence resonance energy transfer (FRET) can improve the specificity and selectivity because of the high photostability of materials. The synthesized CdSe

Table 16.6 Provides an overview of various optical sensors available for detection of As

S. No.	Transducer	Matrix	Linear range	LOD	References
1	Fluorescence sensor	Triple-helix molecular switch	0.01 ng/ml–0.01 mg/ml	0.005 ng/ml	Pan et al. (2018)
2	Colorimetric biosensor	AuNPs/aptamer/PDDA	5–3000 ppb	5.3 ppb	Wu et al. (2012b)
3	Aptasensor	AuNPs/G-T-rich DNA	5–2000 ppb	2 ppb	Liang et al. (2013)
4	Aptasensor	AuNPs/aptamer	1.26–200 ppb	1.26 ppb	Yu (2014)
5	Aptasensor	AuNPs/aptamer/CTAB	1–1500 ppb	0.6 ppb	Wu et al. (2012a)
6	Colorimetric sensor	GSH/DTT/Cys-modified AuNPs	–	1	Kalluri et al. (2009)
7	Colorimetric sensor	LS-modified AuNPs	5–500 ppb	2 ppb	Shrivas et al. (2015)
8	Colorimetric sensor	GNP-MMT@Eu	–	10 ppb	Nath et al. (2018)
9	Colorimetric sensor	PEG-modified AuNPs	5–20 ppb	5 ppb	Boruah and Biswas (2018)
10	Colorimetric sensor	GNR-PEG-DMSA	1–10,000 ppb	1 ppb	Priyadarshni et al. (2018)
11	Optical sensor	GSH/DTT/Cys/PDCA-modified AuNPs	2–20 ppb	2.5 ppb	Domínguez-González et al. (2014)
12	Colorimetric sensor	GSH/DTT/Asn-modified AgNPs	0.4–20 ppb	0.36 ppb	Wen et al. (2018)
13	Colorimetric sensor	Ionic liquid-modified AuNPs	–	7.5 ppb	Tan et al. (2014)
14	Surface-enhanced Raman scattering (SERS) sensor	AgNP functionalized with glutathione/4-mercaptopyridine PDMS	3–200 ppb	0.67 ppb	Qi et al. (2014)
15	Fluorescence sensor	Fluorescence Bioreporter cell *E. coli* PDMS	–	50 µg/L	Theytaz et al. (2009)
16	Fluorescence sensor	Fluorescence Bioreporter cell *E. coli* PDMS		10 µg/L	Truffer et al. (2014)

(continued)

Table 16.6 (continued)

S. No.	Transducer	Matrix	Linear range	LOD	References
17	Chemiluminescence sensor	Luminol and Vanado-molybdoarsenate heteropoly acid	1.0×10^{-7} to 5.0×10^{-5} M	$8.9 \times 10 - 8$ M	Som-aum et al. (2008)
18	Functionalized nanoparticles	Functionalized AuNP		1.0 ppb	Nath et al. (2014)
19	Colorimetric	Polyethylene Terephthalate/Ethylene–Vinyl Alcohol Copolymer/Polyester	5–20 µg/L	1 µg/L	Bonyár et al. (2017)
20	Direct assay	*Enterobacter cloaceae*	–		Rasmussen and Minteer (2015)
21	Direct flow-through	*Shewanella oneidensis*	–		Webster et al. (2014)
22	PL quenching	C-Au-BSA	0–50 ppb	0.004 ppb	Babu and Doble (2018)
23	Fluorescence spectroscopy	MPC/FAM-ssDNA	0–15 nM	630 pM	Muppidathi et al. (2019)
24	Fluorescence spectroscopy	CeO_2 nanowire-BODIPY-ATP	20–150 nM and 150–1000 nM	7.8 nM	Tong et al. (2018)
25	Fluorescence spectroscopy	Fe-GQDs	5–100 ppb	5.1 ppb	Pathan et al. (2019)
26	Fluorescence spectroscopy	L-cysteine capped CdTe Quantum dot	2.0 nM–0.5 µM	2.0 nM	Vaishanav et al. (2017)
27	Flouroscence spectroscopy	Glutathione (GSH) was passivated on the surface of the CDs	2–12 nM	2.3 nM	Yang et al. (2016a)
28	Spectroscopic detection	Aptamer and cetyltrimethylammonium Bromide (CTAB) on gold nanoparticles (AuNPs)	1–100 ppb	16.9 ppb	Thao Nguyen et al. (2018)

QDs has exhibited different fluorescence intensities for various metal ion (Asadnia et al. 2016). GSH-Carbon dots-based multifunctional sensor can detect both As(III) (2–12 nM) and hypochlorite ions (10–90 mM) in drinking water (Radhakrishnan and Panneerselvam 2018). A hydrazine-thiocarbamide probe can detect phosphate ($PO4^{3-}$) and $AsO_3{}^{3-}$ by colorimetric and turn-on fluorometric method with ultralow detection limit (15 nM) (Purkait et al. 2018).

Luminescence-based biosensors are further classified as chemiluminescent and bioluminescent. The signal generates because of the excited state of the target analyte upon exothermic chemical reaction, which emits the light when returns to the ground state. In bioluminescence, the chemical reaction takes place within the bio-organism. Several bioluminescence sensors have been developed based on microbes for very low detection of heavy metals (Jouanneau et al. 2012; Martín-Betancor et al. 2015). The recombinant *Escherichia coli* strains have been adopted due to their fluorescent protein expression, when *E. coli* comes in contact with heavy metals fluorescence expression enhances (Ravikumar et al. 2012; Raja and Selvam 2011). A NIR-emitting inner filter effect (IFE) probe can detect arsenic(III) within detection range (0.067–13.4 μmol L^{-1}), limit of detection (55 nmol L^{-1}) and relative standard deviations (RSD) is 2.1% (Ge et al. 2019).

Enzymatic biosensors work on inhibition effect in presence of heavy metals, some nanomaterials with optical properties have been used to enhance the sensitivity of enzyme biosensors (Biswas et al. 2017). The optical biosensors have advantage of qualitative estimation by naked eye. Synthetic aptamers provide sensitive and selective aptasensors in combination with Au or Ag nanoparticles (Wu et al. 2013; Song et al. 2016; Tan et al. 2016; Yang et al. 2019). Aptamer conjugated silver nanoparticles (Apt-SNPs) based sensor can detect As(III) within the detection range 50–700 μg L^{-1} and LOD (6 μg L^{-1}) (Divsar et al. 2015).

There have been continuous developments for two decades to mend the several features of biosensors. Though, cost-effectiveness, easy handling, portability, and on-field use are still major challenges to solve. Usually, in wastewater heavy metals form complexes with organic materials, and also some organic material interferes, the signal during sensing.

16.5 Commercially Available Sensors for Arsenic Detection

Despite the various conventional approaches and nano-biosensing platforms available for the detection of arsenic in water, developing nations where arsenic contamination is a major issue cannot afford the sophisticated instruments and the large skilled set of workers for analysis. In few instances sampling sites are located at a very far away locations and the logistics for the sampling is not appropriate for timely collection and analysis. Hence, for the real-time analysis and on-site detection, a field-deployable method is required. Considering these factors various commercially available kits have been designed for rapid and field testing.

Most of these kits are relied on over-colorimetric detection of arsenic. Earlier developed test kits are relied on the traditional "Gutzeit" method and its variations. It involves the treatment of the sample with the reducing agent (addition of zinc powder) converting the arsenic compounds present in the water into arsine trihydride (also termed as arsine gas) as depicted in the following equation

$$As(OH)_3 + 3Zn + 6HCl \rightarrow AsH_3 + 3ZnCl_2 + 3H_2O$$
$$H_3AsO_4 + 4Zn + 8HCl \rightarrow AsH_3 + 4ZnCl_2 + 4H_2O$$

The gas diffuses through the sample and gets exposed to a paper imbued with the solution of mercuric bromide and produces a highly colored compound. The intensity of the color is used for determining the As concentration, while As generation coupled to complex formation gives a degree of selectivity. The color generated is commonly determined by comparison with a color chart mainly used for the semiquantitative measurements, or by the attachment with a simple spectrophotometer, especially when colored complexes are formed. When more arsine is formed, a darker color appears, suggesting the formation of a different complex, although its structure is unknown. The coloring developed is roughly proportional to the amount of arsine produced. Though this method is simple and inexpensive this assay has a limitation of interference from tellurium, selenium, and sulfur compounds. However, a major As contamination crisis has occurred in Bangladesh in 1997 where the field test kits were at test and compared with the accepted laboratory methods and most of the kits available have shown false positive and negative results and most of the kits developed have been generating poisonous arsine gas.

Consequently, few other modifications have been made to develop next-generation kits. An earlier modification included the variation of the reducing agent to sodium borohydride. The reaction chamber has been modified to increase the exposure with arsenic, sulfide compounds acting as interferents have been oxidized to sulfates as the preliminary step and electronic readouts as included reduced the chances of error due to manual inspection.

Fe^{2+} and Nickel salts have been added to accelerate this reaction. One of the field test kits based on this concept is the As 75 assay with an LOD upto 10 ppb. Though the problem of sulfide interference has been reduced the test is not benign for the generation of arsine gas that is a potential hazard for the operator and the test strips have been generating mercury solid waste. Another popular low-range As^{+} detection. A Low-Range Test Kit has been developed by Hach USA for detection of total inorganic arsenic in 0–500 ppb range through visual comparison. The kit is easy to handle and appears in the form of doses and interference of sulfide upto 5 ppm is tolerable and the exposure to arsine gas has been minimized. The test kits costs around 180 USD and 100 test samples can be analyzed.

The main challenge in practical usage of these kits is that they require approximately 30 min for the analysis and comparative analysis using the chart questions the accuracy and individual visibility might vary the result. Another disadvantage is that all the kits produce arsine gas that might lead to cancer and presence of hydrogen sulfide may interfere the results. More reliable results can be achieved when the color is read out using an electronic instrument. Akvo Caddisfly Sensor helps to reduce the error that arises due to human interaction while reading/comparing the color with reference chart provided. This sensor uses the smartphone-based application and a color card to find the exact value of the arsenic concentration. The detection can be done using any android Smartphone. A comparative analysis of majorly used commercial test kits is presented in Table 16.7.

Table 16.7 List of commercially available As detection kits

S. No.	Field test kit	Manufacturer	Detection range (µg/L)	Reaction time per test (min)	Cost per box (USD)	Number of tests per kit box	Cost per test
1	Hach EZ (2,822,800)	Hach, Loveland, USA	0–500	21	70	100	0.70
2	LaMotte (4053-02)	LaMotte, Chestertown, USA	4–400	15	208	50	4.16
3	Econo-Quick (481,298)	Industrial Test Systems, Rock Hill, USA	0–1000	15	189	300	0.63
4	Econo-Quick II (481,304)	Industrial Test Systems, Rock Hill, USA	2–100 > 150 > 300	15	158	50	3.15
5	Quick (481,396)	Industrial Test Systems, Rock Hill, USA	0–500 > 500	15	179	100	1.79
6	Quick II (481,303)	Industrial Test Systems, Rock Hill, USA	< 1 2–40 > 50 > 80 > 160	15	231	50	4.62
7	Wagtech (PTH10605)	Palintest, Gateshead, UK	< 10 20–500	21	272	200	1.36
8	Merck (117,917)	Merck, Darmstadt, Germany	5–500	21	202	100	2.02
9	NIPSOM field kit		10–70	10	18	100	1.8

(continued)

Table 16.7 (continued)

S. No.	Field test kit	Manufacturer	Detection range (µg/L)	Reaction time per test (min)	Cost per box (USD)	Number of tests per kit box	Cost per test
10	Palintest arsenator kit PT981		2–100	20		200	

Recently, Reddy et al. have evaluated the functioning of commercially available colorimetric test kits. It has been observed that irrespective of the fact that user-dependent color matching issues arise LaMotte and Quick II kits were both accurate and precise in estimation of arsenic, four kits (Econo-Quick, Quick, Wagtech, and Merck kits) were accurate but not precise. However, two most popular kits (Hach and Econo-Quick II kits) do not show precision and accuracy. It can be concluded that the better quality components of these kits are required for the analysis. Several other comparative studies of the accuracy of these test kits showed that the variation of the reaction condition (e.g., reaction time and temperature) showed different results, e.g., Hach kit on increasing the reaction time (40 min) and temperature 35 °C, however, the manufacturer have provided different operating conditions. Hence stringent mass-scale testing is required for development of better test kits.it is also observed in these studies that most of the kits underestimate the arsenic concentration.

In contrast with spectrometric techniques, electrochemical systems are ideally suited to field determination of arsenic in aqueous media. Owing to their unique advantage offering high sensitivity, selectivity along with low-cost portable instrumentation, low power consumption and feature a unique ability to detect a variety of oxidation states. Voltammetric instruments available for field analysis include a portable system for on-site evaluation (i.e., directly at the collection point), an off-line analyzer for high-throughput samples [a benchtop or in-house system that can be installed in remote areas (i.e., transportable but not portable)], and an online analyzer that performs sampling and analysis for continuous monitoring automatically (e.g., real-time measurement of a wastewater effluent). However, in contrast to colorimetric test kits, very few studies have been done on the reliability of electrochemical field systems for arsenic analysis.

Various electrode materials have been tested, but gold and platinum appear to be the best suited. Environmental Technology Verification (ETV) department of the EPA tested three voltammetric arsenic test kits PDV6000, the Nano-Band Explorer, and the Safeguard system. The PDV6000 is a portable system comprises small analytical cell (electrodes + stirrer) and a handheld potentiostat for field screening of heavy metals. It can be connected to the power supply or a portable battery. It can be coupled with a laptop and specially designed software. The time for analysis is around 30 min.

The Nano-Band Explorer system comprises a WE (array of 100 band electrodes less than 0.5 µm thick), a voltammetric cell, a temperature sensor, an electrode cleaning kit, and a laptop computer, and its costs around $8750 with laptop. The reduction is carried at room temperature within five min. A further improved form (Nano-Band Explorer II) merges the three electrodes (WE, AE, RE) into a single unit (Tritrode electrode). The price of the Explorer II is $8750 without the laptop.

The TraceDetect SafeGuard is a tabletop stream-through system and can be transported to remote areas. It comprises a fluidic (pumps, valves, tubings) system, potentiostat, laptop, and voltammetric cell. The sensor involves an interconnected array of 100 carbon band microelectrodes that are batch-printed on a quartz substrate. The system is designed to detect As over a linear range of 1–100 ppb within 30 min. It can be used for both auto sampling or single analysis. A fully automated online kit OVA5000 provides real-time constant analysis of the arsenic wastewater concentration for 7 days and communicates the results of analysis to an external location. All raw and summary data are accessible and can be archived from the instrument through an intranet or the Internet.

It can be inferred that the existing field testing device ought to be viewed only as a primary mark of potential As contagion. Advantages of such systems are numerous and include quasi-instantaneous understanding of As present, a short response time when facing a contamination problem, and the application of optimized removal procedures (e.g., concentration of coagulant). However, these systems are still in their infancy and few examples can be found where automated online electroanalytical systems provide real-time measurements of arsenic levels in water of interest.

16.6 Conclusion and Future Prospect

Heavy metal ions are essential for human health, however, there exists a borderline permissible concentration beyond that they have a negative impact. They tend to accumulate within vital organs kidney, liver, and Lungs disturbing their normal biological function. Entrance of these heavy metals into human body is through contaminated food and water. Among toxic heavy metals As is naturally present at high concentrations in groundwater bodies and moreover the human activities further become the cause of its elevation. The biggest risk arises from contaminated water when used for drinking posing a great threat to humans. It is documented that at least 140 million people in 50 countries in the world have been drinking water containing As at levels above the WHO provisional guideline (10 μg/L). Adverse health effects have been observed for long-term ingestion of inorganic As including developmental effects, diabetes, pulmonary disease, and cardiovascular disease. As is also associated with pregnancy outcomes including stillbirth, effect on cognitive development, intelligence and Child memory. As has been listed as a potential carcinogen on long-term exposure. All of these have led to precise monitoring of treatment of wastewater to save groundwater.

Apart from the conventional techniques including UV-visible spectroscopy, Mass spectroscopy, and Chromatography Atomic absorption spectroscopy (AAS). Several miniaturized nanomaterial-based sensors (optical, electrochemical, and piezoelectric)/biosensors have been proposed to detect As with improved effectiveness and sensitivity. Though, there is an enormous advancements and innovations these sensors still face challenges due to major limitations that include fluorescence dependence for optical sensor, sophisticated electrode development and dependency of a computerized lab set up for sensing that restricts their real-time applicability for detection of As.

Several Field kits are available commercially; however, these kits require proper validation and can be used for preliminary indication of potential As contamination. Till today, rapid and accurate determination of as in wastewater or groundwater is one of the major translation research concepts.

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Chapter 17
Nanobiosensors and Industrial Wastewater Treatments

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Abstract There have been major advancements in the fields of science and technology, one of them is nanotechnology. Wastewater is a serious issue in society, various methods have been used to tackle this problem. One approach is by use of nanobiosensors are being utilized in the treatment of wastewater because of their advantages like high absorption capability, better membrane filtration, and high monitoring output. Moreover, biosensors are classified into various types which have their associated advantages. This chapter brings an extensive literature collation that gives a systematic understanding of biosensors, nanotechnology, and the utilization of nanobiosensors to manage wastewater treatment with its future aspects.

Keywords Nanotechnology · Wastewater · Biosensors · Treatment · Monitoring · Industrial

17.1 Introduction

Nanomaterial and nanotechnology are the new concentrations in the science field which is utilized across different disciplines (Jiang et al. 2018; Verma et al. 2018,

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2019). Nanotechnology has effectively discovered its application in different areas like computer electronics, correspondence, energy, medication, food sources, and so on. This field is characterized by the formation of materials, instruments, frameworks, etc., useful for managing issues with a 1–100 nm scale (Kuswandi 2018). Nanotechnology alludes to research and innovation improvement at the nuclear, sub-atomic, and macromolecular scale, prompting the controlling influence and examination of designs and nanodevices. Items at this scale, for example, "nanoparticles (NPs)," take on novel properties and capacities that contrast notably from those found in the mass scale (McNeil 2005). Various geometry and pieces are accessible with the use of nanomaterials. The capacity to modify the geometry and the properties of the nanomaterials offer fantastic possibilities to plan to detect frameworks to improve the exhibition of bioanalytical substance examination. Nanomaterials that have unique particle length than the traditional are capable to possess unique scientific and physical sciences phenomenon that is capable to generate materials with unique properties that depend on particle size (Kuswandi 2018). As the size of the construction (sensor) is reduced, the s/v ratio (surface and volume) increases, this decreases in detecting the part size and the transducer present in the sensor further scales down the sensing devices (Sanguansri and Augustin 2006). The conversion of these recently gained capacities, combined with propels in imaging, bioinformatics, and frameworks science (system biology), holds an enormous guarantee for noting a portion of science's most difficult biochemical and genetical demands (McNeil 2005). Today science is likewise profiting by a large group of innovative turns of events, few may ultimately affect fundamental exploration, drug improvement, and clinical medication as nanotechnology (Quintana et al. 2002). Another use of nanotechnology is in nanocompost that can convey the enhancements on-demand while keeping them from imprudently changing over into substance/vaporous constructions that will not be devoured by plants. This is proficient by utilizing nanofertilizers that keep additions from communicating with soil, water, and microorganisms, and delivering additions just when they can be straightforwardly concealed by the plant (DeRosa et al. 2010). NPs can, for example, be utilized as bioactive mixtures in useful food varieties (Chau et al. 2007). Bioactive mixtures that can be discovered normally in specific food varieties have physiological advantages and may assist with diminishing the danger of specific illnesses, including cancer (Sozer and Kokini, 2009). By decreasing molecule size, nanotechnology can add to improve the properties of bioactive mixtures, like conveyance properties, dissolvability, delayed home time in the gastrointestinal lot, and productive ingestion through cells (Chen et al. 2006). Omega 3 and omega 6 unsaturated fats, probiotics, prebiotics, nutrients, and minerals have discovered their applications in food nanotechnology as bioactive mixtures (Watanabe et al. 2005). Other applications of nanostructure materials, like NPs, nanoemulsions, or nanotubules, have been widely used for the advance of new immunomodulatory agents, as such nanostructures can be utilizing to more efficiently manipulate or distribute immunologically active components to objective sites (Smith et al. 2013). Nanotechnology is expanding at a rapid speed and its expansion holds immense potential for beneficial advantages to the growing society. Active research and development have given some of the most promising innovations to the world. Figure 17.1

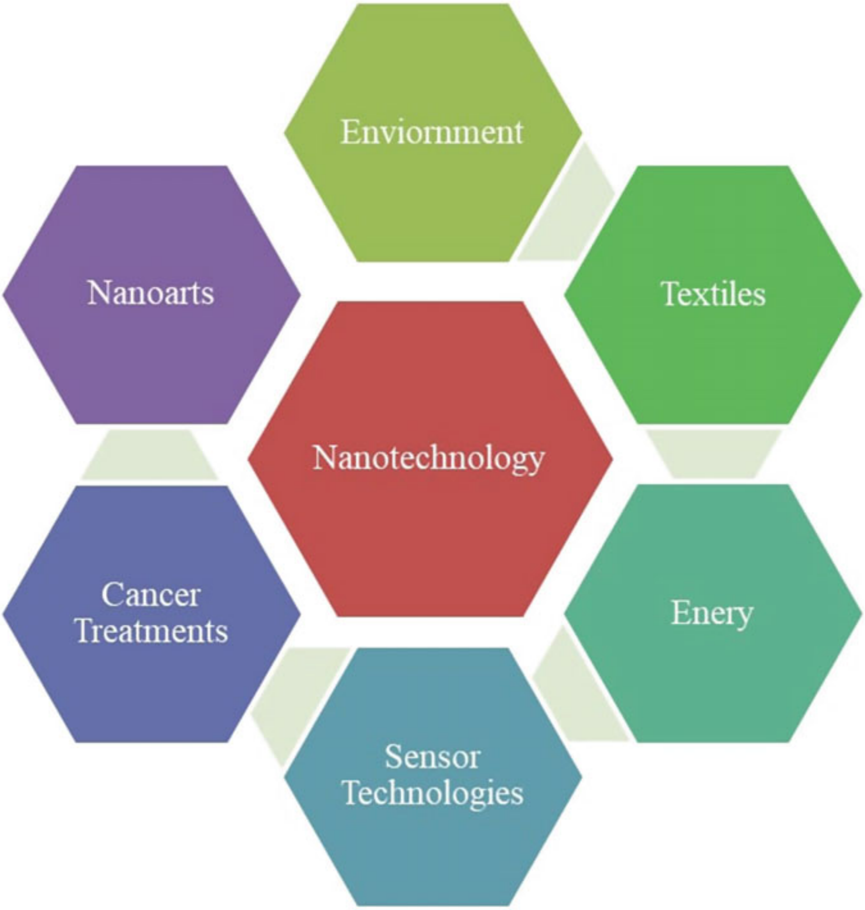

Fig. 17.1 Other areas where nanotechnology is currently utilized

discusses some other areas of nanotechnology where applications and new research are ongoing.

17.2 Nanomaterials for Wastewater Treatment

Water contamination and environmental debasement are the reason for expanding water shortage and decrease in aquatic life (sea). For the past few years, the freshwater quality is reducing, the water demand has kept on expanding especially in territories having parched and semi-bone-dry climates (Ejeian et al. 2018). When the contaminations in effluents of wastewater are monitored, this is the primary step to distinguish contamination territory for decontamination of water. Regular discovery strategies don't comply with following numerous unsafe segments in wastewater because of their fluctuation along with various occasions and sources (Ejeian et al. 2018). Wastewater is made out of various destructive materials that have been antagonistically delivered and released from the household, commercial, factories, or agrarian exercises (Henze et al. 2008; Cornish et al. 1999). They can cause ecological impacts that are unfavorable. For instance, interruption of endocrine and hormones, acceptance of cytotoxicity or potentially genotoxicity, and cancer-causing impacts (Korostynska et al. 2013). The future of wastewater treatment has been revolutionized with Nanotechnology (Jiang et al. 2018). Some examples include rapheme, nanotube, attractive nanoparticle, and silver nanoparticle (Jiang et al. 2018). The introduction of biosensors for the identification of environmental toxins has gotten impressive consideration lately. Such sensors give an extraordinary benefit to recognize the negligible concentration toxin levels in matrices that are complex. These sensors have scaled-down frameworks that empower the advancement of sensors (Tsopela et al. 2016). Two regular boundaries are broadly estimated to examine natural matter convergence of fluid conditions. In the first place, many microorganisms lower the oxygen level to make it capable to be separate naturally biodegradable substances which are termed as biochemical oxygen demand (BOD). It is an estimate of the amount of oxygen that is being utilized for compound responses that are called chemical oxygen demand (COD) (Wacheux 1998). The conventional strategy for in situ evaluations of biological oxygen demand depends on monitoring for five to seven days. These sensors give a lot quicker and exact outcomes for the discovery of these contaminants than traditional strategy (Bourgeois et al. 2001). Substantial metal contaminations in water bodies are generally connected with various sources like industrial waste (effluent discharge, metals processing, etc.), agriculture sources (sludge, pesticides, etc.), and other sources (cosmetics waste, mining, etc.) (Sayari et al. 2005). Life forms rely upon a specific edge level of heavy metals (HMs) like Cu, Ni, Fe, Co, Mn, Zn, Mo, and Se. Some HM like Pb, Cr, Cd, Hg, As, and Sb are harmful and cancer causing, these HM are non-biodegradable. They enter the food chain and get bioaccumulated in the human body and cause toxic manifestations (Jaishankar et al. 2014). Biosensors hold immense potential for customary detecting frameworks because of their high affectability, effortlessness, and recognizable signs (Ejeian et al. 2018). Figure 17.2 other examples of biosensors for detection of heavy metals.

Enormous waterborne microbial illnesses cause remarkable mortality and bleakness worldwide (Henze et al. 2008). Waste (sewage) gives the right development environment to various micro-organisms like protozoa, green growth, parasites, and even harmful infections. Chiefly delivered to wastewater due to anthropogenic and other human habits. (Gerardi 2003). These sensor systems can recognize wastewater microflora, hence becoming an interesting field of research. Other sensors in

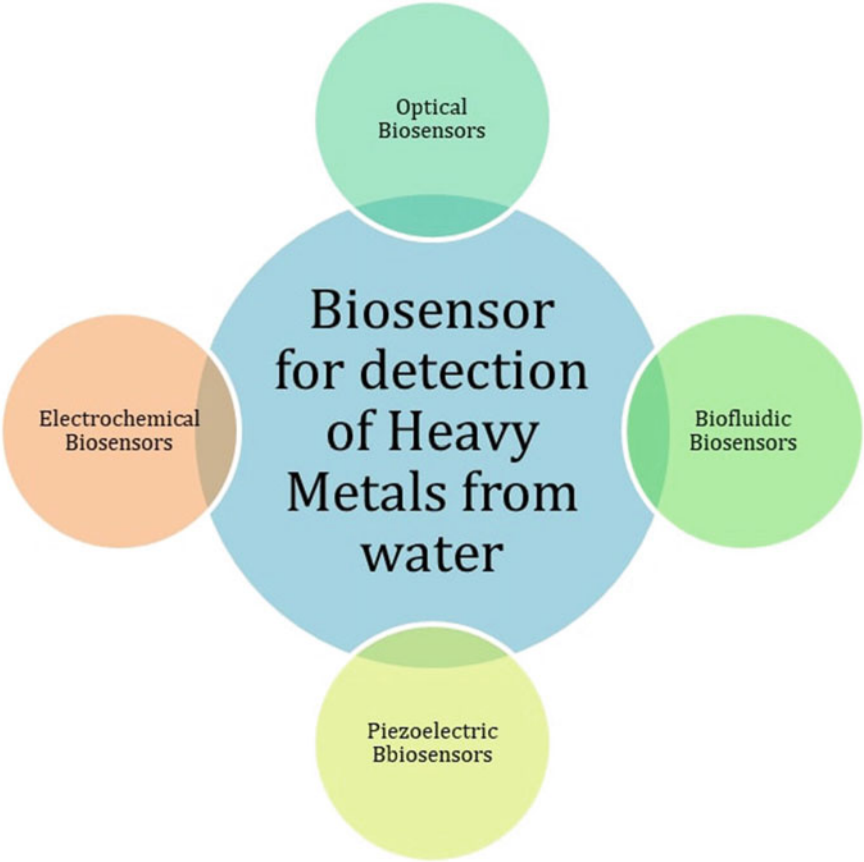

Fig. 17.2 Other examples of biosensor for detection of heavy metals from water

particular the optical biosensors are delicate, to open for continuous observing of poisons, medications, and microorganism microbes in wastewater. Biosensors are likewise intended to amper metrically screening different microorganisms based on their different properties (electrooxidation or electroreduction) which is utilized for water remediation (Bae et al. 2004; Lazcka et al. 2007; Ramírez-Castillo et al. 2015; Kokkinos and Economou 2017; Yu et al. 2017; Rengaraj et al. 2018). Microbial biosensors are insightful devices made out of a microorganism that recognizes an objective substrate and changes based on parameters like physical, electrical, or biochemical aspects for a quantifiable reaction. Another biosensor like the microbial biosensor is capable of detecting and acknowledging instruments for frameworks in terms of electrochemical, optical, and sensory regulated. Guidelines for conducting cell and/or metabolic routes are noticeable because microorganisms not just recognize ecological factors including supplements, temperature, and pH yet, in addition, since their metabolic status (Salouti and Khadivi Derakhshan 2020).

17.2.1 Nano Adsorption and Remediation

The minor dimensions of the sensor empower the reconciliation of a few cycles into a solitary instrument to configuration ongoing checking of different resources, which is valuable for observing the organization of wastewater. Besides, the utilization of nanomaterials positively affects their physical and compound highlights and advances affectability, diminishing examination time, expanding estimation dependability, and showing maximum output discovery (Rodriguez et al. 2006; Kim et al. 2015; Ansari 2017). Nano-biosensors rely upon the selectivity communication of minor biomolecules as biorecognition components and excessive sensitiveness of nanostructures while presented to certain kinds of toxins (Ghasemzadeh et al. 2014). Novel NPs have good photostability, better elimination coefficients, glowing properties with synergist limits (Kaittanis et al. 2010). Adsorption was a favored decision above other water methodologies aimed at its effortlessness inactivity and the comprehensiveness for regular natural and inorganic toxins (Crini 2005). Size-subordinate nanostructures ensured nanomaterials' intrinsic benefits in an equivalent explicit surface territory or dynamic site, which were ancient blocks for traditional adsorbents. Carbon-based nanoadsorbents, normally carbon aerogels (Ling et al. 2011), carbon nanotubes (CNTs) (Wang et al. 2012), graphene (Zhao et al. 2011), and their hybridization states (Yao et. 2012) effective in the treatment of wastewater, and their great affinity towards heavy metals (Jiang et al. 2018). Carbon-based NPs have structure-free totals that diminished powerful surface territory and expanded adsorption energy (Jiang et al. 2018).

17.2.2 Membrane Filtration

Nanomaterials were adding to more proficient water filtration measures. The great energy utilization, lifetime decrease, and separation disappointment direct by film fouling were significant difficulties of layer measures. Changed membranes with practical nanomaterials were viewed as a hopeful chance to confront this problem (Jiang et al. 2018). NPs, for example, alumina (De et al. 2011), silica (Cai et al. 2003), layer hydrophilicity (Bae et al. 2004) was expanded to abstain from fouling. Zeolite NaA NPs inside polyamide layer to shape composite film (Jeong et al. 2007). Also, a critical layer transition upgrade was accomplished contrasted with the basic TFC (slight film nanocomposite layers) layer (Jeong et al. 2007). Multiple polymeric layers-based nano-Ag was capable of contributing towards the development of biofilm (Mauter et al. 2011) and kills infections (De et al. 2011) on the film surface. Other examples include nanomaterials that were based on TiO_2, which depicted useful properties (bimetallic or metallic) impetus to nano-based particles. These were the basic impetus to pollutant debasement, consequently joining them into layers would viably ease build-up retention (example—nano zero-valent iron)

(Jiang et al. 2018). Other instances of film sifting incorporate nanofiber layers and nanocomposite layers.

17.2.3 Nanobiosensing and Monitoring

Water contamination in a very low concentration was triggered by countless manufactured organic mixtures, like PAHs, PBDEs, PCB (Jiang et al. 2018). A significant test for wastewater usage was detecting and distinguishing them quickly and precisely. For some, nanomaterials were great adsorbents; they thought contamination to encounter the identification limit. CNTs have been utilized in genuine water tests for natural mixtures recognition (Cai et al. 2003). Au-TiO_2 nanocomposite showed great straight with following organophosphates (OPs) insect poisons at low levels (1.0 ng/ml) (Qu et al. 2008). Multifunctional nanotube exhibit dependent on TiO_2 was utilized to differentiate herbicide such as dichlorophenoxyacetic ($C_8H_6Cl_2O_3$) corrosive (2, 4-D), methyl-parathion ($C_8H_{10}NO_5PS$), and 4-chlorophenol (ClC_6H_4OH), (Li et al. 2010). Microorganism and infection have likewise been viewed as a drawn-out danger in wastewater. Because of immense surface and volume proportions, nanoparticles can be subjected to biosensors that were on the nanoscale. These biosensors are quick, all-around coordinated with certain microorganisms and infection determination. Quantum specks (Singh et al. 2014), CNTs (Zhan et al. 2014), oxides of graphene-based (Cha et al. 2009), silica (Pang et al. 2015), and metal nanoparticles (Wang et al. 2017) were strong establishment for sensor and discovery innovations.

17.3 Industrial Wastewater Treatment

Biosensors were set up during the 1960s by the innovators Clark and Lyons. Biosensors are characterized dependent on what way the signal is sent from tests to various bunches like electrochemical, optical, temperature, physio-metric, immunochemical, attractive, protein, and DNA base (Mehrotra and Trivedi 2016). For groupings, various strategies can be used.

17.3.1 Based on Bio-recognition Elements

17.3.1.1 Enzyme-Based Biosensors (EBBs)

EBBs had developed as a huge method for subjective and quantitative examination of variable objective analytes in the medicine, food quality control, ecological control, and rural and drug industries (Ispas et al. 2012). EBBs are utilized to account for food fixings (acids, sugars, inorganic particles, amino acids, carbs, and alcohols), toxin (pesticide buildups and substantial metals), food added substances (sorbitol, benzoic acids, and sulfites), and food newness markers (like biogenic amines). Biosensors are additionally utilized on clinical grounds to recognize glucose, urea, cholesterol, and so on (Girigoswami et al. 2019; Salouti and Khadivi Derakhshan 2020). The issue with biosensors dependent on enzymatic restraint is that a restriction enzyme is delicate to heavy metals (Turdean 2011). Updike and Hicks in 1967 have expressed the primary enzyme-based sensor (Mehrotra and Trivedi 2016). Most of the winning EBBs are grounded on either optical or electrochemical transduction, whereas different sorts of transducers are seldom utilized. The two utmost fundamental divisions of electrochemical transducers for biosensing are the amper-ometric and potentiometric/particle specific field impact semiconductor device (Ispas et al. 2012; Salouti and Khadivi Derakhshan 2020). The frequently utilized enzyme for this reason for existing are polyphenol oxidases, peroxidases, L-lactate dehydrogenase, oxidoreductases, amino-oxidases, nitrate reductase, and tyrosinase (Salouti and Khadivi Derakhshan 2020). Adsorption, covalent adhering to strong planes and reinforce thin layers, framed in microencapsulation and polymer hydrogels had been utilized aimed at a protracted epoch to restrain enzyme (Turdean 2011). The selection of chemical/analyte framework depends on the way that these poisonous analytes limit the ordinary enzyme capacity. By and large, the improvement of these biosensing frameworks rely upon a quantifiable estimation of the chemical action afore and after openness toward an objective analyte (Salouti and Khadivi Derakhshan 2020) Typically, the level of subdued compound ($I\%$) that outcomes after divulgence to the inhibitor are quantitatively connected to the inhibitor (i.e., analyte) fixation and the hatching time. Subsequently, the remaining enzyme movement is conversely connected to the inhibitor fixation. Specified that contamination edifices specifically forestall the activities of few compounds, their action, and the subsequent variation in product concentration (Amine et al. 2006).

17.3.1.2 DNA Based Biosensors (DBBs)

Nucleic acid identification methods-based biosensors, DBBs, are being advanced to assess swift, modest, and cost-effective examining of inherited and transmittable diseases. Furthermore, the recognition of definite DNA arrangement is of importance in many parts counting medical, ecological, and food examination (Kavita 2017). These sensors provide benefits as improved affinity with micro-fabrication technology and fabricated through a wide spectrum of conducting/semiconducting media such as conducting polymers, gold, platinum, etc. (Uniyal et al. 2018). Significant kinds of DBBs include acoustic, optical, piezoelectric and electrochemical ones (Salouti and Khadivi Derakhshan 2020). Nucleic acid biosensors are either grounded on the exceptionally explicit hybridization of contrasting components of DNA/RNA particles or else assume the part of a profoundly explicit receptor of biochemical/synthetic species (DNA hybridization, electrochemical DNA, SPR-DNA, mark based or indirect identification, designation unrestricted, or straight discovery). The communication is because of the progression of steady hydrogen connections among the two nucleic acid strands (Salouti and Khadivi Derakhshan 2020). Nucleic acid biosensors are of key focus because of their countless potential for tracking down sequence-specific info in a quicker, modest, and economic way paralleled to the typical ones (Mehrotra and Trivedi 2016; Kavita 2017). Immunosensors are liking ligand-based biosensor solid-state instruments in which the immunochemical response is incorporated into a transducer. The basic of all immunosensors is the explicitness of the atomic acknowledgment of antigens by antibodies to create a steady complex (Salouti and Khadivi Derakhshan 2020).

17.3.1.3 Immuno-Based Biosensors

Immunosensors are classified grounded on the identification standard used. The key advances are electrochemical, optical, and microgravimetric immunosensors (Salouti and Khadivi Derakhshan 2020). Immunoassay procedure executes a reckless, basic, reliable, and delicate examination of various compounds being useful in various spaces of interest like clinical examination for clinical determination, just as in an ecological examination, and food quality control (Balahura et al. 2019). The most broadly utilized immunosensors smear three various types of sign and transduction strategies, succeeding the development of the perplexing antigen–immunizer: optical, in which alters of the optical functions of the encompassing mode are created (e.g., shading, iridescence and changes in the refractive record); electrochemical, given electrical signs (flow, voltage contrasts, and resistances); or piezoelectric, which count on the progressions in quantity identified by piezoelectric instruments (Contreras-Naranjo et al. 2019 and Mollarasouli et al. 2019). Morgan et al. (1996) reported that by and large, the fundamental standards of immunosensors concerning the diverse transducer frameworks comprised electrochemical, heat recognizing, mass distinguishing, and optical immunosensors. Nonetheless, the low substance/actual steadiness restricts the utilization of antibodies in cruel conditions like acids, high temperature, and organic solvents (Uniyal et al. 2018).

17.3.1.4 Cell Based Biosensor (CBBs)

An entire cell-based biosensor is a logical appliance that joins entire building blocks or organelles are accountable for its choosiness, with an actual transducer to make an assessable sign corresponding to the convergence of analytes (Turdean 2011). The cells are efficient, have a broad dynamic period, and are minor touchy to restraint, temperature, and pHvarieties than compounds (Bagde and Borkar 2013). Gui et al. (2017) reported that although entire CBBs are not as subtle to natural varieties as atomic-based ones, these biosensors know how to be altered utilizing unobtrusive genetic designing strategies to recognize a progression of compound reactions inside an active cell (Gui et al. 2017). As per the standard meaning, the full cell should be brought together with a transducer (charge-coupled gadget, luminometer, photometer, and fluid sparkle counter) to perform like a genuine biosensor (Turdean 2011). The principal capacity of an exemplary entire cell biosensor is the identification and advancement of a specific sort of analyte into an electrical and optical sign using a processor. This interaction can be distinguished by control and the utilization of active cells or microbes as a component giving atomic acknowledgment components. Gui et al. (2017) and team as opposed to a typical biosensor, entire CBBs can recognize a more extensive assortment of constituents, building them more vulnerable against variations in a test electrochemical state. Whole-cell biosensors are practical than comparing enzymatic biosensors due to few microorganisms can be refined and disengaged rather essentially, which isn't the situation for various enzymes (Salouti and Khadivi Derakhshan 2020). Whole-cell biosensors for the most part is free, don't need the expansion of cofactors, and are the biorecognition components of the decision once the aggregate sum of risky constituents or toxins is to be resolved (Singh et al. 2014)

17.3.1.5 Microbial Biosensors

A microbial biosensor is analyzing equipment that consists of a micro-organism used to identify an objective matter as well as to transforms the identified impulse into a quantifiable outcome in the form of biochemical, electrical, or physiological. The detection and identification process involves various types of standard optical, sensory-regulated systems, and electrochemical (Salouti and Khadivi Derakhshan 2020). The cell activity regulation or metabolic pathways are identifiable as microorganisms not only detect environmental parameters such as pH, nutrients, and temperature as well as they can only be stimulated on identifying their metabolic status (Lim et al. 2015). Each cell of bacterial biosensors includes genetically modified bacteria inculcating the contaminant-sensing gene which can stain the presence of an analyte and units with the reporter gene that can make a noticeable consequence (Strosnider 2003). For example, *Flavobacterium* sp. or *Pseudomonas diminuta* are in general utilized as organophosphorus hydrolase (direct organophosphate determination) separation in advanced laboratories. The ability possessed by pigments existing in the chlorophyll of flora helps in converting the light (photons) concentrated by absorbing into energy, and a little number of energy molecules are ejected as visible light. Utilizing cyanobacteria in the modification of whole-cell biosensor are few fresh studies appreciated. Wan et al. (2014) proposed that for the identification or detection of heavy metals, biocides, algal biosensors, and BOD are generally used. An algal biosensor for toxicity valuation of water bodies (Campanella et al. 2001). The detector was introduced by combining a suitable algal bioreceptor which is *Cyanobacterium spirulina* sub salsa into an amper-o-metric which is a gas diffusion electrode (Salouti and Khadivi Derakhshan 2020). The analytic equipment permits the supervising of the progress of photosynthetic O_2, as well as the determination caused in enhancement, occurred because of toxic effects stimulated by environmental contaminants (triazine herbicides, carbamate insecticides, and heavy metals) present in a medium (Campanella et al. 2001).

17.3.2 *Based on the Type of Nanomaterial*

17.3.2.1 Nanoparticles Based Biosensors (NPBBs)

Metallic nanoparticle carries distinctive electrocatalytic and electronic functions liable on their morphology and dimension (Park et al 2002; El-Deab et al. 2002). NPBBs are striking as they might be readily made in bulk with typical chemical techniques, and don't need any advanced fabrication methods (Fig. 17.3). They also provide great surface areas due to their very minor size and are usually used as suspensions in solutions (when they intermingle with the analyte) (Kuswandi 2019). Labeling is done with metal nanoparticles in most of the biological molecules deprived of negotiating their biological activities (Hrapovic et al 2004). Gold NPs are a highly explored component for a biosensor as they can rise an electronic sign once the abiotic constituent is sustained in interaction with its nanostructured surface (Liu et al. 2004). The magnetic NPBBs advances the electron transmission through the single-layer particles self-assembled on the planes of electrodes (Zhang 2014). This remark is particularly beneficial in the advance of electroluminescence-based nanobiosensors (Wang 2005). Besides goldNPs copper, platinum, silver, palladium, cobalt, and other NPs are also widely studied in the advancement of nanobiosensors (Oliveira et al. 2013; Hrapovic et al. 2004; Salimi et al. 2009; Xia et al. 2011). In supplementary, plasmonic nanostructures, for example, gold NPs and silver NPs are a charming contender for the headway of exceptionally sensitive biosensors as they are particularly restricted surface plasmon resonances (LSPRs) (Kuswandi 2019). The LSPR of the minus particles are in the apparent and IR light reach and is delicate to the configuration, dimension, figure, encompassing media, and amassing condition of these nanoparticles. This plasmonic conduct conveys the hotspot for the creation of colorimetric sensors for natural examinations. Moreover, the LSPR likewise helps the electromagnetic escaped around the NPs surface, which offers the reason for surface-improved Raman spectroscopy (SERS)-based detection (Kuswandi 2019). Chemical poisons can be recognized and separated dependent on the unique finger impression ranges that climb once they arrive at SERS-dynamic problem areas. These advances to ecological examination dependent on LSPR-based colorimetric and SERS recognition has been concentrated in writing (Wei et al. 2015). Gold NPs-changed DNA has been utilized to propel a miniature cantilever-based DNA nanobiosensor (Su et al. 2003) to spot DNA on its very minor focus over a hybridization response. This response coordinates to the connection of gold NPs and goes about as a nucleating specialist for the advancement of silver NPs once presented to a photographic creating arrangement. The development of silver NPs improves the successful measurement of the miniature cantilever and coordinated to an enhanced recurrence shift (Kuswandi 2019). This technique could recognize the objective DNA at a convergence of 0.05 nM or lower. In nanobiosensors, Micro-pit resonators comprised of permeable silicon are utilized (Kuswandi 2019). These resonators have the particular highlights of line withdrawal and iridescence improvement. Permeable silicon has been employed for distinguishing little natural atoms, like digoxigenin and biotin, 16-nucleotide DNA oligomers, and proteins (antibodies and streptavidin) at pico-and femtomolar-level focuses (Jianrong et al. 2004) Aptamer-based gold NPs as a colorimetric biosensor utilizing mark free and labeled Au NPs are utilized in nano-biosensor (Huang et al. 2011). However, on goldNPs, aptamer adsorbed strongly and advances the consistency of Au particles over the NaCl-incited collection. Aptamers will seriously tie with melamine by the more grounded proclivity which will decline the salt resilience of Au nanoparticles and the outcome is the ensuing build-up of Au NPs (Kuswandi 2019). For this situation, the ssDNA marked Au NPs are utilized for melamine recognizable proof, which depends on the mix of thymine with melamine. The chose ssDNA will initially append to the outside of Au nanoparticles. The DNA-functionalized Au NPs can collect when the oligonucleotides hybridize, which sources the variation of colors (Kuswandi 2019). Melamine could incite hybridization. At the point as soon as added the melamine, the functionalized Au NPs are aggregated after restricting the oligonucleotide to melamine, which brought about the red-to-blue tone variation (Kuswandi 2019).

17.3.2.2 Nanotubes-Based Biosensors

Carbon materials have expected over-the-top responsiveness through the advancement of nanoscience (Rusling et al. 2009). These contain the change of terminals with various nanocarbon, for example, CNTs, carbon powder, carbon cases, and graphene sheets (Zheng et al. 2010; Fang et al. 2008; Iijima 1991). The assessment of the micro-electronic elements of CNTs by Iijima and associates (Hirsch 2002) is a very pinnacle of expressed procedures to portray their acknowledgment capacity. CNTs are shaped by an empty chamber of a distinctive carbon sheet with a solitary walled (SW)-CNT or concentric carbon slips of assorted breadths framing multi-walled (MW)-CNTs with sp^2 holding (Rusling et al. 2009). The specific round and hollow design of CNTs is the essential element that conveys the quantum repression impact in the arranged 1D-microstructured materials (Pumera 2010). These highlights offer the odds to upsurge the substance reactivity and electronic functions of this specific carbon material, which turns into an indispensable point for biosensing instruments (Zhao et al. 2002). Latest examinations have perceived the point that CNTs can build up the electrochemical reactivity of fundamental biomolecules (Zhao et al. 2002; Musameh et al. 2002; Gooding et al. 2003) and can invigorate the electron-move responses of proteins (Matsunaga et al. 2004 and Wang et al. 2003). Notwithstanding improved electrochemical reactivity, CNT-adjusted electrodes had been demonstrated to help collect huge bio constituents (e.g., nucleic acids) (Wang et al. 2003), and mitigating surface polluting impacts (Gooding et al. 2003). The critical affectability of CNTs potential to the surface adsorbates permits the act of carbon nanotube as incredibly subtle nanoscale sensors. These functions make CNT truly alluring for a wide scope of electrochemical biosensors reaching out from amper-o-metric protein electrodes to DNA hybridization biosensors. Such CNT-inferred twofold advance enhancement alleyway (of both the transduction and acknowledgment occasions) permits the recognition of DNA and proteins to 1.3 and 160 mmol, separately, in 25–50 μL

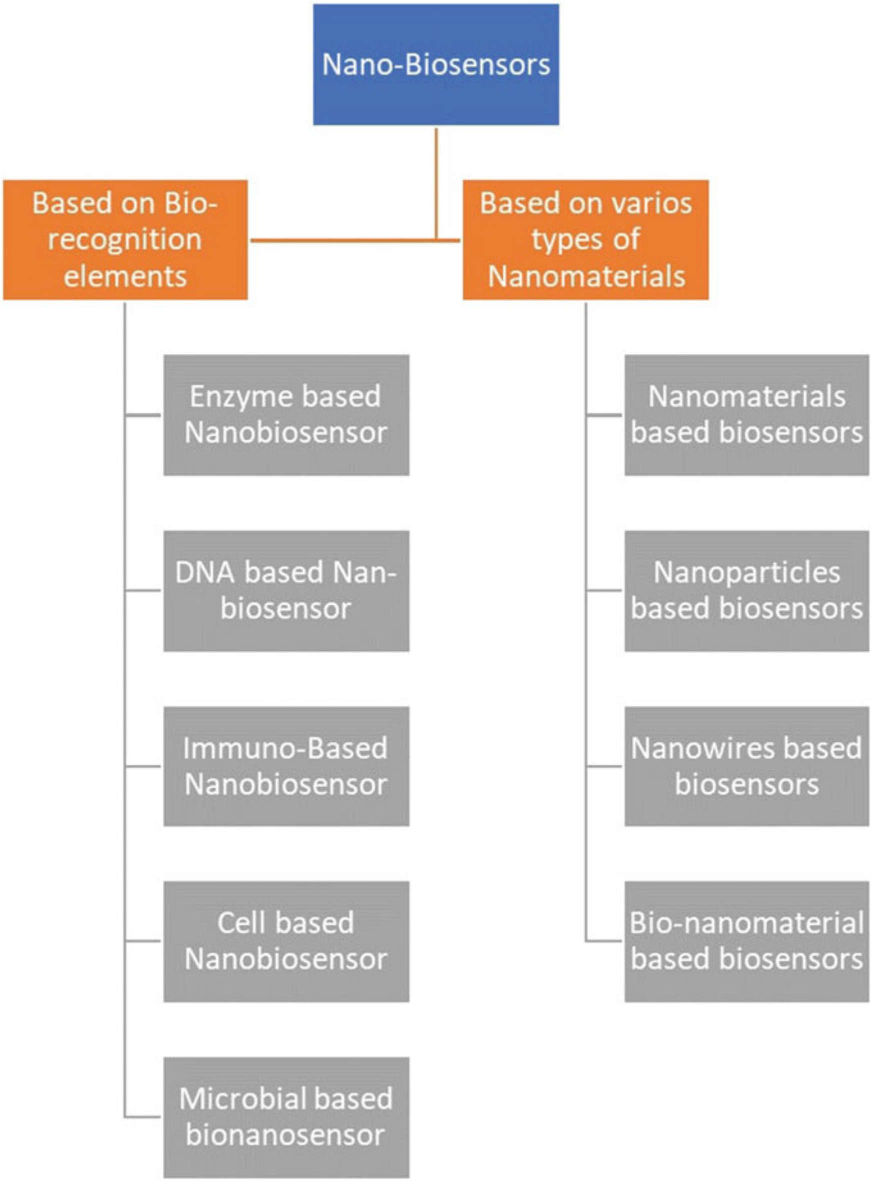

Fig. 17.3 Depicting summarized classification of nano-biosensors

tests and coordinates inordinate potential for sans polymerase chain reaction-DNA examination (Wang 2005).

17.3.2.3 Nanowires-Based Biosensors

Nanowires-based biosensors could be draped with all objectives and resolutions of any chemical constituent or biotic atomic acknowledgment unit, through appropriate fringe properties. The nanomaterials convert the substance restricting episode on their surface into an adjustment of the conductance of the microwire in an extremely delicate, constant, and quantifiable strategy. The conductive TiO_2 microwire packs have been made up and layered with antibodies specific for *Listeria monocytogenes*. At that point, the TiO_2 microwire packs were put on a superficial level among two gold electrodes (Wang et al. 2009). In pollutant tests, microorganisms tie to the antibodies, which root an assessable variety in impedance across the TiO_2 microwire group. Utilizing this training, the nanobiosensor was capable to distinguish as low as 4.7 × 102 CFU/mL L. monocytogenes in 1 h deprived of substantial impedance from other food-borne microorganisms. Hence, has remarkable improvement over exemplary immuno-speck blot examination, which had an acknowledgment cutoff of 2.2 × 105 CFU/mL. The varieties in conductance or opposition through circuits produced using or checking microscale (De La Rica et al. 2008) segments has likewise been utilized to spot individuals from the *Bacillus* (Kuswandi 2019), *Salmonella* (Wang et al. 2009), and *Escherichia* (Liu et al. 2004; So et al. 2008) bacterial genera, just as infections (Guilbault et al. 2004).

17.3.2.4 Bionanomaterial-Based Biosensors

In a biosensor, the bio-receptor is joined with an adept transducer which makes a sign subsequently correspondence with the objective particle of concern. The existence of the biotic components marks the biosensor frameworks, especially explicit and profoundly sensitive, providing a higher advantage over the ordinary techniques. Preposterous, various regular and man-made components had been utilized in biosensors; the mains are chemicals, dendrimers, slight layers, and so forth (Kuswandi 2019) In EBBs, the regular component is the enzymes which react definitely with its substrate (Astruc and Chardac 2001). Catalysts are the furthermost applied biocatalytic constituents, permitting the acknowledgment of analyses differently. Dendrimers are recognized as natural macromolecules with tri-dimensionality and incredibly distinctive design efficacy (Crooks et al. 2001). The ability of these dendrimeric constructions to consistent and sustain the reliability quality of metallic microparticles was stated by Crooks (Lvov et al. 1998). The progress of microelectrodes for the valuation of hydrogen peroxide and oxygen focus is grounded on silicon substrate work through microfabrication advances. Photoresists are utilized to fix compounds or microorganisms on these oxygen-detecting chips (Kuswandi 2019) thin nanostructured layers have delivered the chance, to make biosensors with a high force of identification, with inborn traits related to their measurements at the nanoscale level (Vamvakaki et al. 2007). Pesticide nanobiosensors have been accounted for grounded on liposomes for the distinguishing proof of organophosphorus pesticides (Vamvakaki et al. 2005). In this framework, porins are established into the lipid film grant for the free substrate and pesticide transportation into the liposomes (Kuswandi 2019). Insecticide fixations down to 10–10 mol/L can be supervised by utilizing this restraint fluorescent biosensor. This work characterizes a technique for substrate-incorporating catalyst initiation and relating present reaction at the substrate-bound protein electrode (Kuswandi 2019).

17.3.3 Applications of Nanobiosensors

Advancement in the field of nanotechnology has opened a unique and promising area of biosensors that has appealing affectability and adaptability. The final objective of nanobiosensors aims at distinguishing any biochemical and biophysical associated with particular infection in a solitary atom or event at the smallest unit of life (cell) (Touhami 2014). They can be fused into various advancements at the sub-atomic analysis level and on chip-based lab technologies. Their uses remember the discovery of microorganisms for different examples, checking of metabolites in body liquids and ID of tissue pathology, for example, cancer (Touhami 2014). Their mobility makes it best for the pathogenesis of malignancy applications however they can be utilized in the research facility setting also. The capacity to spotting sickness correlated bio constituents, for example, infection explicit metabolites, nucleic acids, proteins, microorganisms, and cells like flowing tumor cells, is pivotal not only for illness distinguishing proof in the clinical site additionally for biomedical investigations counting drug revelation and progression (Touhami 2014). Nanotechnology, with its improved affectability and diminished instrumentation dimensions, will rapidly propel our present bio-analytic ability for explicitness, quickness, and financial planning. Diminishing in sensor measurement conveys innumerable versatility for mix into multiplexed, compact, wearable, and surprisingly inserted clinical instruments (Touhami 2014). The fuse of nanoscale high-sensitive biosensors with other medical instruments will present the way to creating clinical fields, checking point-of-care diagnostics, and universal medical services systems (Touhami 2014). The biomedical bid of nano-bio sensors is broad; also, the impending impression of nano-biosensor outlines for point-of-care diagnostics will be preeminent. This invention will alter ordinary clinical applies by permitting early identification of ongoing debilitating sicknesses, ultrasensitive site of microorganisms, and long-haul screening of patients using biocompatible bound together clinical equipment (Touhami 2014). Multi-layered NPs outlines are being gathered for the concentrated-on arrangement for single cell-based effective treatment. The approach of biosensing for Cleavable shells, a good methodology for remediation (Touhami 2014). Therapeutic eminence successions are coordinated by biosensor-initiated regulate alterations to offer a sufficient threshold for class treatment on the premises of a single cell.

The main objective of biosensors is too excellent treatment "nano-industrial facilities" exclusive single active cells. Atomic biosensors related to these abilities direct their demeanor. Quality transference is progressing in light of a biosensor spotted issue; quality conveyance is stopped when the cell reaction indicates that more quality treatment isn't required (Touhami 2014). The phenomena of internal coordination of cells in nanocontainers and crystals of the nanoscale are confirmed with mixing shiny colors, similarly, advanced techniques like fluorescent-based/Confocal microscopes, cytometry, are useful for estimation of concentrations. Compelling eminence arrangement has been visualized by the utilization of the GFP reporter sequence (Touhami 2014). DNA binding techniques were applied to flood the degree of enunciation of the gene. Incorporated nanomedical frameworks are being arranged, fabricated, and via testing (ex-vivo and in-vivo) for tiny organisms. Nanomedicine, though at infancy means an alteration in outlook in speculation-on the wreckage of debilitated cells due to therapeutic procedure, irradiation procedures, chemical-based therapies to cellular materials of an organ, and desolation of non-revival cells due to cell death mechanisms (Touhami 2014). Nano-biosensors can be utilized as conservational class checking apparatuses in the valuation of natural/environmental quality or for the substance screening of both inorganic and natural need contaminations (Salgado et al. 2011). The main pros of nanobiosensors are steady checking, better workflow, and the capacity to gage pollutants in complex grids with insignificant specimens. Nanobiosensors can be productively utilized for distinguishing a broad fluctuation of composts, microbes, dampness, various kinds of pesticides and their classes, analytical parameters like pH, coordinated can initiate uphold feasible horticulture to improve harvest efficiency (Sekhon 2014). In addition, crop efficiency is day by day compromised by irritations, weeds, and microbes that impact the similar ranch economy; in this way, botanical specimens must be preserved with fitting activity. Nanobiosensors should also be given splendid cultivating not just by following conditions of the ground and flora, but also by taking into account the pest manifestations and sicknesses arising from it (Antonacci et al. 2018). Utilizing nano-bio sensors, agriculturalists can show ecological conditions completely for plant development and insurance. Nanobiosensors can be productively utilized in cultivating for distinguishing a shifted variety of manures, herbicides, pesticides, microorganisms, dampness, soil pH, and others for improving the harvest yield (Kanjana 2017). In comparison with exemplary chromatography, nanobiosensors were locked in as a substitute for pesticide estimation by the righteousness of its phenomenal selectivity, affectability, outstanding recognition, and reliability just as its speed. Nanobiosensors showed over-the-top capacities on the acknowledgment of biomolecular associations on varying pressure and quantifiable parameters (Álvarez et al. 2016; Elmer and White 2018). Nano-sensors can also be equipped with location tracking devices and screen illness remotely, the wellbeing of crops/plants, ground conditions, and other emerging problems, for example, decrease of soil supplements and water deficiency continuously. Such information and signs contain the best occasions for engraining and collecting floral specimens and water samples with set time parameters, manures, herbicides, pest-resistant chemicals taking into account specific functional, pathology, and ecological conditions of the plant (Kanjana 2017). At the current time, various methodologies for plant microorganism recognizable proof are accessible, yet some of them are moderate like conventional culture-based strategies; others are distinctive of responses, for example, the high measure of polymerase chain reaction techniques (tedious, incapable in distinguishing early diseases), Enzyme-Linked Immuno Sorbent Assay (tedious and low impending), DNA analysis, etc. Splendid nano-bio sensors can uphold, perceive, and treat supplement insufficiencies ground and flora due to the contribution of full-scale micronutrients corresponding with seasonal and supplemental requirements (Kanjana 2017; Duhan et al. 2017). The distinguishing proof innovation grounded in nanobiosensor is an inventive microbial acknowledgment that begins to change horticulture (Álvarez et al. 2016).

17.3.4 Conclusion

Water is an important resource for every human activity and is also the most vital resource in the world. Nanotechnology and its application hold immense potential in the management and treatment of wastewater. Earlier the detection of harmful pollutants in water was a tedious task Nanotechnology-based biosensors have created a revolutionized approach because of their high output of detection, robustness, and highly appreciable sensitivities. The added advantage of nanotechnology is that it is capable of detecting various environmental pollutants because of its improved surface area and better absorption capabilities. Any type of analyte can be detected and treated using this advancement in science and technology. Biosensors and nanobiosensors can deliver quick and definite info on polluted places for environmental screening, toxicity, and monitoring. The growth of novel nanomaterials will show a vital role in guaranteeing acceptable safety and security superior class water to encounter the increasing requirement for consumption water. On the other side, the high accessibility, economical and high antimicrobial activity of numerous nanoparticles create them striking for water purification. Because of transmitted diseases and inadequate drinking water resources, there is a boundless need for progress in the water filtration system. Nanofibers and nanocides can be a beneficial clarification to guarantee harmless and easy access to consumption water.

17.3.5 Future Aspects

Biosensors have flexible uses in medicine, engineering, biomedical technology, toxicology, ecotoxicology, etc., and their reduction becomes beneficial for economical, fast, and sensitive detection. Though environmental biosensors can be fabricated with the upgraded properties of new nanomaterials and nanocomposites, upgraded attention should be made for in-situ and actual observing of contaminants using other technologies. Biosensors ranges recognized bioanalytical techniques, whereas nanobiosensors, with nanotechnology, are developing this area with revolutionary solutions that diminish exposure to conventional contaminants. Laboratory techniques and protocols united with a fast reaction time, better sensitivity, robustness, and portability at the instant of use. The nano-biosensor can determine more variables with advanced understanding and needs a smaller amount of tester material. The usage of biosensors is modest and the practice is quick and inexpensive. Since nano-bio sensors work at the atomic level, they have the maximum throughput and analytical effectiveness, making them simple to practice for the recognition of pathogens, contaminants, and environmental toxicity. The key restriction of the current environmental biosensors is because of their deficiency of bid in physical ecological testers, as a maximum of the recognized "environmental biosensors" for synthetic samples and water from tap sources. Excessive claim to obtain fast, dependable, inexpensive skills for detecting, monitoring, and diagnosing contaminants and poisons in the environment. Though, some confines still delay the widespread usage of this equipment in the real world, counting, for case in point, low-capability loading and working steadiness of biocomponents. The moving novel method of using biosensors on the nanoscale can be equipped with advance robotic systems and location services to generate intelligent management software's to identify, mark, and be capable of working on desired areas of the field, before/through the start of symptoms. Nanobiosensors can decrease the practice of agrochemicals and increase productivity and profits. Breeders and pioneers could achieve on-site diagnostics once wearable devices with biosensors are advanced. A tremendously valued practice of reckless and sensitive sensors are areas of access to isolated pathogens that can be seized more proficiently. On-field testing for mycotoxins and notorious food pathogens is also a promising area of research. A vital trend in nanobiosensors is the advancement of biosensors for applications under extreme conditions, e.g., B. strongly acidic, alkaline, salty environments, extreme temperatures, and organic solvents, as more and more indications comprise such hostile conditions, which are mainly vital because of the rising nano-biosensor industry, the countless requirements for an economical, sensitive, selective, and fast-reacting biosensor on the market.

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Chapter 18
Nanobiosensors Potentialities for Monitoring SARS-CoV-2 in the Environment

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Abstract The worldwide pandemic of coronavirus disease—2019 (COVID-19) is a devastating and distressing scenario that highlights humanity's inability to build fast diagnostic tools for emerging infectious diseases. However, the majority of existing approaches have a significant probability of false negatives, leading in patient diagnostic errors and prolonging therapy. Nanoparticles have shown significant improvement and have the potential to be used as a platform for quickly and accurately identifying viral infection. The relevance of nanoparticles is potential platforms for COVID-19 diagnostics was emphasized in this research. In addition, nanomaterials have surface chemistry, which may be beneficial for the bioconjugation of molecules, large surface potential, and a significant amplification impact on signals. Due to various potential benefits, metallic nanomaterials like gold, silver nanoparticles, and carbon-based nanomaterials (carbon nanotube and graphene), nanogels, and photonic crystals are utilized for biosensing applications. In compared to traditional techniques for identifying severe acute respiratory syndrome coronavirus—2 (SARS-CoV-2), this study covers the most relevant aspects of nanobiosensor-based diagnostics techniques. Additionally, major potential challenges and prospects associated with the advancement of these distinct sensors for SARS-CoV-2 detection are discussed in detail.

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18.1 Introduction

Infectious diseases are the only global risk to human civilization on Earth, apart from a natural failure of cataclysmic nature or an all-out man-made nuclear war. Infectious diseases have afflicted mankind for centuries, serving as the major cause of morbidity and death, and affecting human evolution in the process. Nevertheless, owing to the prevalence and expanded adherence to hygiene and sanitization methods, vaccines, and antibiotics, our industrialized population has grown increasingly complacent about the risk raised by infectious diseases. Epidemiological experts and invasive organizations all over the world have given adequate and often neglected safety hazards of our globally vulnerable infectious conditions and their widespread and unborn effects, despite this unjustified level of complacency and falsified sense of certainty. Since the beginning of the century, the most recent epidemics caused by viruses, including severe acute respiratory syndrome (SARS), Ebola, middle east respiratory syndrome (MERS), influenza H1N1, human immune deficiency virus (HIV), have increased awareness of the major threat to humanity as a whole still posed by viral infectious diseases. The newest threat to world health is the new "coronavirus—2019" (COVID-19) infection of the SARS-CoV-2. In December 2019, COVID-19 was first discovered in China. In March 2020, the World Health Organization (WHO) declared the outbreak to be a pandemic due to its rapid spread across the world. COVID-19 infections may be asymptomatic and display a range of symptoms, from mild flu signs including fever, cough, muscle pain, and exhaustion to serious consequences which may result in death (Singhal 2020; Sheikhzadeh et al. 2020; Giri et al. 2021). Gastrointestinal common symptoms (diarrhoea, nausea, and vomiting) occur in a small proportion of SARS-CoV-2 patients. Asymptomatic persons may spread the disease, and it is thus very vital for them to be identified and isolated.

The current accessible diagnostic techniques of viral infection are including detecting virus or viral biomarkers in human body fluids and computed tomography (CT) for diagnosis based on certain imaging properties. Traditional techniques for viral detection include viral cultural identity, "reverse transcription-polymerase chain reaction" (RT-PCR), and "enzyme-linked immunosorbent assay" (ELISA). The RT-PCR method is suggested as the WHO guideline for SARS-CoV-2 detection (WHO 2021). However, present detection of RT-PCR still involves a collection of samples, sophisticated reagents, professionals, and installations, with limited accessibility and poor results (Sheikhzadeh et al. 2020). Moreover, the latest studies reveal positive RT-PCR testing findings for COVID-19 patients which suggest the misleading results with the molecular bioassays based on genetic elements. In order to address the limitations of traditional diagnostic procedures, new robust techniques for viral detection with excellent accuracy and specificity should be established. Consequently, alternative techniques for the direct detection of viral infections are urgently needed, preferably using a miniaturized sensing device that can diagnose POC systems (Ejazi et al. 2021). Nanotechnology is emerging as a very sensitive technique for detecting viral diseases with enhanced sensing capabilities. Nanostructure-based biosensors, which benefit from the simplicity of their sensing processes and provide advantages such as high sensitivity and specificity, fast label-free, and real-time recognition, particularly attracted the attention of both the nanotechnology and biosensor research groups. The nanomaterial-based biosensor is an analytical framework which is generally integrated with organic elements, such as antibody, enzymes, cell receptors, nucleic, and microorganisms. The equipment can present a variety of signal types (light, electrical, pressure, magnetic, or heat) with the attachment of identified objects to detect low-level, high sensitivity, and direct detection of specific samples. Owing to the present high-surface area, numerous perceptive nanomaterials and nanostructures were utilized to enhance the collection and enrichment of viral particle collection and enrichment in solution and air samples (Xia et al. 2019; Leung and Sun 2020; Ganganboina et al. 2020). For viral detection without labelling, nanoscale biosensors including nanotubes (Palomar et al. 2020; Vadlamani et al. 2020), nanowires (Ishikawa et al. 2009), and 2D materials such as e graphene and molybdenum disulphide (Yang et al. 2018) have been used. In this chapter, we present the current evolution of nanobiosensors for virus detection, particularly those coupled with gold nanoparticles (AuNPs) and magnetic nanoparticles (MNPs), graphene, and carbon materials. We additionally describe the mechanism of nanobiosensors and provide an overview of the efforts of the researchers towards improving the performance of the device. Following the overview of various nanobiosensors, an overview of the challenges and prospects associated with nanobiosensors for virus detection is provided in the following sections. Further, a brief overview of what this chapter is all about is illustrated in Fig. 18.1.

18.2 Origin and Structure of SARS-CoV-2

Viruses are key pathogens that promote death and terrible environmental circumstances globally greatly. The underlying virus structure of rapid transmission and the lack of effective accumulated is a key strategy for managing and treating viral infections. In addition, as a major cause of death in humans, respiratory virus infections also provide enormous environmental, physiological, and public impacts to countries throughout the world. In November 2019, the new kind of virus was detected in Wuhan (Hubei Province—China) which was classified by the WHO as a new coronavirus (2019-nCoV) (Yang and Wang 2020). There are multiple coronavirus variants MERS-COV, SARS-CoV. Swine acute diarrhoea syndrome coronavirus (SADS-COV), newly detected novel coronavirus (2019-nCoV) by the International Committee on Virus Taxonomy (ICTV), called “SARS-CoV-2” (Alberdi et al. 2016;

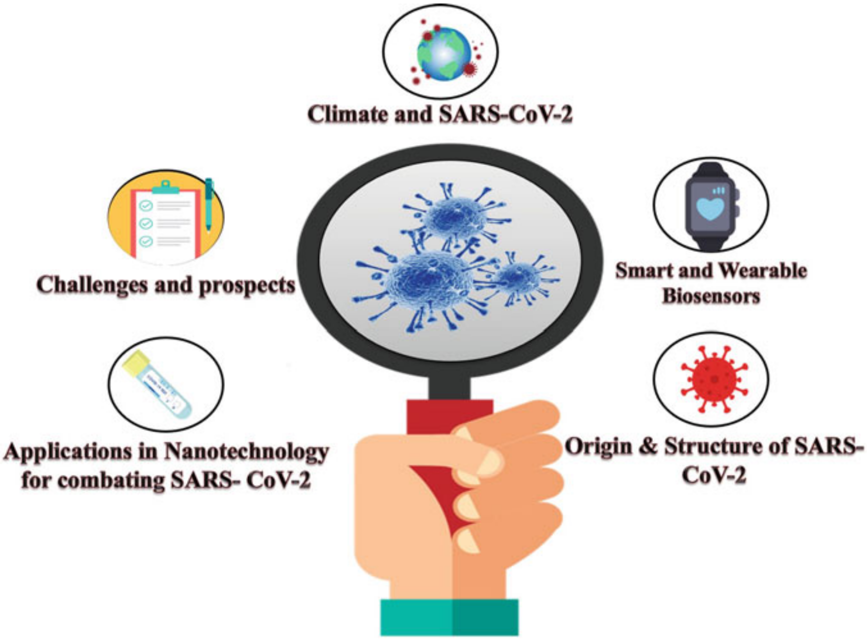

Fig. 18.1 Illustration representing a brief overview of the chapter

WHO 2020; Xie and Chen 2020; Peeri et al. 2021). The spread of SARS-CoV-2 variants, e.g. B.1.1.7 (alpha), which was first detected in the United Kingdom and is the most widespread variant currently circulating in the United States; B.1.351 (Beta), which was identified in South Africa (Tegally et al. 2020); and the P.1 (gamma) variant, in Brazil (Sabino et al. 2021), has all been assigned to the classification by the international organization (Wang et al. 2021; Abdool Karim and de Oliveira 2021; Moelling et al. 2021). In the United Kingdom (UK), 14 mutations and 3 amino acid deletions were identified in the first variant SARS-Cov-2, 20I/501YV1 (B.1.1.7 pangolin lineage) that affects human transmittance of the virus (Rambaut et al. 2020). The Indian "double mutant" variant of SARS-CoV-2, also known as B.1.617, is a "coronavirus variant" found in India and a few other countries. Although the WHO has classified the B.1.617.2 (delta) coronavirus variant as a "variant of concern" it appears that this lineage is composed of at least three sub-lineages: B.1.617.1, B.1.617.2, and B.1.617.3 (Abigail Ng 2021; Mallapaty 2021) related with greater infectivity. As of May 10, 2021, the accelerated pandemic of SARS-CoV-2 and its general, non-specific symptoms had resulted in nearly 56 million recorded cases and more than one million deaths worldwide. Notwithstanding the low mortality for COVID-19 (less than 3% with a range of 2–12% in different countries) comparison with MERS-CoV (~40%) and SARS-CoV (~15%), in order to prevent additional fatalities and potential unknown adverse effects, the rate of community detection and monitoring should be improved due to broad prevalence and reaching more individuals with underlying diseases, e.g. late diagnoses have raised death rates amongst peoples in the United States of America, UK, Europe, Turkey, Spain, India, Latin America and Germany, Iran, and China. In addition, the rapid identification of COVID-19 is amongst the main cases of COVID-19 monitoring and treatment, except for quarantine to prevent infection transmission. Although, the disease is transmitted at an alarming pace, and it was examined within a few weeks of initial reports for the disease symptoms (Huang et al. 2020a) such as evolution (Yu et al. 2020; Tang et al. 2020), transmission dynamics (Lamb 2020; Yu et al. 2020), molecular tests (Shen et al. 2020), and genomics (Zhu et al. 2020). It is clear that rapid identification and diagnosis are essential to the treatment and management of diseases. The estimates of the infection stage and the prediction of infection and recovery are the responsibility for quantifying and monitoring viral loads. At present, the major COVID-19 identification procedures can be mostly divided into three groups in the clinical laboratories: chest computational tomography (CT) scanning, nucleic acid, and antibody testing (Ravi et al. 2020). The use of modern diagnostic methods premised on portable kits, including ELISA kit, may minimize diagnostic restrictions for control and processing through lack of knowledge, high price, an inadequacy of time, and laboratory restrictions around the world.

18.2.1 Biological Properties of the SARS-CoV-2

SARS-CoV-2 is a 50–200 nm diameter coronavirus that consists of a single-stranded ribonucleic acid positive-sense (RNA) envelope characterized by surface glycoproteins including spike (S), envelope (E), and membrane (M) protein (Xia et al. 2020; Verdecchia et al. 2020). The corona has a structure that is approximately spherical in form, containing projections that resemble a crown (Fig. 18.1). The virus is about 125 nm in size and consists of an envelope that is 85 nm in diameter and spikes that are 20 nm in length. This is a single-stranded RNA with a genome that ranges in size from 26,000 to 37,000 bases, making it the biggest recognized genome amongst RNA viruses in terms of size (Weiss and Navas-Martin 2005). The envelope comprises a lipid bilayer linked in 1:20:300 proportions by M proteins, E proteins, and structural S proteins. It is a class I protein complex that is found in S protein. Angiotensin-converting enzyme 2 receptors are responsible for binding to the S1 and S2 subunits, which are separated by two subdomains, namely the C and N-terminal regions of the S1 subunit (ACE2) as shown in the Fig. 18.2 (Zumla et al. 2016). It is glycosylated with an N-terminal side chain, which enables it to penetrate the endoplasmic reticulum (ER) also facilitates its attachment to host receptors by the spike protein. It has been shown that the nucleocapsid protein binds to RNA in vitro despite being highly phosphorylated. Whilst the viral genome is roosted onto the replicase-transcriptase complex, this protein assists in packing the encapsulated genome into viral particles. The membrane protein is the most common structural protein in viruses, and it may exist in two distinct conformations, either of which may enhance the binding of the virus to the nucleocapsid. When present in tiny amounts, the envelope protein

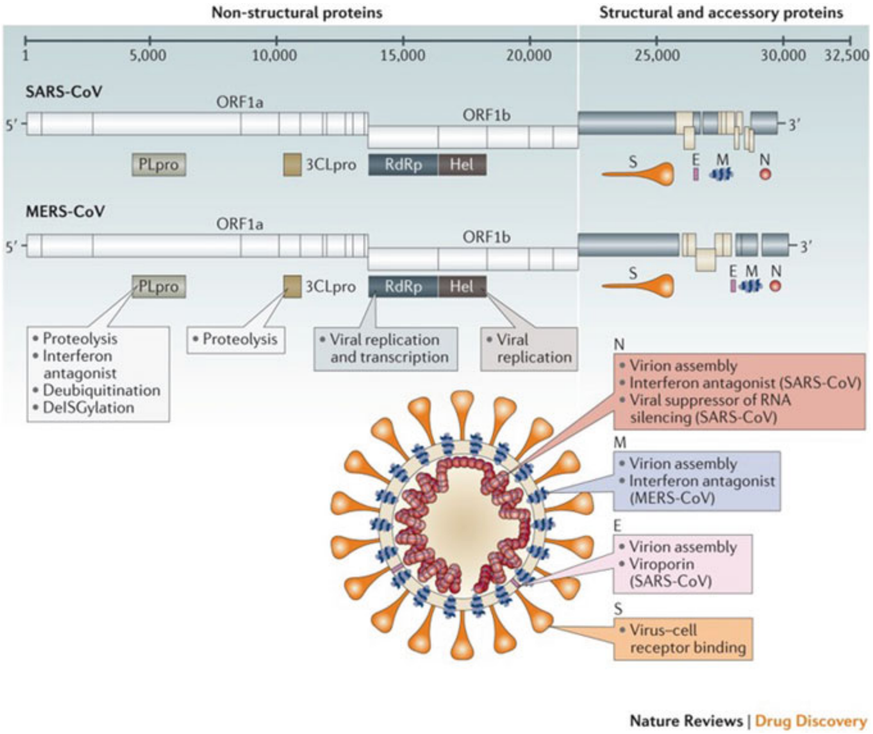

Fig. 18.2 Structure of human coronavirus (reprinted with permission from (Zumla et al. 2016))

behaves similarly to a transmembrane protein in terms of ion channel function (Lu et al. 2020; Hoffmann et al. 2020). Despite N and S2 proteins exhibiting a highly preserved SARS-CoV structure, the S1 component is considerably less preserved and much more distinct from SARS-CoV-2. The above proteins may still be employed as antigen for quick screening (Ward et al. 2020).

18.2.2 Climate and COVID-19

Many factors can affect the pandemic in COVID-19, and the atmosphere has been found to have an impact on its distribution in the world as shown in the Fig. 18.3 (Ukhurebor et al. 2021; Hashmi et al. 2022; Singh et al. 2022). The propagation of SARS-CoV-2, in particular, involves the atmosphere (e.g. temperature, moisture, the velocity of wind, precipitation) and the concentrations of atmospheric pollutants (NO_2), 2.5 and 10 μm particulate matter ($PM_{2.5}$ and PM_{10}) (Gupta et al. 2020, 2021). According to recent reports, SARS-CoV-1 and 2, which have comparable laboratory durability, can endure and be contagious for hours in aerosols and even days on an inanimate surface (van Doremalen et al. 2020). On the other hand, temperature and humidity have a major impact on coronavirus survival and transmissions (Otter et al. 2016). Riddell et al. (Riddell et al. 2020) demonstrated that SARS-CoV-2 can live at 50%—relative humidity (RH) and 20 °C on the surface, at least for 28 days. The use of particulate matter (PM) induces local and systemic chronic lung inflammation (Borro et al. 2020) and may render individual sensitive to SARS-CoV-2 transmission on a lengthy basis and simple exposure to environmental toxins (Cole et al. 2020; Liang et al. 2020). SARS-CoV-2 RNA may also be efficient in outdoor PM, indicating as SARS-CoV-2 can exist in atmospheric high-PM concentrations (Setti et al. 2020). Increased NO_2 levels also may have a significant impact on COVID-19 fatalities, considering persons are more susceptible to acquire SARS-CoV-2 in regions with high-NO_2 levels (Ogen 2020).

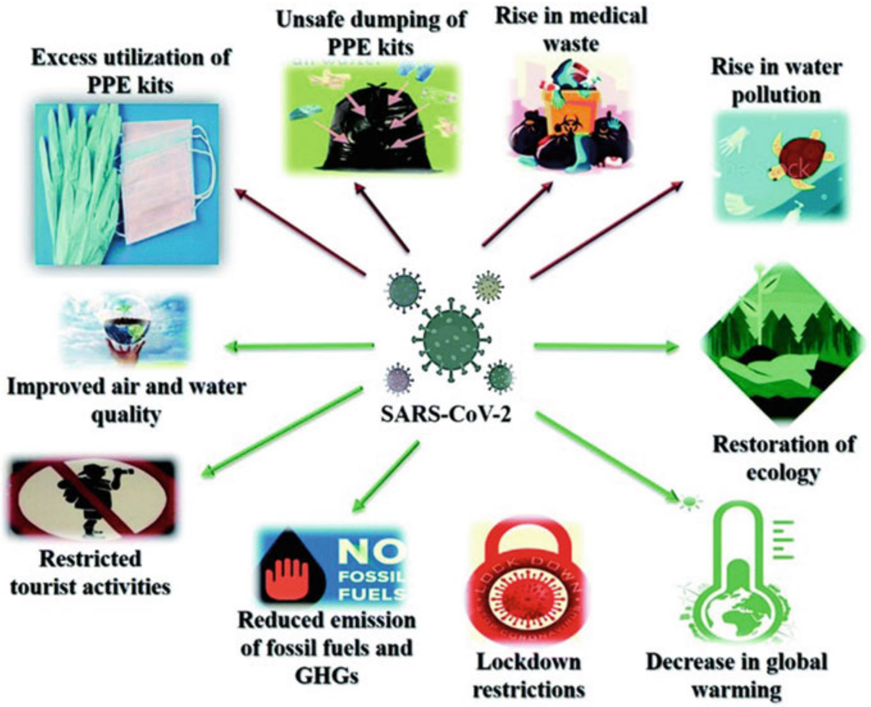

Fig. 18.3 Schematic illustration of various positive and negative impacts of SARS-CoV-2 on the environment (reproduced with permission from (Ukhurebor et al. 2021); permission to reproduce automatically granted by the Royal Society of Chemistry due to authorship)

18.3 Current Applications in Nanotechnology for Combat COVID-19

Nanobiotechnology presents enormous prospects for the design of biosensors for pathogenic microorganism detection, particularly in therapeutic applications. Nanomaterials play a broad range of physical and chemical characteristics, from limiting their growth to developing possible treatments, which can be utilized to fight SARS-CoV-2. Due to their distinctive feature, including such high-volume/surface ratios, SARS-CoV-2 diagnosis is the most commonly used components. Such materials take into account fast and accurate identification and highly sensitive detection owing to large surface interactions between nanomaterials, sensors, and analytes (Mokhtarzadeh et al. 2017). Nanomaterial's nanoscale particle size (1–200 nm) is extremely near to the particle size of the SARS-CoV-2 virus. The very interacting properties of nanoparticles thus greatly affect the electric properties of the nanoparticles. These interactions provide a broad spectrum of selectivity and increased sensitivity. It may be used for the identification, protection, and prevention of diseases.

18.3.1 Biosensors for Detection of Coronaviruses

Point-of-care procedures (POCs) are required to diagnosis patients lacking samples at medical centres, enabling the identification of ill individuals by communities without laboratory systems. Point-of-care diagnosis is a fast, cost-effective, and durable diagnostic method that is highly needed for COVID-19 for early diagnosis and that may be the only option available at this time. The biosensors serve as a feasible alternative diagnostic tool. Virus detection biosensors are also evaluated using compared to conventional testing using a particular transducer. Biosensors are the tools used for testing biological components that may directly include nucleic acid, tissues and physicochemical microsystems, cell-receptors, proteins, and enzymes, including modified proteins, recombinant antibody, aptamers, etc. (Campàs and Katakis 2004). Biosensors like antigen, antibodies, and nucleic acids may be developed using various biological components. Due to their great sensitivity, low cost, and possibilities of miniaturizing, electrochemical immunosensors have now become a compelling choice (Benjamin et al. 2022). This bio-electroanalytical device comes from nanoparticles composed of gold. Nanomaterial is 1–100 nm in size. Using nanoparticles in biosensors helps to control a wide range of limitations and challenges since such materials include nanoscale characteristics like conductance and properties, such as thermal conductivity, chemical resistance, distinctive optical and magnetic repulsion, and increased strength, that are not identified in materials used in other fields of study. Magnetic nanomaterials (iron oxide), metal oxide nanoparticles (gold, silver), quantum dots (QD), and carbon nanostructures utilized in sensor manufacturing may be nanomaterials (Shetti et al. 2019, 2020; Bukkitgar et al. 2020a, b; Ilager et al.

2020). Graphene, gold nanoparticles (AuNPs), and gold nanoinsulas (AuNIs)-based biosensors were utilized to detect COVID-19 Ronkainen et al. 2010. Despite their high sensitivity and ease of use, nanocarbon-based sensors offer a higher promise for microbe identification than other types of sensors (Bajpai et al. 2020; Sharma et al. 2020). A biosensor is a bio-receptor combination measuring biological effects and a signal transducer generating optic, electrical, thermal, and mechanical signalling. The biosensor should not be affected by physical variables such as temperature and pH. The biomimetic function is achieved through a variety of mechanisms, the most common of which are the antigen–antibody (Ag-Ab) interactions, nuclear acid interactions (two complementary layers), enzyme-mediated interactions (enzymatic), cell attachment (microorganisms, proteins), and biomimetic participation. Despite this, each technique has a unique set of difficulties, such as reducing the limit of detection (LOD), enhancing signal to noise—ratio, and evaluating compounds at very low levels. Nanoparticles (NPs) are currently gaining a lot of attention in virus detection due to their rapid sensing and biological activity. As a consequence of various advancements in nanotechnology, the need for nanostructure/material in the design of biosensors has become feasible, resulting in substantial improvements in the performance of the devices. Nanobiosensors are critical instruments in the detection of pathogens that are both accurate and fast. Additionally, there are a variety of other nanobiosensors for virus detection that are classified according to their detection method and can be divided into several categories, including electrical, chemical, optical, piezoelectrical, magnetic-thermal, and biological monitoring. The identification of nanobiosensors derived from COVID-19 infection is described in detail in Table 18.1.

18.3.2 Electrochemical Nanobiosensors

Electrochemical transducers have a cheaper cost, a simpler construction, greater specificity, a sensitivity and are more portable. Their operating concept is based on the electrical signal recording, formed when the bio-receptor interacts with the target analysis selectively (marked or not marked) in proportion to the level of the analyte (Ronkainen et al. 2010). In response to the demand to construct devices to rapidly detect SRAS-CoV-2, several researchers were able to produce and investigate many new electrochemical technologies most often based on nanomaterials from Au, carbon, or graphene. Fan and colleagues developed an entropy-based enhanced electrochemiluminescence (ECL) system to identify the RNA-dependent RNA polymerase (RdRp) gene of SARS-CoV-2. The development of the biosensor involves modifying the DNA tetrahedron (DT) on the electrode's surface and showed a LOD value of 2.67 fM (Fan et al. 2021). A SARS-CoV-2 electrochemical DNA-based sensor based on hybridization of viral RNA in the N gene with complementary DNA-modified electrode was recently described (Alafeef et al. 2020). Lokman et al. (Liv 2021) described a platform for electrochemical immunoassay to identify the spiked antibody for SARS-CoV-2 by using cysteamine, glutaraldehyde-capped gold

Table 18.1 Electrochemical nanobiosensors for corona viral infectious disease detection and their detection methods, target, linear range, and detection limit

Nanomaterials	Biological samples	Detection methods	Target	Detection limit	Linear range	Ref
Cobalt-functionalized TiO_2 nanotubes (Co-TNT)	Nasal and saliva samples	Amperometric detection	S-RBD (receptor binding domain)	14 nM	14–1400 nM	Vadlamani et al. (2020)
Fluorine doped tin oxide (FTO) electrode with gold nanoparticle (AuNPs)	Spiked saliva samples	Cyclic voltammetry (CV), differential pulse voltammetry (DPV)	nCOVID-19 spike antigen (nCOVID-19Ag	10 fM (in-house built device) of nCOVID-19 Ag	1 fM–1 µM in standard buffer	(Mahari et al. (2020)
Carbon screen-printed electrodes with gold nanoparticles	nasopharyngeal	Label-free immunosensor CV, square wave voltammetry (SWV)	SARS-CoV-2 nucleocapsid protein (N protein)	0.4 pg. mL^{-1}	1.0 pg. mL^{-1}–100 ng. mL^{-1}	Eissa et al. (2021)
AuNPs on paper electrode	Nasopharyngeal sample	Biochip	RNA of the N gene	6.9 copies/µL	585.4 copies/µL to 5.854 × 107 copies/µL	Alafeef et al. (2020)
Magnetic beads with carbon black	Untreated saliva and nasopharyngeal sample	DPV	S and N antigens (NAg)	19 ng. mL^{-1} and 8 ng.mL^{-1}	0.04–10 µg/mL 0.01–0.6 µg/mL	Fabiani et al. (2021)
Graphene sheet	Nasopharyngeal swab specimens	Field-effect transistor (FET)	SARS-CoV-2 spike protein	1.6×10^1 pfu/mL in culture medium	2.42×10^2 copies/mL in clinical samples LR:	Seo et al. (2020)

(continued)

Table 18.1 (continued)

Nanomaterials	Biological samples	Detection methods	Target	Detection limit	Linear range	Ref
semiconducting (sc) single-walled carbon nanotube (SWCNT)	Nasopharyngeal sample	FET	S antigen (SAg) and N antigen	0.55 fg/mL—Sag, 0.01 fg/mL—NAg	5.5 fg/mL–5.5 pg/mL 16 fg/mL to 16 pg/mL	Shao et al. (2021)
Redox dye-incorporated silica nanoparticles	RNA from nasopharyngeal sample	Isothermal rolling circle amplification (RCA) sandwich hybridization-electrochemical biosensor	N and S genes	1 copy/µL	1–1 × 10^9 copies/µL	(Chaibun et al. 2021)
Graphene oxide (GO) paper-based electrode	Serum	Label-free detection-SWV	SARS-CoV-2 IgG, IgM and S protein	0.11 ng/mL	1–1000 ng/mL	Yakoh et al. (2021)
Molecular imprinted polymer (MIP)	Nasopharyngeal samples	DPV	N antigen	15 fM	2.22–111 fM	(Raziq et al. 2021)
Magnetic nanobeads	Serum	Amperometric detection	N antigen	230 pg. mL^{-1}	5 × diluted serum samples	Li and Lillehoj (2021)
Carbon nanofibre	Nasopharyngeal sample	Competitive detection using SWV	N antigen	0.8 pg. mL^{-1}	1–1000 ng/mL	Eissa and Zourob (2021)

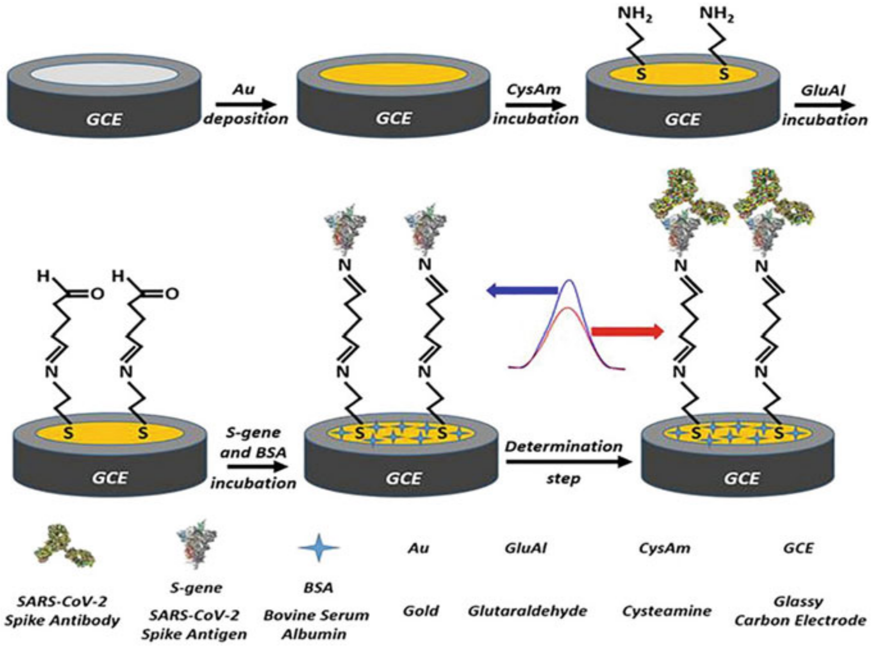

Fig. 18.4 Schematic overview of the diagnosis of COVID-19 using an electrochemical immunosensor framework with gold clusters, cysteamine, and glutaraldehyde modified electrodes (reproduced with permission from (Liv 2021))

clusters, SARS-CoV-2 antigen spike protein, and bovine serum albumin on glassy serum electrode. The Fig. 18.4 illustrates the steps required for preparing BSA/S-gene/GluAl/CysAm/Au/GCE sensor and steps to detect the SARS-CoV-2 spike antibodies. The electrochemical immunosensor developed demonstrated a high sensitivity of 0.1–1000 ag/mL in 0.01 M (pH 7.5) phosphate buffer saline solution, as well as 0.01 ag/mL LOD in saliva and oropharyngeal swab samples.

18.3.3 SARS-CoV-2 Electrochemical Nanobiosensors Based on Gold Nanostructures

Gold nanoparticles, thanks to their optical characteristics, electrical, catalytical, high biocompatibility, and enhanced electron transmission rate, are the most durable metal nanoparticles. As a result, they have a broad range of applications in electrochemical biosensors of different types. Depending on the technique employed gold nanoparticles can y be synthesized by reducing gold salts chemically or electrically (Yeh et al. 2012). The electrodeposition of AuNPs on the properties of carbon electrodes is a highly attractive technique since it is easy, quick, and simple to prepare. In

(Eissa et al. 2021) have developed a new label-free biosensor to identify SARS-CoV-2 viruses in electrochemical samples. Here, screen-printed carbon electrodes (SPCE) have been used to produce the electrodes using AuNPs' electrical deposition. 11-mercaptoundecanoic acid (MUA) was used by the electrodes to inhibit the antibody against SARS-CoV-2 N protein. The designed biosensor was made up of dual AuNPs-modified SPE that consisted of two individual working electrodes. One of the electrodes was used as control electrode that was used for blocking BSA and on the other consisted of immobilized anti-N antibody. Electrochemical signals were generated when the N protein binds to the specific antibody, which are then detected by observing variations in the voltammetric decreasing current, as control electrode produces no signal as illustrated in the Fig. 18.5. This electrochemical immunosensor has a high sensitivity of 1.0 pg/mL–100 ng/mL and 0.4 pg/mL for a PBS (phosphate buffer solution) (pH 7.4) solution. In addition, the initial test for the immunosensor of COVID was highly associated with the RT-PCR end results in positive and negative samples. In (Rashed et al. 2021) have utilized Au-based electrode platforms for the development of a capacitive immunosensor for label-free electrochemical sensing for SARS-CoV-2 antibodies based on the commercialized detection of polyethylene terephthalate (PET). Further, the authors demonstrated the feasibility of utilizing the electrochemical impedance technique with commercially available equipment to detecting SARS-CoV-2 antibodies using commonly produced equipment at significant clinical concentrations.

Label-free electrochemical DNA hybridisation detection has been proposed as a COVID-19 diagnostic method (Tripathy and Shiv Singh 2020). The system design

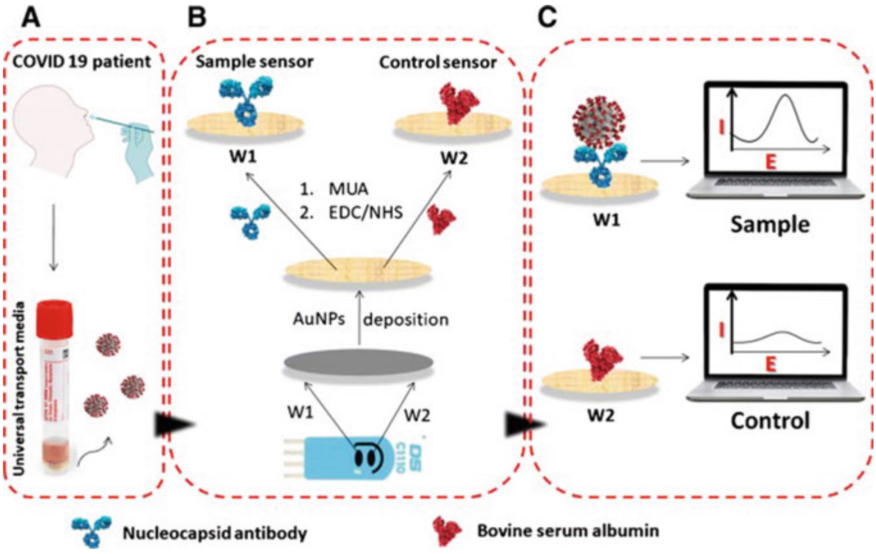

Fig. 18.5 Schematic representation of SARS-CoV-2 nucleocapsid antigen detected using a voltammetric-based immunosensor (reproduced with permission from (Eissa et al. (2021))

of a compact electrochemical biosensor and associated DNA sensing methods is described. The suggested platform can identify and quantify individual viral RNA or c-DNA COVID-19 utilizing the complementary thiolated materials. The resultant current which the authors can detect using amperometry is equivalent to the limit target concentration for a given electrode-bound sensor concentration. However, further study on the sensor's reaction time, limit of detection, and other efficiency characteristics is required. In (Mahari et al. 2020) constructed a sensitive SARS-CoV-2 electrochemical framework on the SPCE surface (denoted as eCovSens). A potentiostatic sensor was manufactured utilizing gold nanoparticles of fluorine doped tin oxide electrode (FTO) with nCOVID-19 monoclonal antibody (nCOVID-19Ab) adsorbed in order to detect changes in electrical conductivity. Likewise, eCovSens was utilized to detect electric conductivity changes by directly modifying nCOVID-19 Ab on the SPCE. Using the DPV methodology, they observed that the two biosensors showed good sensitivity in the concentration range COVID-19Ag from 10^{-15} M to 10^{-6} M. Furthermore, eCovSens' low-detection limit was assessed by 10 fM (fM $= 10^{-15}$ M), whereas the Au-FTO limit was assessed to be 120 fM. Despite its high sensitivity, portability, quick response time (10–30 s), and low cost, eCovSens may be able to compete with Au-based electrochemical biosensors. (Mavrikou et al. 2020) have proposed the combination of membrane engineering and electrochemical analytic fields as a novel technique for electrochemically sensing the SARS-CoV-2 S1 protein model. After S1 antibodies have been deposited in the kidney cells, they have immobilized the Au-screen imprinted carbon electrodes (commercial). Researchers were able to achieve a LOD value of 1 fg/mL for SARS-CoV-2 antigen and excellent selectivity. Moreover, semi-linear behaviour was detected when additional virus-associated proteins were found within the range of 10 fg/mL^{-1} g/mL the sensor response time was reduced at concentrations greater than 10 fg/mL. The capability of the biosensor to recognize the viral antigen without sample processing within 3 min was its main attraction. However, the major drawback of the proposed electrochemical detection technique is the cell production need which demands the employment of specialist personnel and equipment.

18.3.4 SARS-CoV-2 Electrochemical Nano Biosensors Based on Carbon and Graphene Materials

The usage of carbon or graphene nanosensors has increased in the last decade, especially in electrochemical biosensors, as it is observed that the nanostructuring of the transducer electrode greatly enhances all its operational and efficient properties. For example, recently, to enhance toluidine blue (TB) for SARS-CoV-2 RNA detection, In (Zhao et al. 2021) designed a ultra sandwich-type electrochemical biosensor based on the *p*-sulphonated calix(8)arene (SCX8) functionalized graphene (SCX8-RGO) to deposited Au@Fe_3O_4 nanoparticles. Interestingly, no amplification or reverse transcription is required. The SARS-CoV-2 biosensor LOD was determined to be 200 copies/mL. In this connection, an electrochemical immunoassay has been developed to rapidly and effectively identify SARS-CoV-2 coronavirus through saliva samples as illustrated in Fig. 18.6. The electrochemical test was designed to identify S or N protein utilizing magnetic beads to support the immunologic chain and the alkaline phosphatase secondary antibody marked as serological marker. In this case, SPE enhanced with carbon-black nanocomposites detected the 1-naphthol enzyme by-product. The electrochemical immunoassay diagnostic properties were evaluated employing standard S and N protein solutions in PBS and saliva with detection limits of 19 ng/mL for S and 8 ng/mL for N proteins. Its efficiency was evaluated using cultured viruses at level 3 and in saliva clinical samples, comparing findings using real-time PCR-tested nasopharyngeal swab specimens. This analytical instrument has a strong potential for commercial introduction as the first incredibly specific electrical immunoassay for SARS-CoV-2 detection in pretreated saliva samples, owing to data agreement, relatively low-detection value reached, fast analysis (30 min), portability, adaptability, and simplicity of use (Fabiani et al. 2021). The function of a nanomaterial increases surface area to volume ratio and as such electrochemical reaction rate. In order to improve the stability and specificity of the novel biosensor SARS-CoV-2, In (Vadlamani et al. 2020) used titanium dioxide (TiO_2) with cobalt-functionalized carbon nanotubes (CNTs). The catalyst improved the sensor's three main characteristics, according to the authors: selectivity for the S-receptor binding domain (S-RBD), linearity of sensitivity (14–1400 nM), and reaction time (30 s). Electrochemical biosensors are a potential method for fast medical diagnosis of infection—biomarkers leading to early and effective decision-making. In this context, In (Mojsoska et al. 2021) developed an electrochemical immunosensor for SARS-COV-2 spike protein detection. Additionally, the spike protein can be identified with the LOD of 20 μg/ml after 45 min of incubation and a SARS-CoV-2 concentration of 13.75×10^5 and 5.5×10^5 PFU/mL. Meanwhile, the method involving drawbacks following, time-consuming incubation period, and not satisfactory detection values in μg/mL. In order to address the limitations of current techniques, nucleic acid-based biosensors (genosensors) are being developed. Genosensors are a specific and sensitive method for virus detection as advancements in medical diagnostics (Farzin et al. 2020). Electrochemical genosensors have gained a lot of interest amongst genosensors because of their quick reaction, sensitivity, and cost-effectiveness, as well as their compatibility with microfabrication technique and easy operating mode, making them suitable for point-of-care (POC) testing. Leila and co-workers (Farzin et al. 2021) found out to determine the SARS-CoV-2 RdRP sequence (RdRP SARSr-P2) as a potential target for detection of COVID-19 without the need of a label or nucleic acid amplification, and the silver ion-hexathia-18-crown-6 compound modified carbon paste electrode (CPE-HT18C6(Ag)) was used. The proposed genosensor had a satisfactory linear range in sputum samples to SARS-CoV-2 RdRP in the range of concentration 1.0 pM–8.0 nM, with a LOD of 0.3 pM. Researchers at the University of Illinois Chicago (UIC) have utilized graphene sheets in laboratory tests, which can detect variations of the virus, in order to quickly identify COVID-19. UIC reported that the researchers coupled graphene sheets with an antibody intended to target the coronavirus spike protein. The atomizing vibrations of certain graphene

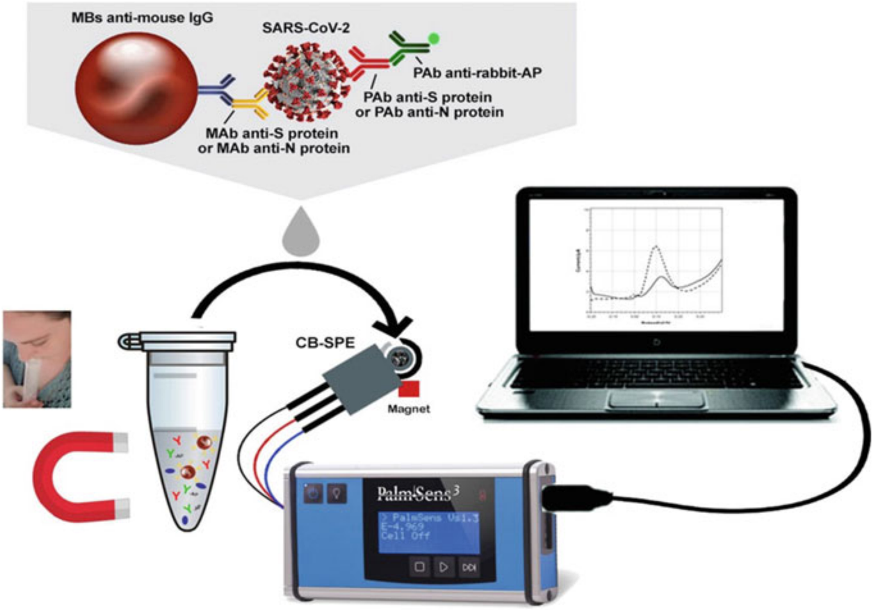

Fig. 18.6 Detection of SARS-CoV-2 in untreated saliva using the MBs-based technique (reproduced with permission from (Fabiani et al. 2021))

sheets were then monitored when introduced in artificial saliva to COVID-positive and negative samples. The sheets are also being tested for other infections, including MERS (Nguyen et al. 2021).

18.3.5 Optical Nanobiosensors

Optical biosensors are generally characterized as analytic instruments that the interaction between analyte and bio-receptor is evaluated in terms of changes to optical measures like luminescence, fluorescence, absorption, and reflectance that correlating with the analyte concentration. In general, gold nanoparticles and gold nanofilms are often used combined with receptor binding domains to construct plasmonic biosensors such as surface plasm resonance sensors (SPR) and located surface plasmon resonance sensors (LSPR) (Qiu et al. 2020); lateral flow immunoassay (LFIA) (Wen et al. 2020; Chen et al. 2020), surface-enhanced Raman scattering (SERS) (Huang et al. 2020b; Soler et al. 2020) are amongst the techniques used in the study. Recently, considerable focus was placed on developing COVID-19 biosensors. The identification of the SARS-CoV-2 N antigen has been reported using a lateral flow assay (LFA) (Grant et al. 2020). Recently, AuNPs are utilized to diagnose SARS-CoV using localized surface plasmon resonance (LSPR). The localized surface plasmon coupled fluorescence coupled optical immunosensor (LSPCF) was utilized to enhance efficiency in the sensing of serum-dilution nucleocapsid protein (N), a SARS-CoV biomarker with a sensitivity of 0.1 pg/mL. This optical nanobiosensor has produced effective early prediction and identification of SARS infection with coronavirus (Chang et al. 2009). Another study utilized a spike protein-specific nanoplasmonic resonance sensor to detect SARS-CoV-2 viruses optically in one step, eliminating sample pretreatment. The sensor was claimed to identify as few as 30 virus particles within 15 min in one phase and detectable viral concentrations ranged between 0–6.0 × 10^6 vp/mL and LOD value of 370 vp/mL (Huang et al. 2021). In this manner, Adrianna et al. (Masterson et al. 2021) fabricated, ultra-sensitive label-free COVID-19 nanoplasmonic screening test that can detect SARS-CoV-2 RNA (4 Ngenes, RdRp Genes & E Genes) and S protein subunits in term of human antibodies (IgG, IgM), all including single biosensor system operate with a detection limit of lower than 89 aM with excellent high sensitivity on each biomarker. The inventors have designed an optical nanosensor in real time to detect active SARS-CoV-2 infection that involves a modular synthesis programme that can be used to detect other viral infections. To detect viral proteins and viral genomic material, the nanosensor to point-of-care diagnostic equipment, single-walled carbon nanotube (SWCNT), functional noncovalently with ACE2, and nanosensors is capable of detecting active SARS-CoV-2 infection and providing accessible and fast testing results (Pinals et al. 2021).

18.3.6 Magneto-Optical Nanobiosensors

Over the decade, magnetic nanoparticle-based sensors have demonstrated remarkable potential for biosensing applications. The nanoparticles are manufactured using magnetic materials such as iron (Fe), cobalt (Ca), nickel (Ni), and manganese (Mg). This is accomplished by labelling magnetic nanoparticles (MNPs) and magnetic beads (MBs) with bio-receptors. These magnetic nanoparticles can also be used to replace the time-consuming filtration process used in biomolecular isolation, hence avoiding the use of large-scale systems including filtration and centrifugation systems. For diverse applications including cancer biomarker, stem cell, pathogens (bacteria and virus), and hazardous metal ions, magnetic biosensors were reported.

A functional magnetic nanoparticle (MNP) containing S protein was proposed to detecting SARS-CoV-2 utilizing a spike and polystyrene bead SARS-CoV-2 mimic. The method is based on the measurement of MNP magnetic response containing without the virus through the magnetic field. SARS-CoV-2 with a LOD of 0.084 nM could be detected quickly and sensitively (5.9 fM) (Zhong et al. 2021). A poly (amino ester) carboxyl group decorated with magnetic nanoparticles (pcMNPs) was used to identify SARS-CoV-2 pseudovirus particles by In (Zhao et al. 2020). By distinguishing two separate sequences of viral RNA (ORFlab and N gene), a 10-copy sensibility and high linearity of 10–10^5 copies of pseudo virus SARS-CoV-2 particles are established. The main benefit of the structure is indeed the high efficiency of extraction in the current diagnosis of COVID-19 pathogenic virus to shorten detection time. In (Tian et al. 2020) employed optomagnetic detection to identify the SARS-CoV-2 RdRp coding sequences and reached a sub-femtomolar detection limit of 0.4 fM.

18.3.7 Piezoelectric Nanobiosensors

Piezoelectric (PZ) biosensor is amongst the extensively studied biosensors for detection, since it is selective, simple process, sensitive, and fast. Piezoelectric crystals are present together with antigens or immobilized antibodies on the surface of a PZ immune sensor. These two biomolecules are connected and used in real time for bio detection, incorporating antigens or antibodies, one generally available in gas phase or solution and the other attached on surface. The piezoelectric biosensor is a system for detecting mass changes on its surface by use of resonance frequencies when mass increase leads to frequency decreases (Zuo et al. 2004). The piezoelectric biosensor was utilized to diagnose SARS-CoV-related coronavirus by the sputum sample. This technique involves binding SARS-CoV horse polyclonal protein A antibody to the surface of the piezoelectric crystals. Frequency changes were captured by changes in the crystal mass via viral binding (Yang et al. 2015). This method may readily cost-effectively test SARS-CoV in comparison with the previous SARS detection methods. In (Zuo et al. 2004) reported the first piezoelectrical immunosensor in the coronavirus detection literature addressing the immunosensor of sputum SARS-CoV. The SARS-CoV horse polyclonal antibodies were immobilized on a PQC surface. The resulting frequency shift was equivalent to the antigen concentration within 0.6 to 4 μg/mL. The biosensor is highly reproducible, stable for over 2 months at 4–6 °C, and may be used 100 times without significant loss of activity.

18.3.8 Wearable and Smart Nanobiosensors

Wearable sensors and nanobiosensors have acquired significant attention during the COVID-19 epidemic due to various contactless healthcare diagnostics. Wearable sensors are capable of detecting crucial physiological signals including blood pressure, heart rate, pigmentation of the skin, body temperature, breath rate, sleep time, and body movements. Recently, Mujawar and co-authors (Mujawar et al. 2020) discussed the nanomaterial biosensor for the diagnosing of COVID-19 in clinically suspected. The nanobiosensors comprise genosensors and immunosensors which were integrated into the chip to evaluate COVID-19 patients. The data collected from the sensors are further analyzed by data gathering and analysis algorithms aided by AI. The interface of the biochip based on nanosensors with IoT is termed as bio-nano things Internet (IoBNT). This IoBNT may be utilized in a number of applications, such as the sharing of data with other medical centres across the world, rapid evaluation of the COIVD-19 contamination, contact tracking, isolation management, including targeting COIVD-19 patient perceiving. Using retrospective smartwatch data, the authors created an online heart rate monitoring technique to evaluate early-stage infection. Additionally, investigated the relationship between type and intensity of symptoms, pulse rate signals, and the influence of infection on sleep and activities (Mishra et al. 2020). In (Seshadri et al. 2020) explored integrating wearables and android apps to forecast and monitor distant changes in the physiological conditions of patients suffering from COVID-19 before clinical symptoms start. In this perspective, biological parameters (cardiac rate, respiratory rate, pulse frequency sleep, temperature) are monitored using commercial wearables to follow COVID-19 infection remotely. In this pandemic, the most common protection package to control the spread of COVID-19 infection is face masks. However, according to surveys, early COVID-19 patients were shown to expel a significant amount of SARS-CoV-2 virus particles by breathing, sneezing, or coughing. A simple but effective method for preventing human-to-human transmission is a suitable face mask. Virus aerosol masks, such as N95 masks, are often used to prevent the inhalation of viral aerosols. These masks include persistently charged electret fibres that absorb 95% of airborne particles in vitro. Exhaled breath screening using a face mask has often been suggested as a less invasive and easy method of sampling pathogens. Unlike nose swabs, breath sampling does not generate highly infectious contaminants and may be done nearly anywhere at any time. Recently, Qiannan et al. (Xue et al. 2021) proposed an integrated facial mask sensor that enables controlled diagnosis by aerosol and can easily detect coronavirus particle aerosols. However, the authors incorporated the nanobiosensor in a real face mask for the identification of a spiked solution of the S protein and evaluated the efficiency of the sample. A compact and lighter antibody-functional sub-100 nm conductive nanowire array with an impedance circuit allows for integration to a standard face mask and consists of a miniature impedance immunosensor. The nanowire was created utilizing a low-cost, mass-producible technique called nanoscale soft printing. The detection of an aerosol mimic coronavirus at 7 pfu/mL required 5 min.

18.3.9 Field-Effect Transistor

The “field-effect transistor” (FET) is a semiconductor that uses the current between one electrode (source) and another electrode (drain). The semiconductor channel between both the source and the drain is regulated by a narrow dielectrical field linked via a third electrode called a gate. Given the availability of existing diagnostics methods, FET biosensing devices provide several potential benefits such as an ability to detect small amounts of the target analyte immediately. Such biosensors may be used for clinical analysis, diagnosis, and POC testing (Zhou et al. 2017). Graphene has high-power mobility and a particular surface exposure to hexagonal carbon atoms that is electro-conductive and has proven ultrasensitive in sensing devices because it detects close changes in the surface and provides an excellent sensing platform (Sanati et al. 2019). Graphene-based FET biosensors have become very sensitive in recent years to electrical disturbances and have reasonably high mobility. Graph-based FET biosensors are thus extremely essential for very sensitive immunological diagnosis. In this approach, Seo and colleagues conducted a clinical SARS-CoV-2 using FET device on clinical specimens (Seo et al. 2020). To develop the biosensor, the FET graph sheets were coupled with specific antibodies for the SARS-CoV-2 spike protein. The biosensor's capability was tested using antigen proteins, an auto-cultivated viruses, and nasopharyngeal swabs from COVID-19 patients. The FET biosensor detected SARS-CoV-2 spike protein 1 fg/mL in phosphate buffer, saline, and 100 fg/ml clinical condition of transport. FET biosensors detected SARCoV-2 in self-grouped medium and nasopharyngeal swab samples with 1.6 10^1 pfu/mL and 2.42 10^2 copies/mL.

The biosensing system developed showed no detectable cross-reactivity with MERS-CoV antigen. For fast screening, the biological sensor described by In (Zhang et al. 2020) may digitally diagnose the COVID-19 virion. In this technique, two receptors such as SARS-COV spike S1—subunit protein antibody (CSAb) or angiotensin-converting SARS-CoV-2 spike S1 (containing RBD) antibody specific antibody-enzyme 2 (ACE2) were immobilized on highly sensitive, highly effective graph field transistors in order to make an immunosensor. The transistor CSAb antibody can recognize a detection limit (real time) of 0.2 p.m. in 2 min.

Gr-FET Immunosensor was developed by immobilizing CSAb or ACE2 (both of which bind especially to S protein RBD) (both of which bind especially to S protein RBD) on graphene surfaces. Both receptors have shown a strong affinity to antibodies, although CSAb has shown more affinity to ACE2. Recently, the existence of SARS-CoV-2 antigens in human nasopharyngeal samples was determined using a high-purity semiconducting (SC) single-wall carbon nanotube (SWCNT) field-effect transistor (FET). S antigen (SAg) and N antigen (NAg), with anti-SARS-CoV-2 spike protein (SAb) antibody and anti-nucleocapsid protein antibody functions, have been identified in our SWCNT FET sensors, reaching a detection limit of 0.55 fg/mL for SAg and 0.016 fg/mL for NAg in sample calibration. FET sensors have shown excellent sensory efficiency by distinguishing positive and negative clinical samples showing the quick-use concept of COVID-19 antigen detection and strong technical good sensitive requirements (Shao et al. 2021).

Experimental results show that antibodies have significant potential to neutralize the SARS-CoV-2 spike protein. Consequently, neutralizing antibodies may be used substantially in the prevention of coronavirus infection by healthy cells. These results are important for fast and easy diagnosis and the development of novel COVID-19 vaccines, medications, and treatments. Gr-FET automated biosensor applications utilized to detect COVID-19 spike protein $S1$ (including RBD) were used sensitivities in comparison with ELISA tests and eliminate complicated enzyme processes or voluminous and expensive optical equipment. FET-based devices typically use biologically recognized antigen/antibody. A full design for technological maturity in

Bio-FET-based devices is yet to be seen. Early detection reduces COVID-19 propagation and death. Although PCR is the standard method for COVID-19 detection, SARS-CoV-2 immunological tests are widely used and play an important role in COVID-19 assessment. Nanomechanical sensors include biosensors that detect a changes in mechanical system response. This work introduces and investigates a graph-based nanoresonator sensor for SARS-CoV-2 diagnosis using a finite element method (FEM). A single-layer graph sheet (SLGS) was coated with SARS-CoV-2 spike S1 antigen to mimic the sensor. The results showed that even with less than 10 viruses per test, suitable nanoresonator design parameters may achieve a high-sensitivity index for SARS-CoV-2 detection (Payandehpeyman et al. 2021).

18.4 Challenges and Prospects

The global health emergency of the COVID-19 epidemic identifies the world's largest major problem with the main emphasis being on improving sensitivity and specificities that current technologies should address the early diagnosis of COVID-19 and future pandemic strains. Electrochemical biosensors are also cheap, cost-effective, easy to use, and have a quick reaction time are ideal candidates for integration into POCTs for COVID-19 detection since they have all of these characteristics. Although attempts have been made to construct COVID-19 electrochemical biosensors, a couple of portable electrochemical biosensors were manufactured. One of the key obstacles in building adequate biosensors is to detect a very little signal between distinct organisms (bio-receptor and analyte). To minimize difficulties, nanoparticles may be employed as labels to substantially enhance the signal such that they are clearly visible. For example, metal nanoparticles (for example, gold or silver NPs) and quantum points may be examined and marked using them on the target DNA/biological recognition sensor. This might considerably increase the electrical signal through a synergetic effect due to the nano-labelling implications and enable ultrasensitive and selective labelling-biosensing systems to be developed. In this respect, further trials should be conducted based on the many appealing physicochemical features of nanoscale material in the construction of nano-enabled biosensing techniques, in particular MERS-CoV, SARS-CoV, and SARS-CoV-2. For this purpose, a study should take place. However, minimal analyzes were conducted for the design of nanomaterial biosensors based on SARS-CoV-2. In place of PCR-based COVID-19 tests, these approaches may be employed with the benefits of simplicity, economic efficiency, quick response, and real-time processes. These biosensors are mainly based on the mediated SARS-CoV-2 detection by nucleic acid and protein (antigen/antibody), although they are not yet 100 per cent accurate due to contamination of such extremely sensitive biosensors and are highly required for ultrasensitive, efficient, and compact SARS-CoV-2 sequence detection techniques. In addition, the CRISPR-Cas methodology or the aerosol-mediated diagnostic methodology for nanomaterial-based biosensors offers the benefits of quick reaction, susceptibility, and absence of sample disturbances. Furthermore, these biosensors demonstrated adequate stability, fast response time, and good sensitivity/selectivity on a lab scale. The outcomes may vary depending on the target antigen/protein/antibody, the nanomaterial or other key biomolecule, and the efficacy of biosensing devices based on nanomaterials. Nanobiosensors must be scientifically developed for detecting SARS-CoV-2 using urine, saliva, serum, and nasopharyngeal swab in different clinical samples for high efficiency. The long-term consumption and expensive isolation and/or synthesis of specific biological metrics/reagents are some of the main obstacles to the development of biologic reagents (such as antibodies, DNA, and antigens). Therefore, it is highly vital to develop nanomaterial-enabled biosensors that do not need extraction methods. Due to the very low level of viruses in patient samples, the detection limit is highly crucial; plasmonic, electrochemical, and magnetic sensors have shown the lowest LOD, demonstrating they are promising technologies. However, a key limitation for both magnetic and plasmonic approaches is that they often need particular instruments for fabrication as well as operation, causing difficult mobility problems. Improved processes are essential in order to make them more portable and also to increase their sensitivity for the usage of POC. Moreover, many procedures based on colorimetric, electrical, and lateral flow tests have shown increased mobility, allowing better functioning on the field since laboratory equipment and instruments are not necessary to acquire findings. The plasmonic and magnetic technologies, on the other hand, require a laboratory structure and also the advantage of maximum throughput that makes it possible to evaluate more samples one step at a time; the electrochemical and plasmonic techniques enable a better and quickest reading than the colorimetric and side flow procedures. In addition, one of the major aspects of the construction of well-organized biosensors is the transport of the targeted molecules to the functional surface. In this respect, robust simulation of these systems (robust, quick, and accurate numerical modelling) and associated with chemical interactions might contribute to improved biosensors. In other relevant biological investigations, for example, validation, design, and proof-of-concept were used for computational fluid dynamics (CFDs). The environmentally safe and reliable POCTs are generally the final obstacle before a POCT biosensor can be used. Many nanomaterials have been incorporated into the electrochemical biosensors with the progress of material science. The possible health and environmental implications of the widespread use of nanomaterials cannot be overlooked. Furthermore, the cost of POCTs for main medical institutions should be cheap. The materials utilized in the production process should thus be as low cost as feasible without impairing performance. Paper-based microfluidic devices are now reasonably eco-friendly and low-cost and hence the most often employed substrate platform. Nanomaterials based on carbon are indeed an excellent green alternative with less pollution. There is still a lot of effort to overcome the challenges of the compact electrochemical biosensor detection for SARS-CoV-2, but we still expect that with the growing tendencies of multidisciplinary integration, ideal nanobiosensors for COVID-19 diagnosis are just around the corner.

18.5 Conclusion

Rapid, simple, and significant diagnosis is essential for COVID-19 care and treatment (particularly in "asymptomatic" and "early-stage patients") emerging out of SARS-CoV-2, and for reducing and controlling their proliferation. Traditional diagnostic methods for viral infections are usually expensive, time-intensive, and labour-intensive and need the use of scientific equipment and experts with extensive disease knowledge. SARS-CoV-2 diagnostic methods and related nanobiosensor-based viral infections possess the promise to be sensitive, selective, and cost-effective. As a result, the low-level detection of specific disease biomarkers can be very useful for the improved assessment, monitoring, and therapy of the development of viral diseases. NPs with structural characterization and modification have the potential to aid in the rapid and more precise detection of SARS-CoV-2 infection, though in the initial stages of the disease's progression. A further technique that might be used to increase diagnosing performance in terms of accuracy is the employment of cascaded diagnosis/detection processes for several types of biomarkers via the use of multiplex nano and bio (immuno) sensors. Researchers are concentrating their efforts on machine learning-based signal processes and the details that are collected as a result of these operations in order to increase the reliability and repeatability of these sensors.

In addition to false positive and negative reports, validation procedures, speed of detection, accessibility, specificity, sensitivity, efficiency, and public use, more comprehensive academic analyzes and research should be carried out consistently to address these challenges. Nanotechnology can significantly improve diagnostic sensor manufacturing, device integration, optimization and validation, and sensing properties at the point of care. Further investigations are necessary to identify and develop new and creative, non-invasive, specialized, economical, and rapid biosensing methods and equipment enabling diagnostic and therapeutic applications, particularly widespread deadly diseases. It is remarkable how much academic attention has been paid to sensing applications with appropriate cost-effectiveness, miniaturized, and simplicity properties. Furthermore, organizations and industrial sectors are focussing on intelligent nanobiosensors (good sensitivity properties) for utilization as strongly certified and smart diagnostic devices. It is critical to highlight that, because of the presence of asymptomatic SARS-CoV-2 patients, nanobiosensors for public and home usage may be able to identify the virus immediately, allowing for improved control and treatment of COVID-19. In this regard, breakthrough smartphone-driven nanobiosensors including colorimetric strips aimed at antigens/antibodies have demonstrated potential for home use and simple point-of-care testing.

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Chapter 19
Recent Trends in Rapid Environmental Monitoring of Toxicants Using Nanobiosensors

Pallavi Singh Chauhan, Neha Sharma, Asha Singh, and Rajesh Singh Tomar

Abstract The previous research has focussed on detecting pathogens in real-world environmental models. The combined use of nanoparticles with devices will facilitate multiplex detection systems, mining techniques, and nanomaterial-based research to simultaneously detect relevant pathogens in a given environment. However, some artefacts associated with these nanoparticles, non-specific binding, aggregation, and toxicity must be administered before they reach their full potential and biosensors. A key advantage is that the fast results, because the signal amplification method instead of the target has revolutionized the detection model. These methods, combined with green nanotechnology, promote safe access to drinking water and reduce global health, as well as accelerating potentially existing methods that provide sensitivity, specificity, speed, visibility, and self-cleaning to complement or replace certain criteria. Dealing with environmental issues at an early stage will bear fruit in the long run.

Keywords Nanobiosensors · Toxicants · Pollutants · Nanomaterials · Biosensors

19.1 Introduction

Pollution, a global concern, negatively affects health of human and socioeconomic development. The existence of environment pollutants, mainly viral, bacterial, parasitic pathogens, and their toxins and chemicals, causes serious public health problems (Manisalidis et al. 2020). Biosensors based on nanoparticle are seen as probable tools for precise, rapid, and sensitive detection by interested analysts (biological and biobiological pollutants) (Mokhtarzadeh et al. 2017). Especially, the traditional detection method of waterborne pathogens has many restrictions due to its low concentration and interaction with a variety of enzyme inhibitors in environmental samples (Toribio-Avedillo et al. 2021). Upgrading the cells to the detection level requires an extended incentive period. This chapter mainly focusses on the present state

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of biosensor nanotechnology, its advantages over traditional sensing methods, and therefore the challenges in analyzing environmental samples. The key is to use of nanoparticles as signal generators to extend output instead of increasing the time. The tendency to develop new sensors within the future and their advantages over other environmental monitoring methods also are discussed (Ullo and sinha 2020).

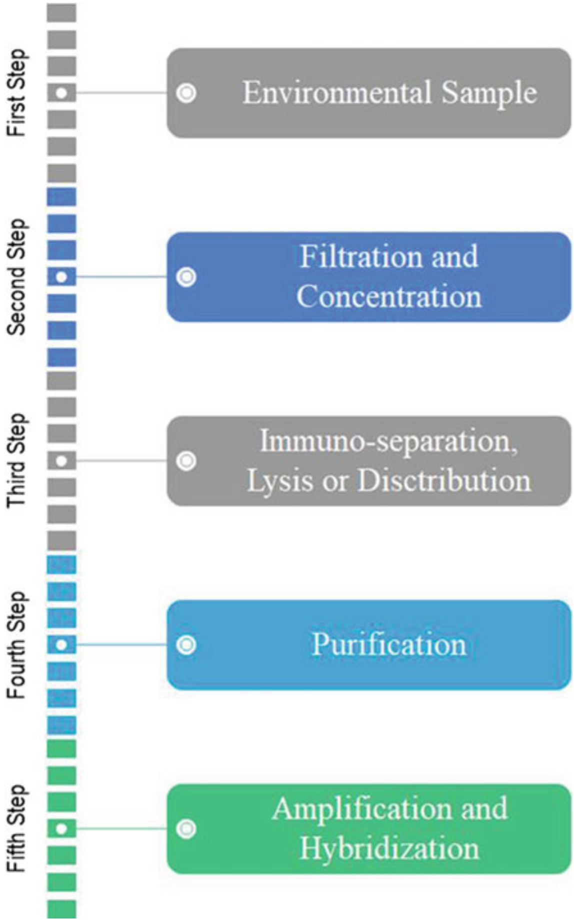
Biosensors have a significant impact on the environment, food, and biomedical applications because they have obvious advantages over traditional theoretical analysis methods, including minimal sampling and processing, real-time sensing, rapid analysis and detection, and efficiency (Maghsoudi et al. 2021). Portable biosensors for food poisoning and environmental pollutants include many biosensor applications in food and the environment, and it provides first-time insights into the latest developments in biosensor technology and chemical sensor technology for researchers (Camarca et al. 2021).

This literature contains useful and up-to-date information for the experts on the latest developments in biological detection tools, development of new technologies, mobile and portable nanosensors (such as dynamic DNA and protein quality) can detect environmental pollutants and pathogens quickly and accurately as seen in Fig. 19.1.

19.2 Noble Metal Nanoparticles in Biosensors

Researchers are more focussed towards biosensors based on precious metal nanoparticles (MNPs) and to explain the basic principles and effectiveness of micronutrients in different classes of biosensors, depending on the mode of transport used (Naresh and Lee 2021). The most important measurements are electrochemical (electrical and potential), optical (surface plasma resonance, chromatography, chemical luminescence, photoelectrochemistry, etc.), and piezoelectricity (Chang 2021). Due to its excellent properties, MNP is attracting attention in biological applications (Zhang et al. 2021). These properties include large surface areas that improve biological concepts and receptor stability, stimulate good interactions and electron transfer capabilities, and provide good biological connectivity. Micronutrient powders can be used alone or in combination with other classes of nanostructures (Jeevanandam et al. 2018). MNP-based sensors can significantly enhance signal amplification, increase sensitivity, and significantly improve the detection and quantification of various biomolecules and ions (Suhito et al. 2021). Several researches on biomolecular sensors with MNP are available, explaining the effects of aristocratic and nanohybrid micronutrient structure, size, and other physical properties on biosensor function (Naresh and Lee 2021).

Fig. 19.1 Showing various steps in environmental monitoring of pathogens and toxicants



19.3 Nanosensors and Nanobiosensors for Monitoring the Environmental Pollutants

Currently, developed and developing countries are concerned about micro-natural resources, especially water resources (Cavicchioli et al. 2019). Therefore, the protection and sustainable use of existing and limited water resources is essential. Agriculture is the most important water use field worldwide. Monitoring and documenting water and soil quality for agricultural projects are done using traditional theoretical analysis techniques that require conventional planning and effort (Manyi-Loh et al. 2018). The need for more precise and general technology is the main driving force behind the use of nanosensors. The ability to incorporate recycled nanomaterials into nanosensors enables different nanosensors to achieve the same goal with high precision without the need for prior model processing (Nagar and Pradeep 2020). New nanosensors are inexpensive and easy to use and can be operated at different precision scales. Nanosensors can monitor and detect physical and chemical parameters or microorganisms in remote areas, as well as environmental conditions (Chelliah et al. 2021). Real-time nanosensors can increase agricultural production by monitoring temperature and humidity at targeted locations and controlling the use of fertilizers/pesticides (Seleiman et al. 2021).

In the digital age, there is a growing need and desire to understand the environment, especially the local quality of water and air. The hurdle in understanding the environment has shifted to the ability to collect all the collected data in order to gather complete information about environmental pollution (Manisalidis et al. 2020). Sensors using nanoparticles reflect a number of technologies developed over the past 15 years to accurately and with high-accuracy detect environmental pollution (Fanti et al. 2021). The ability to routinely quantify nature, which promises simple, inexpensive, and practical technology, becomes a reality.

Researchers, nowadays, are more focussed towards expanding the concepts and values of nanosensors and current developments in applications for the detection of organic and inorganic pollutants, along with statistics for monitoring the environment using nanosensors and nanosensors (Singh et al. 2021).

19.3.1 Nanobiosensors for Assessment of Harvest Index

Physio-chemical characters of horticultural crops (fruits and vegetables) are related with their ripeness. Changes in physio-chemical character and real-time measurement of those characters in crop will guide us the proper harvesting time of crops at maturity stage. Nanobiosensors are used for detection of intrinsic quality characters like total phenolic compounds (Lupetti et al. 2004), vitamin C (Vermeir et al. 2007), and L-arginine (Verma et al. 2015) in horticultural crops. As the crop achieves maturity, it is harvested at proper stage and stored for further processing.

19.3.2 Nanobiosensors for Monitoring of Agriculture Pathogen

Many novel sensors formulated with nanomaterials have been explored in order to achieve high sensitivity and overcome the problem associated with low limits of detection (Rad et al. 2012; Safarpour et al. 2012; Kulia et al. 2011; Pérez-López and Merkoçi 2011; Shiddiky and Torriero 2011). Gold and semiconductive metal oxide nanoparticles-based amperometric biosensors are used to detect diseases causing by different types of microorganism such as bacteria, viruses, and fungi. (Cao et al. 2011; Mandler and Kraus-Ophir 2011; Umasankar and Ramasamy 2013). Biosensors based on nanotechnology can identify issues related to plant health before observable to the farmer like antibody conjugated nanoparticles are used to detect *Xanthomonas axonopodis* causes bacterial spot disease (Yao et al. 2009), optical immunosensors (based on gold nanoparticle), and antibody conjugated fluorescent silica nanoparticles (FSNPs) are used to detect the karnal bunt disease in wheat. It has also been used in detection of *X. axonopodis* pv. *Vesicatoria* causes bacterial spot diseases in *Solanaceae* crop (Yao et al. 2009; Singh et al. 2010). Quantum dots (QD) have unique optical properties and are used for detection of *Candidatus Phytoplasma aurantifolia* (Ca. *P. aurantifolia*) responsible for witches broom disease of lime (WBDL) by using FRET (fluorescence resonance energy transfer mechanism) (Algar and Krull 2008; Frasco and Chaniotakis 2009).

Another approach is that methyl salicylate like volatile organic compounds are synthesized in access quantity during infection by plant, so detection of methyl salicylate like compound which is specific for plant diseases will be more helpful to identify the diseases and to take proper control measures at initial stage itself before forming symptoms (Cao et al. 2011; Mandler and Kraus-Ophir 2011; Umasankar and Ramasamy 2013).

19.3.3 Lipid Membrane-Based Nanosensors for Environmental Monitoring

Recent advances in lipid bilayer stabilization have made it possible to detect a wide variety of compounds from real samples using a variety of lipid film-based biosensor detection techniques (Song et al. 2021). Lipid membrane biosensors have many advantages, such as fast response times (sequences per second) and high sensitivity (i.e., nanomolar detection limits) that can be used to monitor the environment (Naresh and Lee 2021). Because many of these biosensors are inexpensive, easy to use, sensitive, and portable, they can be an expensive, inconvenient, and time-consuming alternative to standard test methods (e.g., chromatographic methods) (Vigneshvar et al. 2016). The double-layer lipid membrane is primarily associated with electronic chemical transducers and uses a number of detection techniques, including ion detection, material transport, electrical activation, fibre channelling, antigen and antibody bonding, phase transition, and modification. Lipid-based platforms are versatile and prone to site, analysis, and modelling (Muller et al. 2019).

19.3.4 DNA-Nanosensors for Environmental Monitoring

Waterborne pathogens, such as *Cryptosporidium paromium*, are serious problems that often require monitoring (Magana-Arachchi and Wanigatunge 2020). The disease affects drinking water, which can lead to serious health problems even at a young age. To prevent the spread of harmful bacteria in the environment, many medical institutes and industry associations check periodically to prevent the spread of harmful diseases. However, these environmental departments require complex testing systems or sensitive, portable, and low-cost packaging (Wang et al. 2017).

Nanoscience researchers have successfully developed microprogenesis, nanosynthesis, and advanced imaging techniques to increase sensitivity and reliability (Jeevanandam et al. 2018). Recently, DNA nanosensors have been used to detect effective germs, toxins, and antibiotics. DNA nanosensors are simple to design and have good quality sensitivity. DNA objects and nanometers can be controlled to detect different types of contaminants (Yang and Duncan 2021). Recent studies focus on the design and structure of DNA nanosensors. Nanosensors could be used for the detection of heavy metals and other hazardous materials (Bahrulolum et al. 2021).

DNA nanosensors, antibiotics, and pesticides are useful for easy and accurate identification of GMOs and other contaminants (Neethirajan et al. 2018). DNA sensors are being researched to find out how many viruses and diseases are there. The DNA nanosensor detects harmless GMOs, lots of antibiotics, and pesticides can be found. Environment analysts on the cost-effectiveness of integrated design and DNA-based nanosensors are most often seen in complex media (Meena et al. 2021) (Table 19.1).

Some medical examinations are performed close to the patient. Electrochemical sensors are designed to measure specific health indicators or to determine whether specific diseases are present in blood or saliva samples (Dave et al. 2021).

19.3.5 Optical Biosensors for Environmental Applications

There is no doubt that the development of biosensor technology over the years has made a significant contribution to protecting human health and biodiversity. However, biosensors are not as good as previously thought, and there are many reasons why it is difficult to make useful and reliable tools (Nam et al. 2021). First, many biosensor systems on sale today are too expensive or too complicated. Second, unlike medical laboratories, natural sample production is complex and varied. The impact of sampling in the case of BIOS should be minimized. Third, long-term monitoring is essential for the protection of bioreactors. Finally, it is important to increase the stability and reliability of biosensors in an effective application (Nguyen et al. 2019).

It has proven to be useful for ophthalmologists on some of the identified types of visual and visual acuity. By combining unique nanomaterials, disease-recognizing properties, and visual modification, it provides a great opportunity to develop next-generation biosensors with new opportunities (Feng et al. 2021). Advances in recent technologies to reduce the number of biological sensors and small wireless communication technologies have greatly improved the long-term awareness of the environment and the neglect of effective pollution mechanisms in local networks (Carlsten et al. 2020). Due to the sensitivity and changes in biosensor errors, visual biosensors are compatible, based on LEDs, and are not suitable for toxic testing, multi-test, and

Table 19.1 Nano-based biosensors for detection of pesticides and chemicals

S. No	Name of NPs	Name of pesticides	References
1	Gold nanoparticles (AuNP)	Herbicide simazine, organophosphate pesticides, methyl parathion, methyl paraoxon, carbofuran, phoxim, dichlorodiphenyltrichloroethane (DDT), rhodamine	Zhang et al. (2017), Wu et al. (2017), Liu et al. (2013), Yin et al. (2009), Lisa et al. (2009), Wei et al. (2016)
2	Fe_3 O_4 functionalized grapheme oxide—AuNP	Catechol hydroquinone	Erogul et al. (2015)
3	4-amini-3-mercaptobenzoic acid functionalized AuNP	Cyhalothrin	Imene et al. (2014)
4	Au-Na dodecylbenzene sulphonate nanoparticles	Methyl parathion	Li et al. (2010)
5	CdTe quantum dots/ AuNPs	Monocrotrophos	Du et al. (2008)
6	Aptamers-based nanoprobes	Acetamiprid	Sassolas et al. (2011)
7	Graphene oxide-copper nanoparticles	Prophenofos, phorate, isocarbophos, omethoate	Fu et al. (2019)
8	Silica nanoparticles	Pyrethroid (3-phenoxybenzaldehyde)	Ye et al. (2018)
9	AuNPs/ERGO-SPCE*	Cyhexatin	Zhang et al. (2019)

* AuNPs/ERGO-SPCE: Gold nanoparticle/electrochemical reduction graphene oxide-modified screen-printed carbon electrode

continuous monitoring of environmental pollution in the field (Dincer et al. 2019). In addition, biosensor researchers can effectively evaluate a variety of environmental factors, including the future and distribution of waste, and provide new information on the production process (Del Valle et al. 2020). The ability to integrate new science and technology into virtual biosensor systems is enormous. I believe that in the future of biosensors will be the most effective way to solve the problems of real life in our daily lives (Dixon et al. 2021).

19.4 Recent Progress in Biosensors for Environmental Monitoring

Analyzing the close link between environment pollution and human health/socioeconomic development, environmental and global monitoring are a priority (Bashir et al. 2020). In this industry, biosensors are used in real-time analytical methods that are cheap and fast. Recent developments in biological

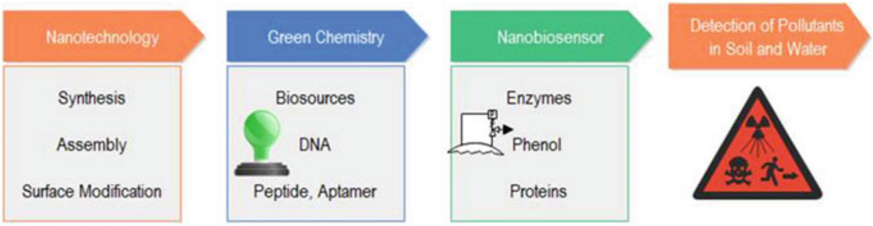


Fig. 19.2 Showing synergy of nanotechnology and green chemistry for the environment monitoring

sensors explain the need for portable, fast, and intelligent biological detection equipment to detect many contaminated components using new nanotechnology-derived communication materials and investments in multidisciplinary professionals as seen in Fig. 19.2 (Mokhtarzadeh et al. 2017). Researchers have examined the latest advances in biological sensors to monitor actual air, water, and soil contaminants such as pesticides, potentially toxic and toxic substances, and glamorous endogenous destructive chemicals (Liu et al. 2018).

19.5 Biosensors a Promising Future in Measurements

A biosensor is an analytical device used to measure the presence of molecules or compounds and convert it into a useful signal (Naresh and Lee 2021). Biosensors use stimuli to transform change into socially acceptable signals. Applications include diagnostic equipment, safety devices, and other critical medical devices. Biosensors can be used to detect pathogens and other bacteria in the food, pharmaceutical, and manufacturing industries (Kaya et al. 2021). Great progress has been made in this area. The research and development of microsystems are associated with new materials such as nanomaterials with biological microorganisms. One of the goals is to create fast and accurate electronic devices (e.g., biofuel cells) for drug use and energy storage (Ramanavicius and Ramanavicius 2021).

19.6 Future Perspectives

The improvement and divergence of nanomaterials have sustained the growth of biosensors for the detection of numerous pollutants. Nanomaterials have extraordinarily applicable and adjustable optoelectronic residences relying on their length and chemical structure. The usage of nanomaterials in fluorescent structures to generate tonnes greater touchy and precise signals. Some of them, which include quantum dots and carbon dots, have excessive fluorescence emission depth and may be used as donors in quenching-primarily, based totally strategies. Meanwhile, nanomaterials which include graphene, graphene oxide, metal nanoparticles, and different inorganic 2D substances which include transition metallic dialcogens and organo-metal frameworks are regularly used as procedure acceptors in fluorescence quenching, with wonderful results. The choice of the maximum appropriate nanomaterial will depend upon the traits of the analyte to be detected, contemplating an element which includes its awareness and chemical nature. To enhance the sensitivity and specificity of biosensors, numerous biomediators had been explored, locating that the aptamer-nanomaterial aggregate generates the excellent results. It need to be borne in thoughts that within side the identical detection machine its miles viable to mix or greater nanomaterials, the usage of those concurrently as donor-acceptors in quenching strategies or as helps for the bio conjugation of different molecules. Environmental monitoring has been a field of interest for the appliance of drones mainly within the monitoring of water and air quality, the surveillance of agriculture, and volcano gas measurements. Sensing systems supported the conjugation of biosensors and drones are required for the environmental monitoring in remote locations thanks to their low-cost, compactness, and low-power requirements. Consequently, there are still few commercial biosensors for environmental monitoring, contrary to clinical applications, which should result to the interdisciplinary context of fabrication additionally as limitations on the in situ operation and on the analytical performance, mainly in reproducibility. Although environmental biosensors are often constructed using the improved characteristics of nanomaterials and novel nanocomposites, an increased attention has been focussed on the in situ and real-time monitoring of pollutants by other technologies.

19.7 Conclusion

Environment pollution is a major problem for the sustainable development of society, health, and economy. The existence of pathogenic microorganism, toxins, heavy metals, pesticides, and organic compound pollutants may be a major environmental issue and public concern. Thus, the environmental sector immediately needs a alternative technique such as nanotechnology-based diagnostic system or test kits analysis which are sensitive, reliable, cost-effective, and portable. Nanoparticles (1–100 nm in diameter) display distinctive properties over the bulk-sized materials and having many important roles in various areas like biomedical, electronic, pharmaceutical, and cosmetic as well as in environment. Agriculture, pollution, food, and natural resources are the component of the challenges to sustainable development. The aim of nanomaterials in agriculture is to reduce the chemical emissions, nutrient deficiencies, and increase productivity by controlling pests, pathogens, and nutrients with the help of nanotechnology. The advantages of nanotechnology have thus a fundamental role keep the environment health safer.

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Chapter 20
Ecotoxicology of Nanomaterials: A Sensor Perspective

Irudhayaraj Savarimuthu, Atirah Tauseef, Adhish Kumar Jaiswal, and Imran Uddin

Abstract Nanotechnology is an emerging field of science and technology with a strong potential to revolutionize the advancement in the field. Various nanomaterials (NMs) have been demonstrated to improve optoelectronic devices, biosensors, batteries, smart fabric, food packaging materials, agricultural yield, etc. No wonder, the consumer products containing NMs are rapidly growing. However, the impact of these NMs with environment, aquatic sources and in particular with humans is largely obscure. The sub-branch of this new emerging field is termed nano-ecotoxicology. The issue has drawn the attention of policymakers around the globe, and there is an increasing attempt to promote such studies by the government agencies. In this chapter, a comprehensive essay is compiled that strings together major aspects of the field. The chapter commences with classification NMs, lists major applications of NMs, and then discusses occurrences of NMs in various consumer products and the projected market size in near future. The major portion in the latter half of the chapter discusses nano-ecotoxicology and the fate of various NMs in aquatic/marine, atmosphere, and human body.

Keywords Nanomaterials · Ecotoxicology · Environment · Sensor · Global market

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20.1 Introduction

“Nano” is a buzzword in the field of science, technology, and engineering. Nanotechnology is a revolutionary step associated with understanding and manipulating materials at scales less than 100 nm to access exotic material properties that are usually not exhibited in materials with over sub-micrometer domain size. Over the past three decades, research in the field of nanotechnology has rapidly advanced. It has yielded a wide variety of engineered nanomaterials (hereafter NMs) that exhibit novel properties at nano-size than their bulk counterparts and thus offer various applications (Cao and Wang 2011).

NMs are making waves in major global economic sectors including energy, electronics, pharmacy, medicine, cosmetics, automotive, agriculture, textile, construction, protective paints, food storage, and defense. NMs make the way to revolutionize various industrial sectors owing to their enhanced mechanical strength, higher electrical and thermal conductivities, highreactivity, increased surface area, durability, antibacterial and antimicrobial properties, catalytic activities, and other traits. Globally, researchers are striving to fabricate novel commercial products incorporated with NMs. Due to the increased production of NMs, there are concerns, in particular, about exposure of humans to NMs that could cause health issues and severely impact the ecosystem (Klabunde and Richards 2009).

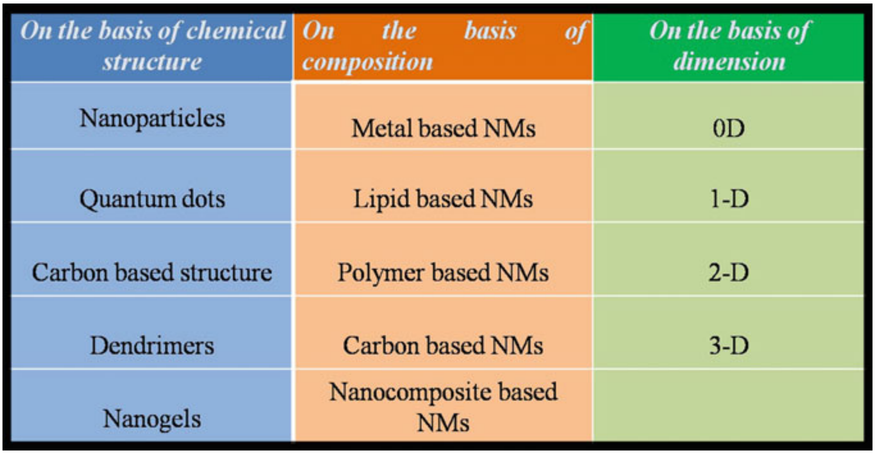
As nanotechnology makes inroads in commercial products, there are increasing concerns about the toxic effects of NMs. In this regard, dedicated attempts are being made to mitigate the toxicological issues. The Organization for Economic Co-operation and Development (OECD) established a regulatory mechanism to address these concerns (Lee et al. 2013). In the US, the NMs are regulated as toxic substances under the EPA’s Toxic Substances Control Act (TSCA) (Wacker et al. 2016). In the European Union, NMs are regulated as hazardous chemical substances under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH), and the Classification, Labeling and Packaging (CLP) regulation. The European Union has imposed regulatory restrictions on the use of carbon nanotubes (CNT) and silver nanoparticles in electronic goods. Thus, the toxicology of NMs has drawn policymakers’ attention to regulate the use and thus limit the release of NMs into the environment. A stringent risk assessment, ideally before introducing NMs in day-to-day products, is urgently required.

Generally, humans are exposed to these nanosized materials either by natural or by artificial sources. Since evolution, humans are exposed to naturally occurring nanosized materials, such as forest fires, dust storms, and volcanoes. However, the ecosystem faces the unprecedented release of NMs through various anthropogenic sources, namely engine exhaust, indoor pollution, building demolition, cosmetics, consumer products, and research laboratories. It is reported that air-borne nano-size particles produced during dust storms cause severe respiratory problems, including asthma. It is found that nanosized dust particles are rich in metals, especially iron, which produces reactive oxygen species (ROS) on the lung surface (Buzea et al. 2007), whereas in anthropogenic sources, the indoor pollution caused by cooking emission, fireplaces, and candles causes severe health effects due to particulate matter inhalation (Morawska et al. 2013). Over the past three decades, the advancement in nano-technological processes and products has been another source of anthropogenic NMs. The research in NMs and its incorporated products continue to evolve, and there exists an uncertainty and concern that these NMs will enter the ecosystem, including water sources, soil, and air and cause health issues in living organisms. Their unique physicochemical properties, reactivity, and catalytic activity may lead to unwanted chemicals, environmental processes, and biological transformation.

Despite exponential growth in facile synthesis strategies and commercialization of NMs, their ecotoxicity is substantially understudied. Some early studies report an alarm call on toxicity and detrimental effects on various aquatic and terrestrial ecosystems (Rocha et al. 2015; McKee and Filser 2016). Ecotoxicology is an interdisciplinary field of science derived from ecology and toxicology. It is devoted to studying toxicants' effects on the ecosystem's structure, function, and biodiversity. Ecotoxicology deals with the migration or transfer of pollutants in air, water, soil, sediments, and the food. The term "Nanotoxicology" reflects the unpredictable effects of NMs interaction with cells, tissues, and organisms (Shvedova et al. 2010). Nanotoxicology is considered as an interface of toxicology and nanomaterial science. As ecotoxicology is dependent on toxicology, the development of the new discipline "nanoecotoxicology" is dependent on nanotoxicology. Nanoecotoxicology is a new subdiscipline of ecotoxicology that deals with two major problems: nanotechnology's safety in the environment and the promotion of sustainable development (Kahru and Ivask 2013). In 2006, the first article on nanoecotoxicology was published; since then, scientific publications about the environmental impacts of NMs have significantly increased. The complexity of NMs lies in their inherent ability to bind with biological materials in the natural environment, their size, unexpected physio-chemical properties, large surface area, etc. The fate and transformation of NMs are global concerns since the interactions of NMs with cells, flora, fauna, water, soil, sediments, air, and humans are involved, which need further studies and sound understanding. Hence, this chapter discusses the occurrence of NMs in the natural environment from various sectors and the behavior of NMs in the natural ecosystem.

20.2 Types of NMs

"NMs" are the key functional elements of nanotechnology. Researchers are developing novel NMs of different morphology, composition, dimension, and functionalities due to the advancements in material science and nanotechnology. The properties of NMs are different from their bulk counterparts because of their extremely small size and shape. The distinct physicochemical properties of NMs can be tuned by tuning the size, shape, functionality, and surface area at the nanoscale. Generally, NMs are classified based on their chemical structure, chemical composition, and dimensionality. Based on the chemical structure, NMs are classified into nanoparticles (NPs), quantum dots (QDs), carbon structures, dendrimers, and nanogels



On the basis of chemical structure	On the basis of composition	On the basis of dimension
Nanoparticles	Metal based NMs	0D
Quantum dots	Lipid based NMs	1-D
Carbon based structure	Polymer based NMs	2-D
Dendrimers	Carbon based NMs	3-D
Nanogels	Nanocomposite based NMs	

Fig. 20.1 Fundamental classification of NMs

(Raghav et al. 2017). Based on composition, NMs are classified into metal, lipid, carbon, polymer, nanocomposite-based NMs. On the basis of dimension, NMs are broadly classified as zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) NMs (Fig. 20.1). 0D NMs include atomic clusters, fullerenes, and cluster assemblies. 1D NMs include nanorods, nanowires, nanotubes, and nanofibers. 2D NMs include nanolayers, nanofilms, nanocoatings, and nanoribbons. 3D NMs include nanoshells, nanoring, and some other complex structures.

20.2.1 Metal-Based NMs

Metal-based NMs include metals, metal oxides/hydroxides, metal chalcogenides, and magnetic substances. Metal NPs are defined as sub-micron-sized particles of pure metal atoms, such as platinum, gold, silver, titanium, iron, zinc, thallium, and cerium or other compounds, such as oxides, hydroxides, sulfide, phosphates, fluoride, and chlorides (Huerta et al. 2016). Silver and gold NPs exist in neutral or cluster forms. Commonly synthesized metal NPs include gold (Au), silver (Ag), nickel (Ni), iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), cadmium (Cd), platinum (Pt), palladium (Pd), etc. Localized surface plasmon resonance (LSPR) is a distinctive characteristic exhibited by plasmonic metal nanoparticles. Bimetallic NPs, a combination of two different metallic elements, have better properties and efficiency than their monometallic NPs counterparts. Few examples of bimetallic NPs are Ag–Ni, Au–Pt, Pt–Ag, Pt–Rh and Ni–Au, etc.

The metal ions of iron, aluminum, titanium, nickel, copper, zinc, tin, cobalt, magnesium, bismuth, cerium, yttrium, etc., provide a wide variety of metal oxide

NPs. These oxide NPs exhibit unique optical and electronic properties compared to their bulk counterparts. Transition metal chalcogenides NMs are another group of chemical entities containing one or more transition metals (Fe, Cu, Cd, Mo, Ru, Ag, Zr, Ta, etc.) and chalcogen (S, Se, Te) as cation and anion, respectively. The chemical and electronic properties of transition metal chalcogenides are intriguing. Copper chalcogenide NMs have unique properties, including semiconductivity, near-infrared LSPR, and non-toxicity.

Quantum dots (QDs) are luminescent semiconductor particles with unique physicochemical properties owing to their highly compact structure (Yadav and Raizaday 2016). Quantum-sized materials of elements, such as silicon, germanium, metal chalcogenides, and metal oxides, generate exotic band structure due to the quantum confinement (Shang et al. 2019).

20.2.2 Lipid-Based NMs

Lipid-based NPs include liposomes, solid lipid NPs, and lipid nanocarriers with the inherent ability to transport hydrophobic and hydrophilic molecules. The liposome consists of a vesicular structure composed of an aqueous phase surrounded by a lipid bilayer. Solid lipid NPs are nanosphere composed of a solid lipid core with a mean photon correlation diameter in the range of 50–1000 nm (Battaglia et al. 2014). Lipid nanocarriers consist of a mixture of liquid and solid lipid matrix instead of just solid lipid NPs. Although there exists a liquid lipid phase, nano-lipid carriers are solid at room temperature.

20.2.3 Polymer-Based NMs

Polymer-based NMs include (i) synthetic polymer NPs, (ii) micelle NPs, and (iii) dendrimers. The natural or synthetic polymeric dispersion or solid particles in the nanoscale are termed as polymeric NPs. These NPs are biodegradable or non-biodegradable, less toxic, and biocompatible, and act as ideal candidates for drug delivery applications. Micelle NPs are core–shell micelle structures made of copolymers in selective solvents. The chemical structure of copolymers dictates the size and morphology of polymeric micelle NPs. Dendrimers are nanosized, highly branched three-dimensional macromolecules with three parts: a central core; an interior surface with highly branched segments; and exterior surfaces with functional groups. Different combinations of these three parts yield end products of different sizes and shapes, which can be optimized for biomedical applications.

20.2.4 Carbon-Based NMs

Carbon is the third most abundant element in the universe, and it plays a vital role in life on the earth. Carbon-based NMs include fullerenes, carbon nanotubes (CNTs), graphene and its derivatives, carbon black, carbon nanofibers, nanodiamonds, and carbon-based quantum dots. Over centuries, graphite and diamond are the two only known allotropes of carbon. In 1985, with the discovery of "fullerene," a third allotropic form of carbon was added to the carbon allotrope family. Kroto et al. discovered the first fullerene having a cluster of 60 carbon atoms by laser-induced graphite vaporization (Kroto et al. 1985). Fullerenes are zero-dimensional, symmetrical nanocarbon cage structures with pentagonal and hexagonal ring arrangements. The most common fullerenes are C_{60} and C_{70}. Carbon nanotubes (CNTs) are another allotrope of carbon, which are cylindrical rolled-up sheets of single-layer carbon (graphene) in nano-size. CNTs exist as single-walled nanotube (SWNT) and multi-walled nanotube (MWNT). CNTs have the inherent ability to attach to various biomolecules like proteins, nucleic acids, and drugs. Interestingly, CNTs exhibit attractive properties, such as ultra-lightweight, high strength, high thermal conductivity, high aspect ratio, and their electronic behavior ranges from a metal to a semiconductor.

In 2004, Novoselov et al. discovered a new allotrope of carbon called "graphene" from graphite. Since then, the application of graphene has been investigated in all areas (Novoselov et al. 2004). Graphene is an atomic thin layer of carbon atoms forming a honeycomb lattice. Some common derivatives of graphene are graphene oxide (GO), reduced graphene oxide (rGO), graphane, fluorographene, graphyne, and graphdiyne. GO is a monolayer sheet of graphite oxide with various oxygen-containing functional groups; this makes it an ideal starting material for graphene mass production. Depending on the degree of oxidation, GO acts as a semiconductor or an insulator, and their optical and electronic properties can be tuned in a wide range. GO consisting of oxygenated functional groups ireduced to obtain new material called rGO. Combining GO and rGO with other materials makes it possible to tailor new material properties for commercial applications. A hydrogenated derivative of graphene, with CH's chemical composition, is named graphane (hydrogenated graphene). Doping of graphene with alkali metals shows semiconductor property, while doping with alkaline earth metals show metallic property (Hussain et al. 2012). A fluorinated derivative of graphene is called fluorographene (fluorinated graphene). A monoatomic layer of carbon hexagons bonded by two linear acetylenic carbon chains is termed graphdiyne. One-third of the carbon–carbon bond in graphene structure is removed and replaced by acetylenic carbon chains to give a new structure, named graphyne. The mechanical properties of graphyne and graphdiyne are influenced by acetylenic linkages (Cranford and Buehler 2011).

20.2.5 Nanocomposites (NCs)

Nanocomposites are multicomponent hybrid materials that have at least one physical dimension in nanoscale. NCs are formed by mixing clays, metals, oxides, inorganic nanoclusters, or semiconductors with organic and organometallic compounds, biomolecules, organic and derived polymers, and enzymes. NCs, which contain polymer or polymer-derived material, are called polymer-based nanocomposites, while those without polymer or polymer-derived material are called non-polymer-based nanocomposites. The enhanced mechanical properties of NCs are widely utilized in automotive and industrial applications (Naskar et al. 2016).

20.3 Environmental Occurrence of NMs

In recent years, rapid advances in nanotechnology have raised concerns on environmental occurrence, behavior, and ecotoxicity of NMs in the natural environment. A critical study of physical, chemical, and biological properties of routinely used NMs is urgently required to address the concerns. NMs are emerging functional materials for various applications, including pharmaceuticals, energy conversion and storage, optoelectronics, sensors, construction, pollution control, etc. (Fig. 20.2).

20.3.1 Nanomedicine

However, modern medicine has advanced at an astonishing pace and has helped in increasing life-expectancy. A cure for incurable diseases, epidemics, pandemics, etc., suffers from disadvantages, such as side effects, drugs administered deaths. It may inadvertently cause sickness rather than promoting health. Nanomedicines can overcome these disadvantages. A wide range of NPs, including metal NPs, silica NPs, lipid-based NMs, dendrimers, polymers, nanogels, carbon-based NMs, etc., are utilized to enhance the pharmacological and therapeutic properties of common drugs.

Metal NPs are considered promising for biomedical research due to their exceptional physio-chemical properties, large surface area, photothermal potential, electrostatic charge, and so on. Metal NPs (Au, Ag, Ni, Pt), metal oxides (Fe_2O_3, Fe_3O_4, ZnO, TiO_2), silica NPs, etc., are used in biomedical research and applications. In the future, medicines based on nanotechnology have significant potential to increase the growth of the pharmaceutical market, targeted drug delivery systems, and bioimaging. However, there is a substantial gap between the results at the research laboratory level to an actual hospital setting. Table 20.1 shows the list of nanomaterials and their possible potential applications in diverse fields.

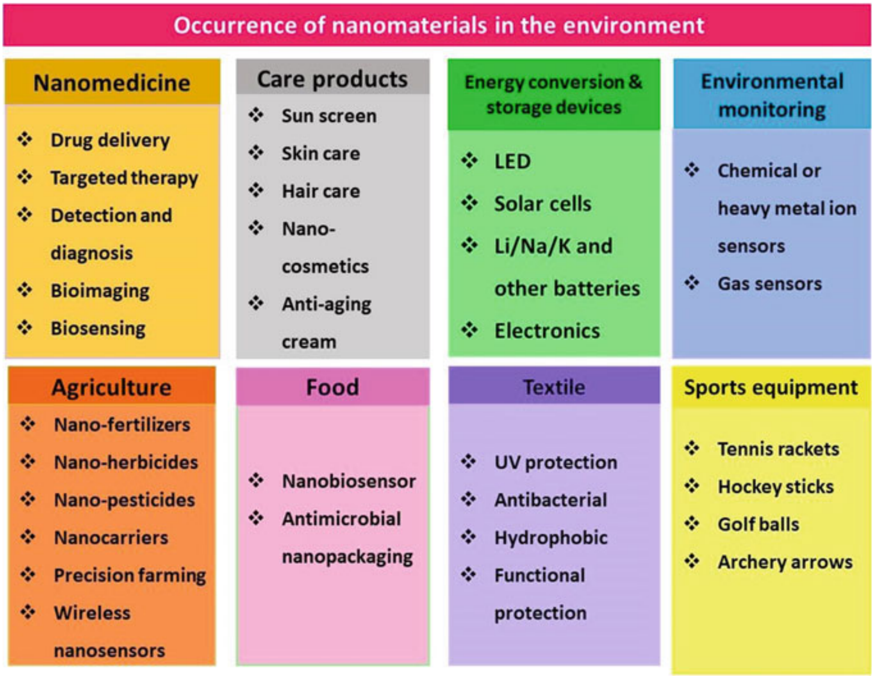


Fig. 20.2 Occurrence of NMs in the environment

Table 20.1 NMs and their potential applications

NMs	Potential applications	Refs.
Ag NPs	Drug delivery systems, catheters, dental applications, bone healing, wound healing, and other medical applications	Burdușel et al. (2018)
Superparamagnetic iron-oxide nanoparticles (SPION)	Targeted drug delivery, molecular and cellular imaging, oncology therapy, and other biomedical applications	Dulińska-Litewka et al. (2019)
ZnO NPs loaded mesoporous silica nanolayer	Treatment of drug-sensitive breast cancers cells and applied in drug delivery system and imaging	Ruenraroengsak et al. (2019)
Silicon NMs	Biosensing, bioimaging, lesion detection, cancer staging, and treatment in humans	Phillips et al. (2014)
Lipid NMs	Drug delivery system	Naseri et al. (2015)
Dendrimers	In vitro and in vivo bioimaging	Yue et al. (2019)

20.3.2 Care Products

The use of small-sized particles in personal care products is not new to the cosmetics industry. In recent years, there is a substantial increment in the personal care products claiming to utilize different NMs. NMs provide an additional benefit in many dermatological applications. Ultra-small spherical vesicles called liposomes are already in the market for advanced clinical studies (Lasic 1998). The size of liposomes is between 100 nm to several micrometers. They are used in drug delivery, therapeutic drugs, and cosmetic materials in body lotion. The standard product based on NMs is sun care products. The nano-formulation sunscreen products of ZnO or TiO_2 are used as new physical filters of harmful UV radiation. The nano-formulation improves reflection and lowers refractive index; therefore, modern sunscreens containing ZnO or TiO_2 NPs are relatively muddier compared to micron-sized formulations that are usually bright white. The whitening effect is undesirable; hence, the ZnO or TiO_2 NPs smaller than the wavelength of light is used to eliminate the whitening effect. Both ZnO and TiO_2 incorporated sun care products from different manufacturers are already available in the market.

Furthermore, nanoemulsions with penetration effect and biophysical properties are used in sunscreens, deodorants, skin and hair care products, and cosmetics delivery to the skin (Khezri et al. 2018). Hydrogels are hydrophilic polymeric networks that swell in water without dissolving. The hydrogels are used as cosmetics delivery systems for skin. Niosomes, a non-ionic surfactant-based vehicle, is used as a nanoparticulate drug delivery agent. Lipid NPs act as a promising candidate for dermatological applications. Solid lipid NPs (SLN) and nanostructured lipid carriers (NLC) play a significant role in their sub-micron size and pearl-like nature. These lipid particles show adhesiveness; when in contact with the skin surface, they form a thin layer, having a size-dependent surface area. By manipulating the size of lipid NPs, different effects can be obtained. The pearl-like shape of lipid NPs gives a lubricant effect to the cosmetics. The lubricant and mechanical barrier properties of NPs help in irritation and allergic reactions.

Further, highly acidic or basic cosmetic formulations will act as irritants; in such cases, dispersions of lipid NPs with optimum pH are used for topical application (Souto and Müller 2008). Other vital features of SLN and NLC are their solid matrix, which helps to stabilize active ingredients. Moreover, lipid NPs formulation provides a whitening effect on the skin surface and acts as a promising pharmaceutical delivery agent.

A nanotech-based approach, to in-home pregnancy tests, uses gold nanoparticles' unique optical properties resulting from surface plasmons. In the early 1990s, the first Au NPs-based in-home pregnancy test kits to produce a pink color when exposed to certain hormones secreted by pregnant women was marketed by Carter-Wallace and are still available through Church and Dwight (Jans and Huo 2012).

20.3.3 Environmental Monitoring

Nanosensors are nanostructured materials employed for monitoring chemical and biological phenomena in the environment. Based on the application, nanosensors are classified as chemical sensors and biosensors, and others.

20.3.3.1 Chemical Nanosensor

Chemical nanosensors are applied to analyze a chemical species, namely heavy metal or molecules in the environment. The term “Heavy metals” are coined for metals in the form of elements or ions or complexes with a density of 4 g/cm^3 or the density five times greater than that of water (Ugulu 2015). The classification of most toxic heavy metals, as per Environmental Protection Agency (EPA) of the USA, include arsenic (As), mercury (Hg), copper (Cu), lead (Pb), nickel (Ni), cadmium (Cd), and chromium (Cr). The presence of heavy metal, including of a low concentration, causes adverse environmental and health issues. Although numerous traditional instrumentation methods are available to estimate different heavy metal ions, they suffer from the need of sophisticated instrumentation, complex sampling processes, and high cost. Chemical sensors have the merit of on-site detection of multiple heavy metals. In particular, NMs based chemical sensors are prominent in detecting heavy metals due to their unique physicochemical properties, such as size, surface area, catalytic efficiency, reactivity, and absorption capacity. Various NM-based chemical sensors include optical and electrochemical sensors.

Optical chemical sensors include colorimetric, fluorescent, plasmonic, and SERS sensors applied for environmental applications. Electrochemical sensors are applied in health, environmental, and food safety monitoring applications. Irrespective of the type of nanosensor, commonly used NMs are metals, plasmonic metals, metal oxide, metal chalcogenides, and carbon-based NMs. Metal NMs of gold (Au), silver (Ag), copper (Cu), platinum (Pt), palladium (Pd), cobalt (Co); rare earth metals; metal oxides of Zn, Ti, Cu, Sn, In, and W; and carbon-based NMs of CNT and graphene, have been employed for optical and electrochemical sensing of heavy metal ions (Maduraiveeran and Jin 2017; Ullah et al. 2018).

20.3.3.2 Gas Nanosensors

Gas nanosensors are applied to detect various gas species, namely NH_3, NO_2, and humidity, in the environment. For example, Ag nanocluster/polyaniline functionalized MWCNTs have been developed for rapid, trace-level confirmation of NH_3 at room temperature (Abdulla et al. 2017). NO_2 is a prominent toxic gas released in the environment from the automotive and industrial sector. Metal oxides and two-dimensional transition metal chalcogenides are widely applied for the rapid sensing of NO_2 gas (Kumar et al. 2020). Semiconductor metal oxide gas nanosensors have shown remarkable gas sensing efficiency (Aroutiounian 2019). Graphitic carbon nitride (g–C_3N_4)-based NMs have demonstrated prominence as metal ion sensors, gas sensors, biosensors, and humidity sensors (Wang et al. 2019).

20.3.4 Agriculture

NMs have immense potential to influence the agricultural sector. Nanotechnology is highlighted as cutting-edge technology for sustainable development, food safety, security, and agricultural waste recycling.

NMs in agriculture has immense potential for (i) preparing nano-fertilizers/nutrients for greater nutrient uptake efficiency by plant pores, (ii) developing nanocarriers for reducing the amount of pesticide consumption, (iii) fabricating nano-biosensors for controlled release of fertilizers and other agrochemicals, and (iv) enhancing water uptake efficiency in the plant by nano-silicon (Dwivedi et al. 2016).

In recent years, it is believed that precision farming based on small micro-electromechanical systems (MEMS) called "smart dust" is considered as emerging nanotechnology for agriculture. Smart dust is a wireless computer network consisting of sensors, robots, and transponders that can detect light, temperature, magnetism, vibration, and chemicals. This smart dust can be sprinkled on the farm, connected to farm equipment and computers to monitor the crops, soils, and livestock.

Increased food grain production depends on several factors, such as proper irrigation, good quality of seed and fertilizers, etc. Imbalanced availability of soil nutrients or fertilizers is often very challenging in agricultural production. Nano-fertilizers are breakthroughs in agricultural applications. Nano-fertilizers are nanoporous materials coated with thin polymer film where nutrients can be encapsulated and delivered in a slow or controlled manner (Rai et al. 2012). Conventional herbicides and pesticides are more expensive and cause water pollution, whereas nanoherbicides and nano-pesticides are considered eco-friendly ways to solve weed and pest problems. Various NMs, such as nano-alumina, nano-silica, NPs of copper, TiO_2, CeO_2, and silver, are widely applied in agricultural applications due to their antibacterial and antimicrobial properties.

20.3.5 Antimicrobial Nanopackaging Material

In today's world, the food packaging material is the outcome of several years' evolution, and during this time, various types of materials have been used, namely paper bags, cardboard boxes, wooden and glass containers to modern polymers. Traditional packaging materials are used as inert and passive barriers to prevent moisture, oxygen, and contaminants from interacting with the food product, thereby enhancing the shelf life of food. These traditional packaging materials suffer from demerits, such as lack of recyclability, average mechanical, and barrier properties. The biggest challenge faced by food producers and suppliers is to increase the shelf life of their products, while retaining the freshness, nutritive qualities that are acceptable to the consumers. In order to address these food packaging issues, nanotechnology has been utilized in academia and industry. NMs can improve the packaging materials' properties in terms of antibacterial, antimicrobial, mechanical strength, UV resistance, stability, transparency, biodegradability, flexibility, and improved barrier properties. Various NMs are employed for food packaging and safety, such as polymer-based nanocomposite, active and intelligent packaging, nanocoatings, nanosensors, nutrition, and nutraceuticals.

Antimicrobial and antibacterial properties of metal NPs (silver, gold, zinc, or metal oxides) are utilized in food packaging applications (Mihindukulasuriya and Lim 2014). For example, ZnO NPs are effective against foodborne pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus cereus* (Kim et al. 2020). In recent years, a lot of attention has been devoted to nanocoating in food packaging in order to exploit their antimicrobial and barrier properties. In nanocoating, the NMs or nanocomposite can be applied or incorporated on the surface of packaging materials. For example, silica NPs and metal oxide NMs, such as ZnO, Fe_3O_4, and TiO_2-based coatings, are being explored for food packaging applications.

In recent years, the efficiency of food packaging materials made of polymeric composites has improved by adding NMs as fillers to form nanocomposites. Various NMs employed to improve the properties of the polymer include clay, nano-clay, montmorillonite (MMT)-based organoclay, silica, nano-silica, $KMnO_4$, graphene, nitrocellulose, nanofibrillated cellulose, silicon carbide, and CNTs. For example, the incorporation of fillers, such as nanoplatelets into the polymer, improves the barrier properties (Vasile 2018). Generally, the nanofillers are impermeable to less permeable for gases and moisture than the pure polymer matrices. Moreover, nanocomposite for food packaging applications improves thermal stability, fatigue, barrier properties, stiffness, tensile strength, optical properties, and antimicrobial properties.

The goal of active and intelligent packaging is to enhance the monitoring of packaged food in the environment by dynamically reporting the food's quality through signals. In these systems, food materials' shelf life is enhanced by incorporating active NMs with inherent properties, such as antimicrobial, water vapor and oxygen absorbers, and ethylene removers.

20.3.6 Textiles

The history of natural fibers and fabrics dates back to ancient times. The textile industries are one of the principal industries for consumer goods generating apparel, household textiles, furnishing textiles, and technical textiles. Fibers are the building blocks from which fabrics are made with different characteristics. Nowadays, technology has made its way to revolutionize the textile industry that could improve or add special functions and properties to the fabrics. NMs have tremendous potential to improve the existing characteristics or add novel functionalities to textiles, namely water, and dirt repellency, UV protection, wearable electronics, wear and wrinkle resistance, flame retardant, antibacterial, or antifungal activity, etc. NMs are new functional materials added to the textiles either during the fiber production or during the finishing. Nano-TiO_2 is currently used for its functional benefits, namely UV protection, antibacterial, photocatalytic, conductive, and hydrophobic properties (Zahid et al. 2018; Yu et al. 2019). Nano-SiO_2 is incorporated into coating materials for making the fibers superhydrophobic, flame-retardant, and abrasion-resistant (Lahiri et al. 2019; Zhou et al. 2020). Nowadays, integrating functional electronic materials or devices, such as mobile communication technology, audio players, display, and light effects into conventional textiles, is also being explored. For example, several wearable fabric-based piezoelectric nanogenerators and triboelectric nanogenerators are integrated into fabrics to endow smart bestow smart textiles applications in artificial intelligence (Dong et al. 2020).

20.3.7 Sports Equipment

The incorporation of NMs in sports equipment provides several benefits, namely sturdiness, lightweight, and efficiency. Sports equipment, such as tennis/badminton rackets, golf balls, archery arrows, hockey sticks, fishing rods, etc., are a few types of equipment where the use of NMs enhances the performance of equipment. CNTs are the most promising NMs exploited for sports equipment because of its higher specific strength, strength, and stiffness (Zhang et al. 2016). Furthermore, fullerenes, nano-SiO_2, and nanocomposites are being explored in manufacturing nanotechnology-based sports instruments.

20.4 NMs in the Global Market

Rapid advancements in nanotechnology have resulted in numerous nanotechnology-based consumer products. NMs have been extensively incorporated into consumer products. However, the potential effects on the environment and human health are either unknown or lack proper investigation.

The project on emerging nanotechnology (PEN) created the Nanotechnology Consumer Product Inventory (CPI) in 2005 with the support of Woodrow Wilson International Center for Scholars, listed 54 nanotech-based consumer products (Vance et al. 2015). In 2010, the CPI listed 1012 consumer products marketed by 409 companies in 24 countries. In 2007, the National Institute of Advanced Industrial Science and Technology created a similar inventory that tracks “nanotechnology consumer products” marketed in Japan (Michelson 2013). In 2012, “The Nanodatabase,” an inventory of NMs claimed consumer products available in the European market, was created by the Danish Consumer Council and Ecological Council and the Technical University of Denmark's Department of Environmental Engineering. The nanodatabase inventory is continually being updated, and it currently lists 4443 products.

Research studies forecast that the global nanotechnology market would exceed US$ 125 billion by 2024. The share of nanoparticles to the global nanomaterials market is projected to be 85%. NMs applications in energy, electronics, and biomedical would account for 70% of the global NMs market by 2024 (Liu and Xia 2020). It is expected that the most extensive application of NMs would be in the electronics sector. It is projected that nanotechnology advancements will make way for the next industrial revolution, and commercial products containing synthetic NMs would increase exponentially in the near future.

Consumer Products Inventory (CPI) listed "1814 consumer products from 6222 companies in 32 countries." Allied Market Research projects that the global market for nanomaterials consumer products is expected to grow to half a billion US$ by 2022. In Fig. 20.3, a bar chart is presented as the number of products versus application. Nanotechnology Products Database (NPD) listed 8860 products from 2453 companies in 62 countries in various applications.

The global NMs market is expected to grow in several industrial subdivisions, such as sensors, masonry materials, skincare, pharmaceuticals, water, wastewater treatment, maintenance, laser, circuits, and processors. Because of its antimicrobial and antibacterial properties, nanosilver is among the most popular NMs used in various fields, namely electronics, personal care products, and textiles. Global Market Insights Inc. analysts estimated that Ag NPs are the most commercialized NPs, accounting for over 50% of the global NM incorporated consumer products in 2015

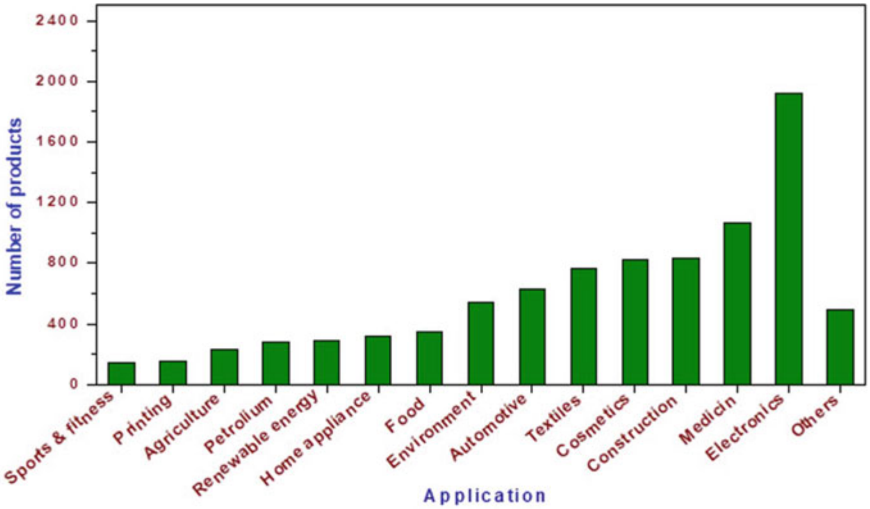


Fig. 20.3 Number of NMs incorporated products in various applications (Rocco et al. 2015)

(Inshakova and Inshakov 2017). Based on mass, TiO_2, SiO_2, and ZnO are the most produced NMs worldwide, and the global annual production of (i) TiO_2 is up to 10,000 t/year, (ii) CeO_2, FeO_x, AlO_x, ZnO, and CNT between 100 and 1000 t/year, (iii) SiO_2 more than 10,000 t/year, and (iv) Ag NPs are only 2% of that of TiO_2 (Piccinno et al. 2012). The industrial application of nano-clay and nanocellulose is expanding. The Transparency Market Research (TMR) studies estimate the global market revenue of nanoclay is expected to grow by 24.9% and of the nanocellulose market by 19% by 2020. Moreover, geographical location plays a significant role in the commercialization of NMs. For example, in the USA, active food packaging is applied to increase shelf life. It is acquiring at a relatively slow pace in Europe because of the legislation restrictions and lack of knowledge about consumer acceptability and environmental and economic impact.

20.5 Behaviors of NMs in the Environment

20.5.1 In the Aquatic Environment

The use of NMs in numerous research laboratories and industries, therapeutics, storage devices, electronics, agriculture, and consumer products is increasing at an alarming rate. There is an uncertainty about environmental toxicity, occupational and public exposure, organism exposure, and the behavior and fate of NMs in the ecosystem.

20.5.1.1 Bioconcentration, Bioaccumulation, and Biomagnification

In an aquatic ecosystem, three phenomena, namely bioconcentration, bioaccumulation, and biomagnification, are commonly used in ecotoxicology. When the concentration of toxic substances or pollutants in an organism becomes higher than its concentration in the surroundings (air or water), it is classified as bioconcentration. The uptake and accumulation can be hazardous to the organism and other organisms in the food web. Bioaccumulation is a process of enrichment and accumulation of a pollutant in an organism, relative to that in the environment. Biomagnification is a process by which pollutants magnify as they move from one trophic level to another. However, there are several differences between the uptake of various NMs and traditional dissolved toxic substances.

One fundamental concern is transformations of NMs surface by chemical and biological reactions with the environment. In an aquatic ecosystem, the formation, behavior, and fate of corona on the surface of NMs are poorly understood. Further, the fate and behavior of NMs depend both on their physicochemical properties and characteristics of the receiving environment, including the concentration of natural organic matter, water hardness, ionic strength, salinity, pH, temperature, and so on.

In an aquatic ecosystem, the formation, behavior, and fate of corona on the surface of NMs are poorly understood. The unique physicochemical properties and environmental processes may alter the fate and toxicity of NMs. Ecotoxicity studies revealed that several NMs, including metal NPs, carbon-based NMs, nano-ZnO, nano-SiO_2, and nano-TiO_2 are toxic to the ecosystem, even at low concentrations. Hence, nano-ecotoxicology research began to focus on: (i) environmental fate on interactions with contaminants; (ii) biological fate in living organisms; and (iii) ecotoxic effects on living organisms. Adsorption, dissolution, transformation and aggregation, oxidative peroxidation, production of ROS, and surfactant or capping agent properties are essential environmental processes for determining the fate of NMs. The safety of NMs encompasses both human health risk assessment and ecological risk assessment of NMs.

20.5.1.2 Biophysicochemical Behavior and Fate of NMs in the Aquatic Environment

The environmental behavior of NMs is decided by environmental processes, including adsorption, aggregation, dissolution, and surface transformations. Various other factors, such as natural organic matter, ionic strength, pH, dissolved salts, hardness, light, other contaminants, microorganisms, and electrolytes, dictate the physicochemical interactions between NMs and the released environment. Adsorption is a critical phenomenon at the NMs–water interface because of the large surface area and other physicochemical properties. This process can adversely affect natural organic matter's mobility and fate (e.g., humic acid, fulvic acid, amino acids, proteins) and pollutants (e.g., heavy metals, organic compounds). The molecular interactions responsible for the interaction of NMs and environmental adsorbates (e.g., natural organic matter, inorganic ions, organic molecules, micro- and macromolecules, mineral particles) include π bonding, electrostatic interaction, hydrogen bonding, Lewis acid–base interaction, and hydrophobic effects. Further particle–particle interactions and particle–substrate interactions are responsible for the aggregation and deposition of NMs. For example, in a natural environment, competitive adsorption inevitably occurs between NMs and natural organic matter (NOM). The NOM forms a corona layer over the surface of NMs and thereby decreases adsorption capacity and blockage of pores. The NM-NOM is the new active entity that will influence the fate of NMs.

Next, the fate, transport, and biotransformation of NMs in the aquatic environment are highly dependent on their aggregation state. The surrounding environment of NMs affects the colloidal stability of NMs, mostly metal NPs, and causes dispersion, aggregation, disaggregation, and sedimentation in an aqueous medium. The process of aggregation of NMs plays a significant role in the dissolution of NMs. The NOM, dissolved oxygen, and metal ions play a vital role in the dissolution of metal and metal oxide NPs (Wang et al. 2016). Further research studies have demonstrated that the sedimentation of NMs depends on various environmental factors, including pH, electrolytes, ionic strength, temperature, and NOM. For example, electrolyte concentration and type and NOM concentration influence the interaction and stability of ZnO NPs. Table 20.2 illustrates the fate of NMs in the aquatic environment.

20.5.1.3 Biophysicochemical Behavior Between NMs and Microorganisms

The physicochemical characteristics of NMs dictate the interactions between NMs and microorganisms. In particular, alga and bacterium are the two common vulnerable microorganisms in nanotoxicity studies and the food chain. Generally, microorganisms carry negative charges on their surfaces; hence, adsorption may occur when they meet positively charged NMs, such as CeO_2 NPs, SiO_2 NPs, etc. The adsorption process is because of the electrostatic force of attraction. For example, (i) positively charged Fe NPs interact with negatively charged cyanobacterial cells; and (ii) metal oxide NPs (Al_2O_3 and CeO_2) interact with negatively charged bacterial cells. In general, NMs may not directly attach to microorganisms' cell walls; instead, they interact with the extracellular substances, such as polysaccharides and proteins. The bacterial cell walls and extracellular substances consist of carboxyl, amide, phosphate, and hydroxyl functional groups, which act as active sites for molecular-scale interactions. The molecular interactions are highly influenced by interaction energy, including van der Waals forces, electrostatic interactions, specific interactions, and acid–base forces. These forces vary with the environment and subsequently influence the adsorption. The adsorption of NMs on microorganism surface could result in physical, biochemical, and oxidative damage, blockage of nutrient uptake, and affect cell metabolism. Internalization of NMs occurs depending on bio physiochemical characteristics of NMs and the physical property of cells. Table 20.3 shows the nano–bio interactions between NMs and microorganisms.

20.5.1.4 Biophysicochemical Behavior Between NMs and Hydrophytes

Hydrophytes refer to the plants that live in or on the water, including emergent plants, floating-leaved plants, and submerged plants. In an aquatic ecosystem, hydrophytes are the primary producer, the uptake of NMs may affect the food chain and food web through the trophic levels and harm the entire aquatic photosynthetic system. These plants possess a layer of ceraceous substances or cuticles on the leaves' surface, which acts as a useful adsorption site for NMs. Further, the roots are the most vulnerable part of the plant where adsorption and internalization of NMs can occur. Once adsorption occurs, the internalization process starts, and NMs would likely be transported to various other parts of the plants, such as shoots, epidermis, vacuoles, mesophylls, and gas spaces. Table 20.4 highlights the nano–bio interactions and NMs based toxicity to microorganisms.

Table 20.2 Fate of NMs in the aquatic environment

NMs	Chemical factors	Mode of interaction	Environmental process	Fate	Refs.
Ag NPs	NOM, pH, Au^{3+}	Galvanic replacement	Precipitation and alloying	Bimetallic Ag–Au alloy	Sharma et al. (2019)
Ag NPs and TiO_2 NPs	Under dark	Inter- and intramolecular bridging effect	Hetero-aggregation	Ag/TiO_2	Sharma et al. (2019)
	Under light	Illumination generates reactive oxygen species (ROS)	Dissolution	Release of Ag^+ ions	
TiO_2 NPs	Humic acid (HA) and Pb^{2+}	Complexation (HA-Pb^{2+})	Aggregation	Aggregated TiO_2 NPs	Sharma et al. (2019)
Cu NPs	NOM	Complexation	Dissolution	Release of Cu^+ ions	Wang et al. (2015)
Au NPs	HA and electrolytes	Electrostatic and/or steric repulsion	Adsorption	Au-HA	Stankus et al. (2011)
Graphene oxide (GO)	Minerals	Electrostatic attraction	Hetero-aggregation	GO-Mineral	Zhao et al. (2015)
GO	Sunlight	Photo-disproportionate	Reduction	Reduced GO	Hou et al. (2015)
CdSe/ZnS QDs	Aphotic	Complexation with chelators	Dissolution	Release of Cd and Zn ions	Paydary and Larese-Casanova (2020)
MWCNTs	HA	Steric repulsion	Adsorption	MWCNTs-HA	Saleh et al. (2008)
C_{60} fullerenes	Environment conditions and sunlight	Van der Waals forces	Degradation	Degraded products	Sanchís et al. (2018)
Nanoplastic	NOM, Fe_2O_3, alienate	Coulombic attraction	Hetero-aggregation	Hetero-aggregates	Oriekhova and Stoll (2018)

Table 20.3 Nano–bio interactions between NMs and microorganisms

NMs	Microorganisms	Mode of interaction	Environmental bioprocess	Toxicity	Refs.
Ag and ZnO NPs	*P. aeruginosa*, *B. subtilis*, *B. barbaricus*, *K. pneumonia*, and *M. luteus*	Electrostatic attractive force	Aggregation	Growth inhibition	Dhas et al. (2014)
CuO NPs	*S. coelicolor* M145	Electrostatic attractive force and internalization	Dissolution	Oxidative stress, ROS, and dissolved ions	Liu et al. (2018)
ZnO NPs	*B. subtilis*	Van der Waals and hydrogen bonding electrostatic repulsion	Adsorption and internalization	Oxidative stress, ROS, and dissolved ions	Leareng et al. (2020)
TiO_2 NPs	*Scenedesmus*sp and *Chorella*sp	Electrostatic attraction	Adsorption	Growth inhibition	Sadiq et al. (2011)
CeO_2 NPs	*Pseudokirchneriellasubcapitata*	–	Adsorption and internalization	Oxidative stress	Rogers et al. (2010)
SiO_2 NPs	*Scenedesmus obliquus*	–	Sorption	Shading, photosynthetic activity inhibition	Wei et al. (2010)
Al_2O_3 NPs	*Escherichia coli*	Electrostatic repulsion	Adsorption and internalization	Transfer across peptidoglycan layer	Simon-Deckers et al. (2009)
Zero valent Fe NPs	*Cyanobacteria*	Electrostatic attraction	adsorption	Oxidative stress, shading effect	Chen et al. (2011)
CdTe/CdS QDs	*Chlamydomonas reinhardtii*	–	Adsorption and internalization	Oxidative stress and release of Cd^{2+}	Domingos et al. (2011)
GO-waste water	*Chlamydomonas reinhardtii*	Hydrophobic interactions	Adsorption	Mitochondrial ROS formation	Martín-De-Lucía et al. (2018)
MWCNTs	*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*	sorption	Adsorption	Membrane damage	Hartono et al. (2018)

Table 20.4 Nano–bio interactions and NMs based toxicity to microorganisms

NMs	Aquatic plant	Environmental bioprocess	Toxicity	Refs.
Ag NPs	*Lemnagibba*	Adsorption and internalization	Growth inhibition due to intracellular ROS generation	Oukarroum et al. (2013)
CuO NPs	*Eichhorniacrassipes*	Adsorption, internalization, and transformation to Cu_2S and Cu species	Damages root caps and meristematic zone	Zhao et al. (2017)
ZnO NPs	*Hydrillaverticillata* and *Phragmitesaustralis*	Adsorption and internalization	Phytotoxicity	Song and Lee (2016)
CdTeQDS	*Lemna minor*	Adsorption and internalization	Growth rate inhibition and biomass inhibition	Modlitbová et al. (2018)
TiO_2 NPs	*Spirodelapolyrrhiza*	Adsorption and internalization	Growth inhibition and damages plant defense system	Movafeghi et al. (2018)

20.5.1.5 Biophysicochemical Behavior Between NMs and Fishes

Fish, the primary consumers of the aquatic ecosystem, are vulnerable to NMs. NMs enter the fish from their surroundings and also through NMs-exposed microorganisms and invertebrates. Adsorption and internalization are the two primary pathways of NMs for fish. Compared with other microorganisms or invertebrates, fish are likely prone to accumulation of NMs because of their larger body. Skin, the outermost layer of the surface, contains an epidermis and dermis, where the former secretes mucus that covers the fish's body.

Table 20.5 shows the nano–bio interactions of NMs with fishes and their toxicity. The various physicochemical behavior between NMs and fish include; (i) NMs, especially nonspherical-shaped NMs, are likely to get tangle with strands of mucus; (ii) scales can also act as adsorption sites; (iii) gills the respiratory organ can allow NMs in addition to water, and air (iv) mucus layer on gills can act as trapping sites (Handy et al. 2008).

20.5.2 *Biophysicochemical Behavior and Fate of NMs in Soil*

Since civilization, the soil has suffered from various classes and types of pollutants. Due to rapid advancement in nanotechnology and its products, the presence of NMs is inevitable in the soil. NMs enter the soil through different sources and pathways, such as nano-fertilizer, nano-insecticide, nano-pesticide, agrochemicals, wastewater from different laboratories and factories, consumer products, etc. Owing to their small size and high surface area, NMs pass through the soil pores and adhere to soil particles and organic matter. The mobility, interaction, and toxicity of NMs in

Table 20.5 Nano–bio interaction and toxicity of NMs to fishes

NMs	Fish	Environmental bioprocess	Toxicity	Refs.
Ag NPs	*Piaractusmesopotamicus*	Adsorption and internalization	Oxidative stress and genotoxicity	Bacchetta et al. (2017)
CuO NPs	*Poeciliareticulata*	Adsorption and internalization	Affects reproductive traits	Forouhar Vajargah et al. (2020)
ZnO NPs	*Oreochromisniloticus*	Adsorption and internalization	Bioaccumulation and oxidative stress	Kaya et al. (2015)
TiO_2 NPs	Teleost *Danio rerio*	Adsorption and internalization	Genotoxicity	Rocco et al. (2015)
Fullerene C_{60}	*Anabas testudineus*	Adsorption and internalization	Oxidative imbalance and histomorphological changes	Sumi and Chitra (2019)

the soil are dependent on the physicochemical properties of NMs. The NMs harm soil characteristics, soil microorganisms, enzymes, nutrients, and plants. Table 20.6 provides a list of NMs, their interaction, and toxicity to the soil.

20.5.3 Biophysicochemical Behavior and Fate of NMs in Air, Human, and Animals

The main routes of entry of various NMs include ingestion, inhalation, dermal penetration, and blood circulation. The entry route of NMs is through consumer products and applications (Fig. 20.4).

Natural mineral or inorganic NPs can have an atmospheric, biological, anthropogenic, or geological origin. Colloidal aerosol in the atmosphere is considered NPs, which are the precursor for more massive particles that strongly influence the global pollutant level, global climate, visual field, and atmospheric chemistry. Ultrasmall particles (< 100 nm) are randomly and unintentionally released from vehicular emissions. For example, platinum and radium particles are released by catalytic converters of cars; carbon black from exhaust combines with aerosol, and iron oxide particles are produced by brake-wear and exhaust emissions (Gonet and Maher 2019). Generally, exposure to fine particles of less than 2.5 μm in an outdoor or indoor atmosphere is reported as the largest environmental risk factor leading to cardiovascular mortality and morbidity (Rajagopalan et al. 2018). These ultrafine particles are considered toxic and potent to gain access to anybody via ingestion, inhalation, and circulation. For example, humans living in an urban environment are exposed to iron-rich air-borne NPs, and they are highly prone to cardiac mitochondrial dysfunction and cardiac stress from earliest childhood (Maher et al. 2020). Hence, in recent years, researchers are formulating reliable and facile methods for measuring the concentration of various NPs in indoor and outdoor environments to determine the workers' levels of exposure. The NPs cause biochemical damage by interactions with cells of the human body. It has been reported that NPs gain access to the body mainly via airways, the skin, or ingestion.

20.5.3.1 Inhalation of NMs

The two parts of the human respiratory system are the upper and lower respiratory tract, where the former includes the nasal cavity, pharynx, and larynx, while the latter includes the trachea, bronchi, and lungs. Depending on the size, the inhaled particles remain in the nasopharyngeal region or tracheobronchial region, or alveolar region. These particles are prone to cause cardiovascular diseases. For example, it is reported that healthy volunteers on exposure to Au NPs by acute inhalation, the Au NPs were detected in the blood and urine within 15 min to 24 h, and were present till three months of exposure (Zhu et al. 2006; Elsaesser and Howard 2012; Larese Filon et al. 2015). On exposure of Sprague–Dawley rats to Ag NPs affected the lungs and liver of male and female rats.

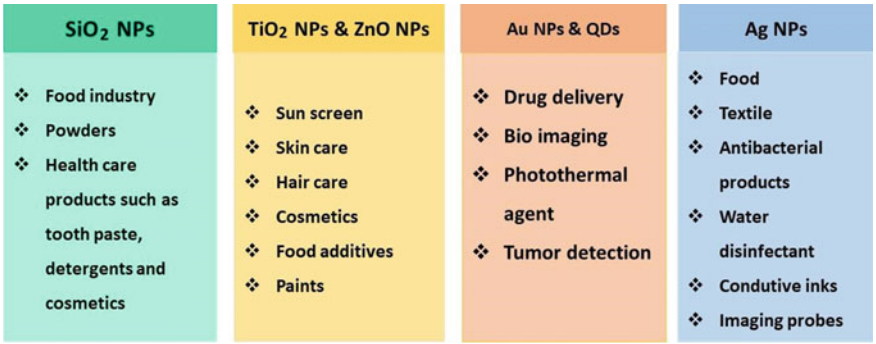
Table 20.6 NMs interaction and toxicity to the soil

NMs	Study conducted	Mode of contact	Fate and mode of uptake of NMs	Toxicity	Refs.
AgNPs	Soil	Soil treated with AgNPs mixed sewage sludge	Sorption of Ag_2S by organic matter and formation of Ag^+sorbed/complexed species	Formation of Ag_2S and hetero aggregates with soil components	Del Real et al. (2016)
	Soil microbes	Ag NPs and soil sample	Dissolution of Ag^+ ions	Ag^+ ions cause adverse damage to the cell wall, disintegrates the nucleoids of *N. europaea*. The decrease in nitrate-oxidizing bacteria counts	Wang et al. (2017)
	Lactuca sativa (lettuce)	Ag NPs exposed to plant root	Bioaccumulation, dissolution, and internalization	Ag NPs blocks nutrient transport, whereas Ag^+ ions induce excess ROS generation	Wu et al. (2020)
CuO NPs	Soil	CuO NPs were mixed into the soil	Dissolution of CuO NPs	Dissolved CuO NPs have a greater affinity for plant roots than Cu ions	Gao et al. (2018)
	Aerobic granular sludge	CuO NPs were mixed into microbial aggregates	Production of ROS	The abundance of nitrogen-removal and phosphorous-removal were increased and decreased, respectively	Zheng et al. (2017)
	Triticumaestivum (wheat)	CuO NPs were mixed into the soil	Dissolution of CuO NPs	Reduction in maximal root length	Gao et al. (2018)

(continued)

Table 20.6 (continued)

NMs	Study conducted	Mode of contact	Fate and mode of uptake of NMs	Toxicity	Refs.
ZnO NPs	Soil	ZnO NPs were added to soil	Negatively charged clay bind with dissolved Zn^{2+} ions	Zn accumulation and a decrease in pH	Zhao et al. (2013)
	Soil enzymes	ZnO NPs were added to soil	Dissolution of Zn^{2+} ions	The decrease in the bioactivity of soil protease, catalase, and peroxidase	Du et al. (2011)
	Wheat	ZnO NPs were added to soil	Dissolution of Zn^{2+} ions	Reduce in biomass	Larue et al. (2018)
TiO_2	Soil	TiO_2 NPs were added to soil	High clay content and organic matter decreases NPs mobility and bioavailability	TiO_2 accumulation in leaves	Du et al. (2011)
	Soil enzymes	TiO_2 NPs were added to soil	Agglomeration of TiO_2 NPs and production of ROS	Decreased bioactivity of soil protease, catalase, and peroxidase	(Du et al. 2011)
	Wheat	TiO_2 NPs were added to soil	Agglomeration of TiO_2 NPs and production of ROS	Reduce in biomass, accumulation in a cell or on the cell wall	Du et al. (2011)
MWCNTs	Soil	Treated with MWCNTs for one year	Sorption	Reduced soil DNA and induced bacterial community shift	Ge et al. (2016)
	Soil bacteria and microbes	Treated with MWCNTs for one year	Sorption	Affects soil bacterial taxa and microbial biomass	Ge et al. (2016)
	The seed of *Cucurbita pepo* L	Culture	Absorption	Reduction in germination percentage, root and shoot length, biomass accumulation	Hatami (2017)



SiO_2 NPs

- Food industry
- Powders
- Health care products such as tooth paste, detergents and cosmetics

TiO_2 NPs & ZnO NPs

- Sun screen
- Skin care
- Hair care
- Cosmetics
- Food additives
- Paints

Au NPs & QDs

- Drug delivery
- Bio imaging
- Photothermal agent
- Tumor detection

Ag NPs

- Food
- Textile
- Antibacterial products
- Water disinfectant
- Condutive inks
- Imaging probes

Fig. 20.4 NMs routes of entry through consumer products and applications

20.5.3.2 Skin Penetration of NMs

The skin, a dynamic sense organ protects from UV radiation and harmful external agents, plays a crucial role in regulating human body temperature and immunological response. The skin has three layers, namely epidermis, dermis, and hypodermis. Since nanotechnology advancements, various NPs, especially TiO_2, ZnO, lipid, and Ag NPs, are incorporated into cosmetics. The size and other physicochemical properties mainly influence the penetration of NPs into the skin. Research studies have shown that NPs of size: Less than 4 nm can penetrate intact skin, between 4 and 20 nm can permeate both intact and damaged skin, greater than 45 nm cannot penetrate nor permeate the skin. The NPs promote ROS production and growth inhibition leading to acute toxic effects in living organisms and penetrating the skin (Yoshioka et al. 2017).

20.6 Conclusions

There is no uncertainty that nanotechnology is rapidly growing, and it could turn out to be a serious issue for the environment and society. The incorporation of NMs in consumer products is growing exponentially, and their potential environmental and health risks are of great concern to researchers and policymakers. Moreover, the adverse effects of NMs are under studied or underestimated. Although our knowledge of the toxicity of various NMs in the ecosystem has increased over recent years, the issues such as the mechanism of action and exposure concentration remain largely obscure. Nanoecotoxicology is a new hybrid discipline and is most likely going to make an essential contribution to sustainable and safe nanoproducts. However, an improved understanding of nanomaterials' risk factors to the environment, various terrestrial, aquatic, terrestrial organisms, and humans is critical.

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Chapter 21
Legal Implications of Nanobiosensors Concerning Environmental Monitoring

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Abstract The international community has been facing significant challenges posed by pollution, depletion, and degradation of the environment mainly due to the introduction of toxic substances or waste by man. However, to ensure the existence, safety, security, and healthy living of humanity, there is a need to guarantee a safe and effective protected environment, free from pollution and substances that could cause the depletion of the ozone layer and hazardous for human being. Though, there have been reported recent cases of the utilization of nanotechnological and scientific methods such as nanobiosensor (NBS) which are developed for detecting toxic substances for environmental, biomedical, and other applications. NBS has been proven to be one of the most useful scientific methods for environmental monitoring and management. NBS has been an effective scientific method for environmental monitoring and is meant to complement the international legal framework that ensures effective monitoring and prevention of the introduction of hazardous substances into the environment. Despite the attractive advantages that NBS tends to provide in the environmental domain, there are still some legal implications and challenges of utilizing NBS, owing to some potential NBS risks in environmental monitoring and management. Consequently, this chapter adopted a doctrinal analysis of NBS for environmental monitoring, its prospect in complimenting some international legal framework in environmental monitoring, the legal implication, and the challenge of using NBS for environmental domain.

Keywords Environment · International community · Legal framework · Nanobiosensor · Pollutants

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21.1 Introduction

Given the growing concern over the deteriorating environmental and atmospheric conditions of the earth and the depletion of the ozone layer ensuing from both natural and human actions, there has been an emergence of the international regulatory regime that tends to rectify some of these environmental or ecological challenges (Adedeji and Eziyi 2010; Riget et al. 2016; Aidonojie et al. 2020; Ukhurebor et al. 2020a, b). This is given the fact that the preservation and conservation of the environment, biological diversity, and promotion of sustainable development are of utmost importance to humanity (Susan 2009; Anderson et al. 2004; Sundström et al. 2014). However, despite the efforts of both the local, national, regional, and international community to curtail the continuous contamination of the environment and depletion of the ozone layer, there is still the release of persistent organic and inorganic pollutants (such as carbon-based compounds) that consist of synthesized substances (such as pesticides and other harmful chemicals) (Falkner 2016; Ladychenko et al. 2019; Nwankwo et al. 2020) and such other related hazardous substances from both domestic and industrial activities which are the by-products from human and natural activity (Yamin and Depledge 2004; Bodansky et al. 2017; Ukhurebor et al. 2021a, b, c, d, e). Scientific studies have revealed that persistent release of dangerous pollutants on the atmospheric environment will cause a depletion of the ozone layer and therefore lead to environmental catastrophe (Bridges and Oldeman 1999; Githeko et al. 2000; Garret et al. 2011; Ukhurebor et al. 2020a, b).

In order to scientifically resolve these environmental abnormalities, there have been some scientific innovations in the field of nanotechnology such as nanobiosensor (NBS) that has been developed, evolved, and accepted as a useful scientific method for environmental monitoring and management (Rapini and Marrazza 2017; Willner and Vikesland 2018; Ukhurebor and Adetunji 2021; Onyancha et al. 2021). NBS are essentially sensors that comprise nanomaterials (Muller and Nowack 2008, Dai et al. 2017; Vogel 2019; Ukhurebor 2020). NBS is used or can be used in detecting substances such as detecting of mycotoxin in foodstuff (Pérez-López and Merkoçi 2011; Ramezani et al. 2017; Liu et al. 2018), detecting of toxic substances for environmental monitoring, monitoring, and detecting of detrimental environmental constituent that may cause environmental pollution or depletion of the ozone layer as well as some biomedical applications (Qin et al. 2016; Alahi and Mukhopadhyay 2017; Ghoto et al. 2019; Ukhurebor 2020; Kerry et al. 2021).

However, the extent of the legal framework that is developed in regulating environmental pollution and waste management may pose some challenges in using or developing NBS for environmental monitoring. This is concerning the fact that some NBS may allegedly contain some substances or chemical that could pose a number of risks or threat to the environment during or at the expiration of their life cycle, and this may be subjected to legal control or maybe truncated from being used for environmental monitoring (Franco et al. 2007). This legal implication is stem from the fact that there is a need to ensure sustainable development of any scientific method in preventing, controlling, and monitoring the introduction of substances or waste that may be hazardous to the environment and humanity (Xu et al. 2014; Selvilini et al. 2018; Zhang et al. 2019); albeit, NBS are been reported and scientifically proven to be environmentally friendly and relevant in several scientific domains (Adetunji et al. 2021a, b; Kerry et al. 2021; Ukhurebor and Adetunji 2021; Onyancha et al. 2021). Given the above, this chapter tends to adopt a doctrinal research method in examining NBS for environmental monitoring. The applications of NBS as one of the most effective scientific methods seem to complement the existing environmental legal framework for environmental monitoring. The legal implications or challenges in this aspect are also discussed.

21.2 The Concept of NBS in Environmental Monitoring

The twentieth century has experienced an increase in population and a great industrial developmental stride. Various studies have reviewed that the major hazardous substances polluting the environment (land, water, and air) are majorly caused by human activities in the industrial exploitation and exploration of natural resources (Nwankwo and Ukhurebor 2020; Ukhurebor et al. 2021a, b). This is concerning the fact that the need to increase the level of production has led to undue exploitation and exploration of natural resources, developing chemical to improve food production (McCullum et al. 2003; Mackey and Montgomery 2004; Ukhurebor et al. 2020b, 2021b), genetically modifying plants or animals (Chenet al. 2012; Adetunji et al. 2021a, b). These human activities do not only lead to a high rate of food production, but are also resulting in some reported cases of hazardous and harmful to human health and environmental consequences (Childers et al. 2011; Cordell et al. 2009; Nwankwo and Ukhurebor 2019), and this is in affirmation to the report of Kumar and Guleria (2020) that environmental pollution that directly affects human health account for over 9 million deaths in the world in 2015, which is far higher than other death-causing agents.

However, researchers have been researching on the effective scientific method (especially in the nanotechnology domain) that can be used in detecting hazardous substances that can cause pollution to the environment (Malik et al. 2013; Darsanaki et al. 2013). NBS is one such scientific method, which consists of nano and biosensor machinery (Pandit et al. 2016; Ukhurebor and Adetunji 2021). Nanotechnology has to do with the designing and making of useful devices and systems ranging from 1–100 nm scale (Ma et al. 2018; Andreescu et al. 2005; Ukhurebor and Adetunji 2021). The relevance of utilizing nanotechnology is based on sensing (Touhami 2014; Ukhurebor 2020; Kerry et al. 2021). Given this, the use of nanotechnology in the context of biosensors for environmental monitoring involves the use of nonmaterial in detecting pollutants and hazardous substances within the environment (Fahimi-Kashani and Hormozi-Nezhad 2016; Muenchen et al. 2016).

Biosensors utilize biological components derived from a sensitized component associated with a physicochemical transducer (Gosset et al. 2018). The essence of a biosensor is to generate a digital electronic indicator that is relative to the concentration of an exact analyte or set of analytes. NBS for environmental monitoring can be used in detecting hazardous and pollutant substances that may be harmful to the environment (Malhotra et al. 2017). NBS can be group into nanosensors, which is a biological sensory process used to transmit information concerning nanomaterials to the macroscopic world (Patolsky et al. 2006). Research has shown that the ability to modify the size and composition of nanomaterials can provide tremendous prospects for scheming novel sensing devices or systems and therefore improve the performance of bio-analytical assay or chemical (Devreese 2007; Duhan et al. 2017; Vogel 2019). Furthermore, NBS can be incorporated into miniature devices for speedy screening, monitoring, and detecting a large variety of hazardous substances that can cause pollution of the environment (Bellan et al. 2011).

Up till now, several NMS have been fabricated and reported, ranging from largely utilization gold nanomaterials to novel nanomaterials that are either carbon-built or transition-metal dichalcogenide-built. These nanomaterials could be exploited either by themselves or via the combination (hybridization) with other nanomaterials for the improvement of highly sensitive NMS (Yoon et al. 2020). Some of the utmost contemporary categories and subcategories of NMS and the prominent historical outline of some of the reported evolutions of NMS mechanisms are shown in Figs. 21.1 and 21.2, respectively, as adopted from Kerry et al. (2021).

Given the above, it is apt to state that the concept of NBS is of great relevance in environmental monitoring, given its scientific sustainable means of preserving the climate earth from pollution and hazardous substances.

21.3 NBS for Environmental Monitoring Complimenting Some International Environmental Legal Framework

The growing quantity of possibly harmful contaminants in the environment calls for fast and low-cost investigative procedures to be utilized in all-embracing environmental monitoring plans. Furthermore, over the previous years, a rising quantity of creativities and legislative arrangements for controlling and monitoring environmental contamination have been implemented in parallel with growing scientific, technological, social, and public apprehension in this domain (Rodriguez-Mozaz et al. 2005; Rogers 2006; Silva et al. 2011; Ukhurebor et al. 2021a).

There is a vast request for fast, consistent, and cost-effective systems for the controlling, monitoring, detection, and analysis of impurities in the environment (Salouti and Derakhshan 2020). Numerical investigation of environmental samples is typically carried out via the traditional investigative approaches such as spectroscopic and chromatographic procedures in identifying various contaminants or impurities in the environment. These approaches, though precise and sensitive, still need sophisticated and exclusive instrumentation, skilled personnel for their process, and multi-phase as well as complex sample research (Salouti and Derakhshan 2020).

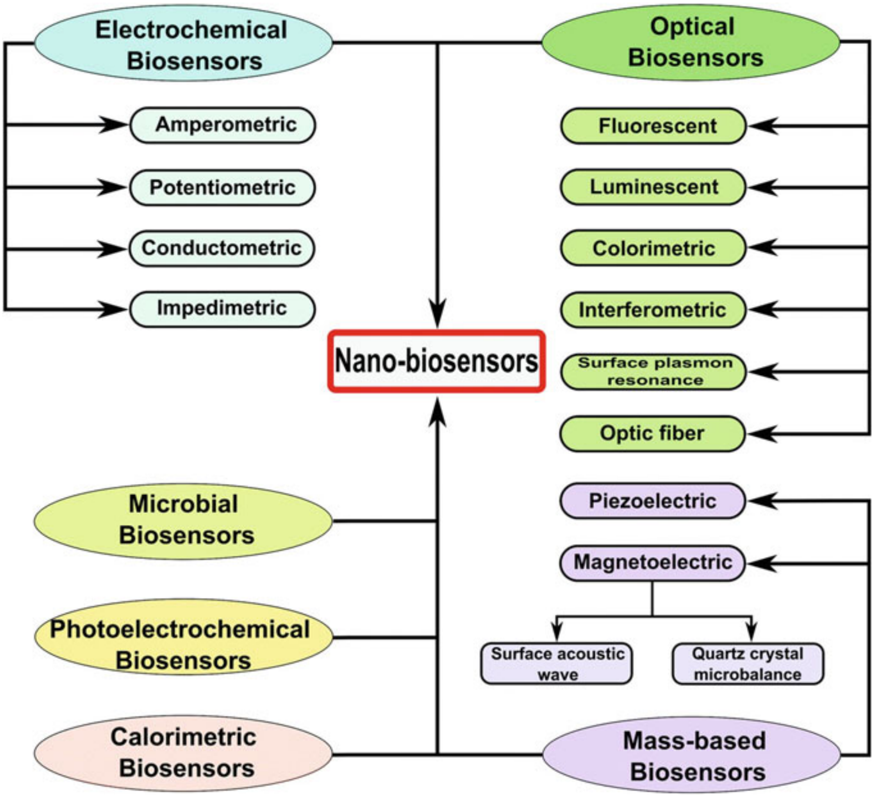

Fig. 21.1 Some of the utmost contemporary categories and subcategories of NMS (Kerry et al. 2021). Permission to reproduce automatically granted by the Royal Society of Chemistry due to authorship

Most of these procedures are also reportedly labor-exhaustive and time-intensive, as well as some difficulties in the monitoring of contaminants on location, in actual duration, and at the high occurrence. To stunned most of these issues connected with the existing environmental investigative and monitoring procedures, several innovative biosensors (an investigative mechanism for the quantifiable monitoring and detection of analyte with an organically active component) are being established, developed, and evolved. Most of these biosensors depend on nanotechnological tendencies (Salouti and Derakhshan 2020; Ukhurebor 2020; Kerry et al. 2021).

Biosensors are typically classified owing to the bioreceptor components involved in the organic recognition procedure (such as the immune-affinity recognition components, enzymes, whole cells of microbes, living organisms (animals or plants), or fragments of DNA), or owing to the physicochemical transducer employed (such as thermal, piezoelectrical, optical, or electrochemical). The foremost kinds of bioreceptor components that are utilized in the environment monitoring and analysis are the whole cells of microbes, enzymes, antibodies, and DNA. Also, from existing

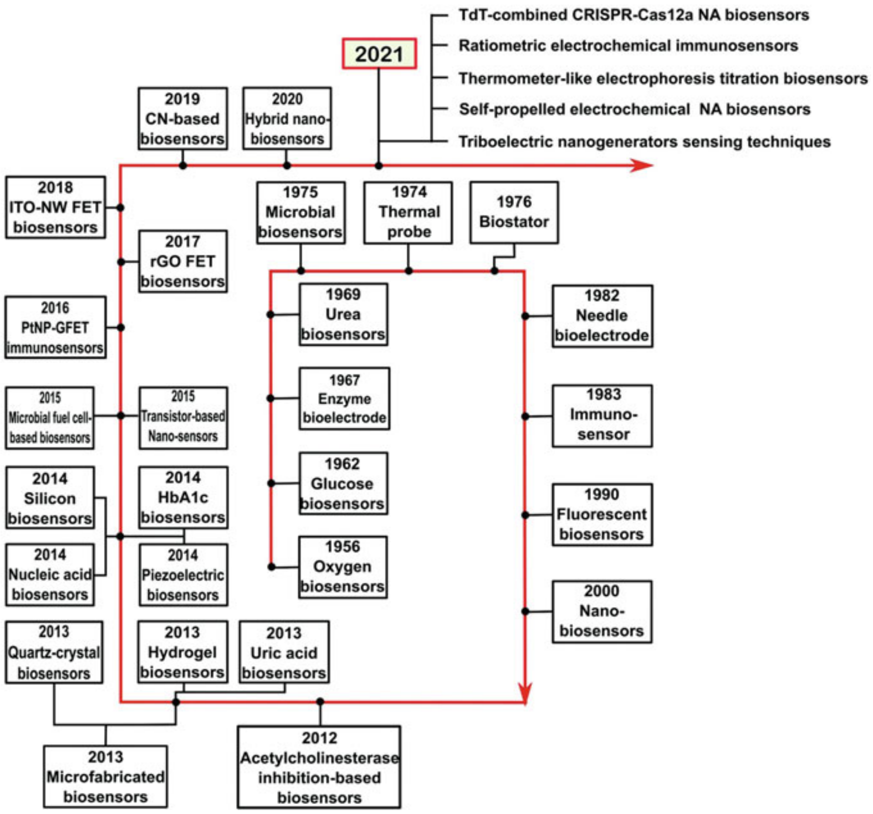

Fig. 21.2 Prominent historical outline of some of the described evolutions of NMS. Where *FET* field effect transistor, *GP* graphene, *PtNP* platinum nanoparticle, *rGPO* reduced graphene oxide, *ITO-NW* indiumtin oxide nanowires, *CN* carbon nanotube, *TdT* terminal deoxynucleotidyl transferase (Kerry et al. 2021). Permission to reproduce automatically granted by the Royal Society of Chemistry due to authorship

works of literature the utmost of the biosensors for environmental, monitoring, analysis and applications are that of the electrochemical transducers (Salouti and Derakhshan 2020).

The foremost benefits offered by biosensors for environmental monitoring and applications over the conventional investigative procedures are the prospect of transferability, miniaturization, work on-site, and the capability of detecting and measuring pollutants in multifaceted matrices with negligible sample research. Though several of the established and developed systems can hardly compete hitherto with conventional investigative procedures in terms of precision and reproducibility, they could be utilized by regulatory establishments and by industries to offer adequate information for routine screening, monitoring, and testing of environmental samples (Rodriguez-Mozaz et al. 2005; Rogers 2006; Salouti and Derakhshan 2020).

NBS has been scientifically proven to be relevant in various scientific research (Gharaatifar and Hasanzadeh 2017; Viswanathan et al. 2009), however, the relevance of NBS for environmental monitoring cannot be overemphasize, given the fact that the essence of introduction of the scientific concept of NBS for environmental monitoring is aimed at ensuring prevention and control of the introduction of hazardous substances or constituents that may contaminate or pollute the environment (Wang et al. 2014; Miao et al. 2016). It must be noted that the introduction of NBS for environmental monitoring is further aimed at scientifically complimenting and strengthening the already existing environmental management procedures as well as the legal framework for environmental monitoring. This is concerning the fact that the development of international, regional, national, states, and local environmental laws was triggered by the need to protect the life of humanity and preserve the ecosystem from hazardous substances that may deteriorate the health of man and cause pollution to the environment (Crutzen et al. 2008; Xiang et al. 2019). Furthermore, it may lead to environmental problems such as pollution, acid rain, deforestation and desertification, the destruction of the ozone layer, and climate change (Auta et al. 2017; Nwankwo et al. 2020). The introduction of hazardous substances to the environment and depletion of the ozone layer is mainly caused by human activities via industrial activities or in the cause of exploiting the earth's natural resources (Barnes et al. 2009; Barboza et al. 2019). In the 1950s and early 1960s, the international community was more inclined with the issue of nuclear damage (a by-product of the atoms for peace proposal) and marine pollution from oil/petroleum resources (Andrady 2015). However, it was Rachel Carson's (1962) famous book *Silent Spring*, the story of silent spring that exposed the hazards of the pesticide Dichlorodiphenyltrichloroethane (DDT) and other harmful substances that causes pollution of the environment and depletion of the ozone layer (Carson 1962). Concerning these environmental abnormalities emanating from the activities of man, she questioned humanity's faith in the development of technological apparatus and industrial activities that are hazardous to the environment. Given this, the stage and quest for an environmental legal framework in environmental monitoring were set rolling within the international community. Several international conferences were held for the negotiation of international environmental treaties and conventions for environmental issues. These give birth to several international environmental legal frameworks that tend to ensure effective environmental monitoring and management within the international community and member states.

The essence of the *Stockholm Convention* on persistence of organic pollutants was aimed at the prevention, controlling, and monitoring of the use of persistent organic pollutants (Lallas 2001; Thanh et al. 2009) and other related substances which are the by-products from human and natural activities, which is also one of the major aims of NBS for environmental monitoring. *Article 3 of the Stockholm Convention on persistence of organic pollutants* emphasize the need for member states to take measures to reduce or eliminate and monitor the releases from intentional production and use of substances or chemicals that may be hazardous and harmful to the environment (Godduhn and Duffy 2003; Henry et al. 2016). Furthermore, *Article 7 of the Stockholm Convention on persistence organic pollutants* required each party or member state to develop and implement plans for the implementation of its obligations under this *Stockholm Convention* within their nation. Also, member states to the *Stockholm Convention* are required to establish the means to integrate national implementation plans for persistent organic pollutants in their sustainable development strategies where appropriate (Lamon et al. 2009; Torres et al. 2013).

Also, the *Rio Declaration on Environment and Development* was adopted to mitigate, control and monitor the systematic examination of patterns of production, specifically the production of toxic substance or components, such as radioactive chemicals and lead in gasoline, or poisonous waste that may be harmful and hazardous to the environment (Aidonojie et al. 2020). *Principle 3 of the Rio Declaration* emphasize the need to effectively monitor the activities of man as it relates to the environment, it also stated that man must engage in sustainable development for the preservation and conservation of the ecosystem which is one of the major reasons for introducing scientific and technological means (such as NBS mechanisms) for environmental monitoring. Furthermore, there are other relevant international environmental legal frameworks such as the *United Nation Framework Convention on Climate Change* (UNFCCC), the *Kyoto Protocol and United Nations Convention to Combat Desertification*, the *Basel Convention*, *and the Bamako Convention* that also emphasize the need for environmental monitoring and prevention of the ecosystem from pollution and depletion caused by hazardous constituent or substance. In this regard, given the above, NBS for environmental monitoring further seems to scientifically complement and enhance environmental legal framework which tends to ensure the effective control and monitoring of the environment from pollution, deterioration, and depletion by means of the introduction of hazardous substances into the environment. This is concerning the fact that the introduction of NBS for environmental monitoring is a perfect scientific sustainable development plan and strategies contemplated by *Article 7 of the Stockholm Convention on persistence of organic pollutants and the Rio Declaration*; that emphasize the need to effectively control and monitor the environment from pollution, degradation, and depletion.

21.4 Legal Implications and Challenges Concerning NBS for Environmental Monitoring

In the cause of utilizing nanomaterials or other substances in developing NBS, waste (such waste could be hazardous and non-hazardous) could easily or possibly be generated in the process or at the expiration of the life cycle of such NBS products (Hrapovic 2004; Kuswandi and Mascini 2005; Kuswandi 2018). Also, utilizing NBS for environmental monitoring may also release certain organic compound (carbon-based) that consist of or are made of synthesized substances such as pesticides and polychlorinated biphenyls (PCBs) that may be an agent of pollution to the environment (Nagatani et al. 2007; Liu et al. 2010). Given this, it is relevant when for using or utilizing NBS for environmental monitoring, to determine the effective process of handling, treating, and disposing of such wastes or organic pollutant contain in the nanomaterials or substances that encompasses NBS for environmental monitoring (Tchounwou et al. 2012; Liu et al. 2013). This is given the fact that failure to ensure appropriate utilization and applications of NBS for environmental monitoring does not only constitute more danger to the ecosystem but to humanity as well (Luo et al. 2006; Guo et al. 2011); possibly or maybe strictly faced with the legal implications of prohibiting the use of NBS. However, some of the relevant environmental legal frameworks that posed a challenge or affect the use of NBS for environmental monitoring will now be briefly discussed.

21.4.1 The European Union Directives of 2008/98/EC with Regard to Waste

It is apt to opine that the European Union (EU) Directives of 2008/98/EC with regard to waste is a policy framework on waste prevention and control. The EU Directives classified waste into hazardous and non-hazardous substances or waste (Ukhurebor and Aidonojie 2021). Although, no specific reference to waste generated from NBS for environmental monitoring was mention in the EU Directive of 2008/98/EC. However, when a waste generated from NBS becomes harmful to the environment that is, doing more harm to the ecosystem and humanity, it can be legally classified as hazardous.

Given the above, where the EU Directive of 2008/98/EC on waste can be applied in categorizing harmful waste generated from nanomaterials or substances in developing NBS for environmental monitoring, it may be categorized as hazardous. This is concerning the fact that substances such as zinc oxide and some of the substances mentioned above are regarded or classified as dangerous and very toxic to the environment, most especially to the aquatic environment. In this regard, when a NBS comprises substances such as zinc oxide or other substances which are considered harmful to the environment (aquatic to be specific), the EU Waste Directives of 2008/98/EC may limit, control or truncates the use of such NBS for environmental monitoring, most especially with regard to an aquatic environment.

21.4.2 The Stockholm Convention on Persistence Organic Pollutants

The United Nations has via several treaties and conventions prohibit the use of synthesized substances that may be a potential environmental pollutant (Heidelore 2007). In recognition of the fact that certain substances or chemicals possess harmful and toxic properties that may resist bioaccumulation, degradation and they can easily contaminate or pollute the air, water, and other environ (Weiguang et al. 2013). *The*

Stockholm Convention on Persistence Organic Pollutants 2004 via *Article 3 and 5* encourage member states to eliminate or control the use of substances or chemicals contain in Annex A, B, and C to the convention. These chemicals include Heptachlor, Aldrin, Hexachlorobenzene, Chlordane, Dieldrin, Endrin, PCB, Mirex, Toxaphene, Dichlorodiphenyltrichloroethane (also called DDT), Hexachlorobenzene (HCB), dibenzofurans, Polychlorinated biphenyls, and Polychlorinated dibenzo-p-dioxins. These chemicals can only be used to the extent permitted by the *Stockholm Convention on Persistence Organic Pollutants in Article 6 and Annex A*, *B*, *and C*, which NBS was not expressly mentioned or among the listed purpose for which these chemicals can be used. Furthermore, *Article 3(3)–(5) of the Stockholm Convention on Persistence Organic Pollutants* also required member states to ensure that the development of new industrial chemical or substance must be adequate screen in accordance with Annex D to the Convention to determine the toxicity level to the environment and human health. Given the above, the *Stockholm Convention* on Persistence Organic Pollutants encourages the use of minimal waste technology and substances or chemical those are less harmful and hazardous to the environment (Karlagans et al. 2001).

The implication of the above provision of the *Stockholm Convention on Persistence Organic Pollutants* with regard to NBS for environmental monitoring is that if any of these chemicals contain or use as a nanomaterial in developing NBS or are directly use in NBS, the usage of such NBS for environmental monitoring may be disallowed if consider harmful to the environment and to human health, *given Article 3*, *5*, *6*, *and Annex A*, *B*, *C*, *and D of the Stockholm Convention on Persistence Organic Pollutants.*

21.4.3 The Basel Convention

The *Basel Convention on the Control of Tran Boundary Movement of Hazardous Waste and their Disposal* is an international treaty that has a similar provision like the European Union Directive of 2008/98/EC on waste (Ukhurebor and Aidonojie 2021). The major aim of the Basel Convention is to ensure an effective control or prevention of waste that may be adversely hazardous to the environment and human health (Kummer 1992). *Article 2(1) of the Basel Convention* provides that waste can be classified as an object or chemical or substances which are hazardous in nature (that is the hazardous nature is determined based on their origin and composition) and are meant to be disposed of (Sejal 2001; Kempel 1999). *Article 1 of the Basel Convention* specifically stated that substances that fall under the category of *Annex I to the Basel Convention* are classified or regarded as hazardous waste, except they do not have the characteristic or nature of substance contained in *Annex III to the Basel Convention.* Some of these substances include; Waste water/oils, water mixtures/hydrocarbons, emulsions, selenium compounds, compounds that contain Inorganic fluorine, mercury compounds, a substance that contains phenol compounds, and chlorophenols, substances containing thallium, Metal carbonyls substance. Also, wastes that contain substances such as polychlorinated terphenyls, PCBs, cyanides, Zinc, Arsenic, Selenium, Cadmium, and several others as contained in *Annex 1 to the Basel Convention* are considered hazardous. Provided they exhibit one of the hazardous characteristics contained in *Annex III to the Basel Convention*, in other words, it must both be listed and contain some characteristic such as being explosive, flammable, toxic, or corrosive.

Furthermore, *Article 2* and 4 of the *Basel Convention* required state parties to ensure that steps are taken for an overall reduction, prevention, and control of any hazardous waste or substance generated within members' states. *Article 12 of the Basel Convention* further directs state parties to adopt a protocol that establishes liability rules and procedures appropriate for damage resulting from the movement of hazardous waste across borders. Given this, waste can pollute the environment via air and water, they capable of moving from one boundary to another. In this regard, where a NBS for environmental monitoring contain any of the above substances or there is a likelihood that waste that may be generated from a NBS during or after its life cycle may constitute a hazardous waste, the process of scientifically utilizing such a NBS for environmental monitoring may be truncated.

21.4.4 Bamako Convention

The *Bamako Convention* is also known as the *Bamako Convention on the Importation and Control of Trans-Boundary Movement of Hazardous* waste within Africa (Ukhurebor and Aidonojie 2021). This convention is similar to the *Basel Convention* (Shearer 1993; Ovink 1995), given the following reasons:

(i) Both conventions share the same objective of protecting human health and the environment from the potentially harmful impacts of the trans-boundary movement and disposal of hazardous waste.
(ii) Both conventions recognize the sovereign right of states to ban the import and export of hazardous waste.
(iii) Both conventions call for cooperation in the development of appropriate technical guidelines and codes of practice.

Given the above, it means the use of NBS for environmental monitoring may also be truncated by the *Bamako Convention* if in the circumstances such NBS could pose a threat of being a hazardous waste. However, the origin of the *Bamako Convention* is traceable to the alleged failure of the *Basel Convention* as regards two pertinent issues (Murphy 1994; Kitt 1994) which are:

(i) The realization that the *Basel Convention* had failed to prohibit the trade of hazardous wastes to less developed countries like those in Africa
(ii) The realization that many developed nations were still exporting waste to Africa
(iii) The *Basel Convention* states that illegal hazardous waste traffic is criminal but it contains no enforcement provisions.

In order to address the above concerns, the *Bamako Convention* placed a complete prohibition on the import or any activities that may lead to generating hazardous wastes (Eguh 1997; Dzidzornu 2004; Gutierrez 2014). Given this complete ban of waste, *Section 1, of Articles 1 of the Bamako Convention*, defined waste as harmful materials or substances which are meant to be disposed of or are intended to be properly disposed of in accordance with members' state provisions of national law (Ijaiya et al. 2018; Fatsah 1993). Furthermore, *Sections. 2, of Article 1 of the Bamako Convention*, defined hazardous wastes, as wastes that have been so-referred to as hazardous by virtue of the provision of state legislation, harmful substances that is banned, canceled, and declared as harmful by government regulation action, wastes which are subjected to the international regulation mechanism as a result of being radioactive (Kaminsky 1992; Eze 2007). In this regard, where a developed NBS for environmental monitoring life cycle has expired or may possibly pose a threat to be hazardous, such developed NBS may falls within the definition of substances that are hazardous as provided for in the *Bamako Convention.*

Also, *Article 4 of the Bamako Convention* further identified substances that are regarded as hazardous wastes as provided for in *Annex I, II of the Bamako Convention* and nations or members' state legislation. Some of material or substances referred to as hazardous as contained in Annex I to the *Bamako Convention* are as follows; any substances containing radionuclide as a result of human activities; substances that generated into waste as a result of the fact it was formulated and produce from biocides; wastes that emanate in the treatment of heat and operation that involve cyanides; substances that degenerate into wastes as a result of the use of organic solvent; substances that contain polychlorinated biphenyls, terphyenyls, and polybrominated biphenyls; wastes emanating from the development of an invention and research that are new and their effect to the environment are unknown, of which any waste emanating from a NBS may fall into this category. Furthermore, substances may also be regarded as hazardous if it contains any of the following constituents; arsenic composite, mercury element, leads element, beryllium composite, cadmium composite, tellurium, thallium compound, and hexavalent chromium substances (Ukhurebor et al. 2021c). Furthermore, *Annex II to the Bamako Convention* also stated that a substance may be regarded as hazardous if it possesses the following characteristic; being flammable, explosive, organic peroxides, toxic, ecotoxic, corrosive, and poisonous to man and the environment.

Given, the above provision of Annex I and II to the *Bamako Convention*, there is no doubt that if a NBS for environmental monitoring contains any of the substances as provided for in Annex I to the *Bamako Convention* or possess any of the characters as provided for in Annex II to the *Bamako Convention*, such NBS may be prohibited for being used for environmental monitoring. This is concerning the fact that; the *Bamako Convention* blacklist such substances use in the formulation and development of the NBS. Furthermore, the inventor and user of NBS may be discouraged from utilizing NBS for environmental monitoring, given the fact that *Article 4(3) (C) of the Bamako Convention* required that the generation of hazardous wastes within the area under its jurisdiction be reduced to a minimum and in ensuring the reduction of hazardous wastes parties must take into account social, technological, and economic aspects.

Article 9(4) of the Bamako Convention, provide that a trans-boundary movement of hazardous wastes is deemed to be illegal traffic and that proceedings according to the provisions of the convention are taken against the contravener(s) (Wordsworth 1993; Donald 1992; Kaya 2012). *Article 4(3) (b) of the Bamako Convention*, further, imposes strict and unlimited liability, as well as joint and several liability on anyone, involve in generating hazardous waste.

Given the above, it suffices to say that the *Bamako Convention* covers a wider spectrum of what constitutes wastes or substances that may be categorized as harmful to the environment and man. It covers and regulates the trans-boundary movement of both hazardous and radioactive waste. Furthermore, it also categorizes wastes emanating from the development of an invention and research that are new and their effect on the environment is unknown. In this regard, NBS being a current scientific invention for environmental monitoring could fall into this category where possess substances that may harmful to the environment or in the circumstances after the life cycle of NBS it becomes a threat to the environment, such NBS may be truncated from being used for environmental monitoring, given the *Bamako Convention.*

21.5 Conclusion and Recommendations

The study has been able to evaluate the concept of NBS for environmental monitoring, and how the concept of NBS tends to compliment some of the environmental legal frameworks concerning environmental monitoring in mitigating and reducing the effect of atmospheric pollution and hazardous substances in the environment. Furthermore, some of the international environmental legal frameworks such as the European Union Directives of 2008/98/EC with regard to waste, the *Stockholm Convention on Persistence Organic Pollutants*, *Bamako*, and *Basel Conventions* that tend to regulate how man relate to its environment was also considered. These environmental legal frameworks were evaluated with regard to the relevant provisions that may limit or truncate the use of NBS for environmental monitoring. This is concerning the fact that some substances that may be used in developing NBS for environmental monitoring may possibly fall within the category of substances that are considered harmful and hazardous within the *Stockholm Convention*, *Basel*, and *Bamako Convention.*

However, in order to effectively utilized NBS for environmental monitoring, is hereby recommended that it will be relevant to always conduct an environmental impact assessment on the possible risk of using such NBS for environmental monitoring, where such NBS contain substances that are possibly classified as hazardous under the legal framework. For a better, accessible, and sustainable environment, further investigation on the use of NBS and other smart systems for environmental monitoring needs to be urgently carried out. To achieve this mission, biosensors are emerging and evolving as a crucial investigative mechanism for effective investigations for environment quality analysis and evaluation. Progressions in sensing constituents' growth together with enhanced integration made NBS very smart, which meets environment quality analysis and evaluation requirements. In addition, the interfacing of biosensing models with other systems as well as the internet of things (IoT) and AI made biosensing-based investigations accessible in the environmental domain.

Incorporating NBS for environmental monitoring into international environmental treaties or conventions will also be of great relevance in ensuring effective monitoring and usage of NBS for environmental monitoring. This recommendation is concerning the fact that NBS being a recent scientific method was not within the contemplation of the drafter of most environmental legal framework. Also, in incorporating the concept of NBS into environmental treaties and convention, there is a need to also establish monitoring-based agencies that will ensure effective compliance with the provisions of the treaty or convention that provide for and regulate the use of NBS for environmental monitoring, receive periodic reports, and advisory opinions from states parties concerning the use of NBS for environmental monitoring within their jurisdiction.

Furthermore, it is also required that members state of the international community incorporate NBS for environmental monitoring into their local and national environmental laws so as to ensure effective compliance of the use of NBS for environmental monitoring.

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