

Preface

A chemical sensor is a device that transforms the chemical information about a specific sample component to total composition analysis into an analytically useful signal. Chemical sensors usually contain two basic components connected in series: a chemical recognition element (“receptor”) and a physicochemical transducer. The recognition system translates the chemical information (i.e., concentration of the analyte) into a chemical or physical output signal. The transducer (i.e., a physical detection system) serves to transfer the signal from the output domain of the recognition element to the electrical, optical, or piezoelectric, etc., domain. A biosensor device is capable of providing specific quantitative analytical information using a biological recognition element (e.g., enzymes, antibodies, natural receptors, cells, etc.), which is retained in direct spatial contact with a transduction element.

Although sophisticated techniques such as chromatography and spectrometry provide accurate and conclusive results, screening tests allow a much higher throughput of samples at a lower cost and with less operator training, so larger numbers of samples can be analyzed. Biosensors combine a biological recognition element (e.g., enzyme, antibody, receptor) with a transducer to produce a measurable signal proportional to the extent of interaction between the recognition element and the analyte compound. The different uses of these devices available today are extremely varied, with food and environmental analysis as an emerging and growing application. The advantages offered by biosensors over other screening methods such as radioimmunoassay, enzyme-linked immunosorbent assay, fluorescence and luminescence immunoassays, with respect to food and environmental analysis, include automation, improved reproducibility, speed of analysis, real-time analysis, but most importantly, the main advantage is that the device can be regenerated and used again, whereas most of the screening methods are only single-use techniques. The main areas of development common to these groups of contaminants include multiplexing, the ability to simultaneously analyze a sample for more than one contaminant, and portability. Biosensors currently have an

important role in food safety; further advances in the technology, instrumentation, reagents, and sample handling will surely reinforce this position.

The new biosensor technology has significant technological advantages compared to that of traditional non-sensor-based detection methods. Portable and handheld biosensors, such as dynamic DNA and protein arrays for rapid and accurate detection of pathogens, are typical examples of how biosensor technology can contribute to the defense against bioterrorism. For example, vesicles for use in biosensors have both high specificity and sensitivity, where the vesicles include a receptor specific for the intended analyte and a signal generating component.

A large class of chemical and biological sensors were based on the physical characterization of interfaces. More specifically, electronic (bio)chemical sensing is often related to the characterization of interfaces between ion- and electron-based conductive materials by means of electrical variables such as voltage, current, and charge. Also, recent trends in integrated electronics and the development of nano technology have started a revolution in the field of biosensors allowing the shrinking of very complex electronic systems into millimeter square sizes and this has prompted the development of nanosensors. This would allow implementing complex and sophisticated instrumentation in cheap and portable devices for fast detection of harmful and toxic agents.

The aim of this book is to bring into focus this important research area and advances of biosensors and more specifically those related to the rapid detection of weapons of bioterrorism. The object is related to present advances in the development of portable chemical sensors for the rapid detection of chemical weapons of terrorism; the scope is related to provide a comprehensive review of the most recent research topics most pertinent to the advances of devices that can be used for real-time detections of toxicants such as microbes, pathogens, toxins, nervous gases such as botulinum toxin, *Escherichia coli*, *K. Pneumoniae*, sarin, VX, listeria monocytogenes, salmonella, marine biotoxins, staphylococcal enterotoxin B, saxi toxin, gonyautoxin (GTX5), francisella spore virus, *bacillus subtilis*, ochratoxin.

Biosensors have found a large number of applications in the area of environmental, food, and biomedical analysis. Recent advances include portable devices for rapid detection of insecticides, pesticides, food hormones, toxins, carcinogenic compounds in the environment, such as polycyclic biphenols, etc. Despite public anticipation that biosensors with real-time detection will be able to monitor biological and chemical weapons, the technology has not caught up with the expectations. Presently, biosensors in environmental monitoring stations nationwide can detect compounds like anthrax—but detection can take 12–24 h. The best ones on the market take 20 min. The detection of explosives and especially of biomolecules is an important part of security and defense activities. Technology has enhanced the possibility of combining the functions of drugs and explosives through different biocells in the same analysis module. A testing platform has been developed with a built-in configuration flexibility that allows it to be used for different applications such as chemical, explosives, drug, and biological agents detection. The characteristics of detection are determined by the combination of biocells. It is a new

biosensor technology and helps users to combine a number of different detection tasks within the same test, without having to reprogram the instrument.

Biosensors have not yet made a large impact in the area of rapid detection of chemical and biological threats. Biosensors come in thousands of forms and types based on a wide range of physical and chemical principles with varying types of usable outputs. However, the field applications of sensors have been adequate. The diversity of research has been limited mainly to glucose as a mass market. The drawbacks of chemical sensors can be summarized as follows:

- a. They are not robust
- b. Insufficient for complex analytes
- c. Extra laboratory testing is not possible by non-skilled personnel.

Most reports in the literature have suggested that biosensors were at a pre-competitive stage, but highlighted the laboratory proof-of-concept. Presently, we are looking into portable and handheld biosensors, for example, dynamic DNA and protein arrays for rapid and accurate detection of pathogens. A few challenges for biothreat detection had high sensitivity—*detect* very small amounts of pathogens, toxins, and chemical agents; high selectivity—*discriminate* targets from other materials, massively parallel to detect multiple pathogens, minimize false positive, have rapid response, without sample preparation, and inexpensive. To have high spatial resolution, time resolution, selectivity, and sensitivity to chemicals and biosensors, nanowires and potentiometric measurements were used. Techniques which in principle give some nanometer resolution of the area where we want to measure were presented in the literature.

Electrochemical systems based on inhibition of acetylcholinesterase suggest that the detection of nerve agents can be accomplished with fast speed and sensitivity down to femtomolar levels. Detection routes using antibodies and DNA provide many advantages such as high sensitivity of detection, selectivity, and can be used by non-skilled personnel. A wealth of ideas for portability of the sensors was recently presented in the literature. Electroanalytical and optical strategies involving exploiting methods based on the use of immunosensors and genosensors were presented. The combination of screen-printed electrodes with functionalized magnetic beads constitutes a powerful and efficient strategy for the development of disposable magneto-biosensors for the rapid and ultrasensitive detection of many analytes of bioterrorism significance. Magnetic micro- and nanoparticles have a large active surface area which makes possible the immobilization of a high concentration of biomolecules onto the solid phase of the transducer as well as a decrease of matrix effects.

Protein and even cell detection methodologies with interest for various applications were based on nanotechnology (i.e., nanoparticles, nanochannels). Nanoparticle-based immunosensing systems were offered as excellent screening alternatives to sophisticated and high cost equipment that require well-prepared professionals for their use, including data treatment, prior obtaining of final results with interest for further decisions taken in analysis/screening scenarios.

Development of a sensitive and specific biosensor for rapid detection of microorganisms and protein toxins often requires information about the identity of the analyte of interest. On the other hand, recent advances in microbiology and biotechnology have led to the possibility of creating new microorganisms as well as production of new protein toxins with completely or partially unknown DNA or protein sequences. Thus, rapid identification of microorganisms and protein toxins not only enables the detection of the bio-agents but also facilitates the development of highly portable biosensors for use in the field.

The book is targeted at the development of new routes for construction of devices for the rapid detection of chemical warfare toxic agents and therefore for bioterrorism prevention. New trends in methodology are provided, such as advances in microfluidics, which now offer a realistic means for simplified, practical handling with the facility for compressing existing analytical platforms in biosensing. New perspectives for the construction of biosensor devices are described and include novel routes in the high-throughput screening of toxic proteins using immunochemical tools for the construction of electrochemical, optical, and piezoelectric DNA biosensors for rapid detection of weapons of bioterrorism. Recent works with electronic tongues (that is, analytical systems formed by an array of chemical sensors) featuring high selectivity plus a chemometric tool to process a complex multivariate data are also presented. As the generic application covered is related to security, the described systems are those devised to identify and detect explosive compounds. Magneto-actuated biosensors for bacteria and infectious diseases affecting global healthcare are also described in the book.

Since the introduction of modern chemical warfare agents (CWAs) at the beginning of the twentieth century, there has been a continuous interest in the development of robust and reliable analytical tools for the detection of these agents, to provide early alarm in case of terroristic attacks, as well as to monitor their presence in the environment and prevent contamination. Nevertheless, powerful analytical techniques, including chromatographic methods and mass spectrometry are not suitable for field applications and fast early warning, due to the lack of portability, power requirements, long response time, and expensive procedures. In this context, electrochemical (bio)sensors offer advantages in terms of portability, high sensitivity, miniaturization, integration, low cost, and power requirements. The aim of the book is to highlight the important issues of electrochemical (bio)sensors for fast and cost-effective detection of CWAs in the field, considering the main advantages and limitations of this technology, and the latest trends in nanotechnology, lab-on-chip, and functional materials.

There is a growing demand for rapid and reliable methods of determination of microorganism contamination of waters and food products to ensure quality assurance and to improve the healthcare system in general. The majority of the available analytical methods for determination of microorganisms are time-consuming and expensive. In recent years, different approaches have been attempted to develop alternative procedures for determination of microorganisms. A chapter that summarizes the recent achievements in the development of synthetic recognition systems-based devices for monitoring the presence of bacteria,

bacteriophages, and viruses, in water and food products is included herein. Molecular imprinting has been most successful in devising relevant synthetic receptors. Application of these recognition systems for determination of microorganisms is described in the present book in detail.

A chapter that is devoted to construction of the immune optical, calorimetric, and piezoelectric biosensors for detection and control of toxic agents such as pesticides, nonylethoxylates, mycotoxins: T2, aflatoxins, patulin, etc., is included. Special attention is given to methods for control of genotoxicity. Cell biosensors are considered as possible types of chemical sensors and their integration into sensor elements is investigated. In general, sensitivity in the field of application of the existing approaches for the control of total toxicity and genotoxicity are thoroughly described.

Due to the structural complexity of marine toxins and the difficulty to produce the corresponding biorecognition molecules, the development of assays and biosensors for their detection has become a challenge. Compared to traditional detection techniques, biosensors can provide advantages in terms of sensitivity, specificity, design versatility, portability, and multiplexed configurations. A chapter that provides a critical overview of the immunosensors, receptor-based biosensors, cell-based biosensors, and aptasensors developed for the detection of palytoxins (PITXs), brevetoxins (PbTXs), and tetrodotoxins (TTXs) is included. Although only few biosensors for these emerging marine toxins have been described to date, the chapter reflects the promising advances made in this field.

Aptamers are defined as a new generation of nucleic acids which have recently presented promising specifications over antibodies. They can be produced *in vitro* by Systematic Evolution of Ligands by EXponential Enrichment (SELEX), and have the ability to recognize selectively and sensitively their targets (protein, toxin, drug, or cell). Thus, they have a wide range of applications in different areas, such as drug delivery, imaging, and biosensing. Accordingly, an increasing number of studies related to aptamer-based sensors “aptasensors” have been introduced in the literature. The recent studies on development of aptasensor technologies, which were applied for toxin detection, have been overviewed.

Mycotoxins such as ochratoxin A and aflatoxins are dangerous food contaminants that usually occur in trace amounts from nanograms to micrograms per gram of food. Therefore, highly sensitive methods are necessary for their detection. Conventional analytical methods such as high-performance liquid chromatography (HPLC) and mass spectroscopy are expensive and time-consuming, therefore biosensor technology is promising for rapid detection of toxicants in the field conditions. Among biosensors, those based on monoclonal antibodies and DNA/RNA aptamers are of special interest, because they provide sensitivity of detection that is better than allowable quantities of toxicants in food. While antibodies are traditional receptors in biosensors, aptamers are novel biopolymers with affinity comparable to that of antibodies. However in contrast to antibodies, aptamers are more stable and the biosensors based on aptamers can be regenerated, allowing their multiple use. A contribution reviews the recent achievements in

development of aptamer-based biosensors for detection of selected mycotoxins by electrochemical and acoustic methods.

Recent developments of analytical strategies for determination of potentially hazardous adulterants and allergens achieved to date are presented in the book, highlighting the general considerations and potential prospects for the future. The variety of electrochemical biosensors that have appeared in recent years shows that it is a booming research area with still many challenges, but also great opportunities to develop sensitive, reliable, robust, and cost-effective allergens and adulterants biosensing methodologies.

There is an urgent need for the development of fast and reliable sensors for detection of most toxic compounds that are formed in natural and industrial processes and can have severe consequences on human health. Among many other species, inhibitors of acetylcholinesterase are the focus of many investigations due to a variety of chemical structures and large scale of industrial production. Some anticholinesterase agents, e.g., sarin, soman, and VX gas, were specially developed as chemical warfare with extremely low toxic exposures and lethal consequences for the soldiers and the civil population. Although the accumulated stockpiles of chemical weapons are mostly destructed following the Chemical Weapons Convention, some incidents related to the use of anticholinesterase agents have been reported during the civil wars in Iraq and Syria. The use of homemade sarin in Tokyo subway by Aum Shinrikyo in 1995 is the most known incident related to nerve gases after the Second World War. Meanwhile, threats related to the production and application of anticholinesterase agents by terrorists exist up till today. In addition to chemical warfare, organophosphorus and carbamate pesticides irreversibly inhibit cholinesterase activity and can cause poisoning of agriculture workers and contamination of some foodstuffs. These hazards call for further efforts in the development of appropriate biosensors devoted to detection of anticholinesterase agents in the levels allowing the use of personal protection equipment and hence decrease in the number of potential victims. In this review, progress in the detection of anticholinesterase species based on biosensing technologies is considered with particular emphasis on the results obtained within the past 10 years.

Recently, the diagnosis and treatment of a poisoned person can be done only in specialized centers. Furthermore, currently used clinical methods of intoxication diagnosis are not sufficient for early detection. Conventional laboratory tests based on urine and blood require professional, high-skilled staff, and high cost-equipment as they are arduous and lasting analytical procedures. There is a need to elaborate relatively cheap and easy to use tests, which can simplify and shorten the process of diagnosis of intoxicated patients as well as simplify monitoring of patients from high-risk groups (firemen, miners, security, policemen, soldiers, etc.) having contact with toxic gases. A chapter of the book is focused on novel, early detection sensors for rapid diagnostics of environmental toxicity in the blood of people intoxicated with carbon monoxide.

This book brings together expert scientists with large experience in biosensor technology, some well-recognized environmental analytical chemists, institutes that have large experience in validation, testing, and measurements. A large number of

scientists with previous exciting achievements in constructing novel biosensors are authors in the present book. Stimulated paragraphs are focused toward achievements to the construction and application of biosensors to directly monitor toxic agents (i.e., in the field) and set targets for the future development of such devices having pronounced advantages, e.g., portability, cost-effective, real-time, fast response times, etc. Such developments will lead to rapid (near real-time), low cost devices for in situ monitoring of toxic weapons of bioterrorism. It would also serve to solve many problems of detection of analytes that cannot either be detected with the existing instrumentation or due to the high cost of this instrumentation.

The volume brings together contributions from the most eminent international researchers in the field, covering various aspects of work that have not been published in many scientific journals beyond the “state of art” in this field or even commercial units that are available in the market. Very low detection limits, e.g., 10^{-17} M are reported and novel detection schemes for existing or new pathogens, toxins, and other bioterrorism weapons are presented. Inexpensive, robust, portable miniaturized highly selective biosensing systems that were able to detect multi pathogens without sample preparation are the targets of the present review articles; this will give the opportunity to learn new technological schemes that will lead to the construction of devices against bioterrorism that will minimize the risk of weapons of terrorism.

Work on rapid identification of microorganisms and protein toxins by proteomics is an issue that is well presented and the participants of the volume have the opportunity to present their techniques on how to bind the transduction element into the physical sensor unit. Novel techniques of transduction such as nanowiring, nanoparticles, or molecular imprinted polymers are presented and some of these transducers are able to be implanted in the human body. Target analytes detected include a wide range of microbes, pathogens, toxins, nervous gases such as botulinum toxin, *Escherichia coli*, *K. Pneumoniae*, sarin, VX, listeria monocytogenes, salmonella, marine biotoxins (such as palitoxins, spirolides, etc), staphylococcal enterotoxin B, saxitoxin, gonyautoxin (GTX5), francisella spore virus, *bactillus subtilis*, ochratoxin A, cholera toxin, etc. Emphasis is given to the simultaneous analysis of multiple species screen-printed low density microelectrode arrays that were used to develop genosensors to detect *Salmonella* SSP and *staphylococcus aureus*. Scanning electrochemical microscopy is used to monitor listeria monocytogenes. Chemical transduction biological elements acting as “receptor” such as antibodies, enzymes, DNA, RNA, lipids, natural, and artificial receptors were immobilized on the physical sensor to recognize the target analyte.

In preparing the book, we have relied on the timely contribution of authors and without their motivation and commitment the publication of this volume would not have been possible. We, thus, extend appreciation to all the authors. We also convey our thanks to Springer for affording us the opportunity to publish this volume.

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