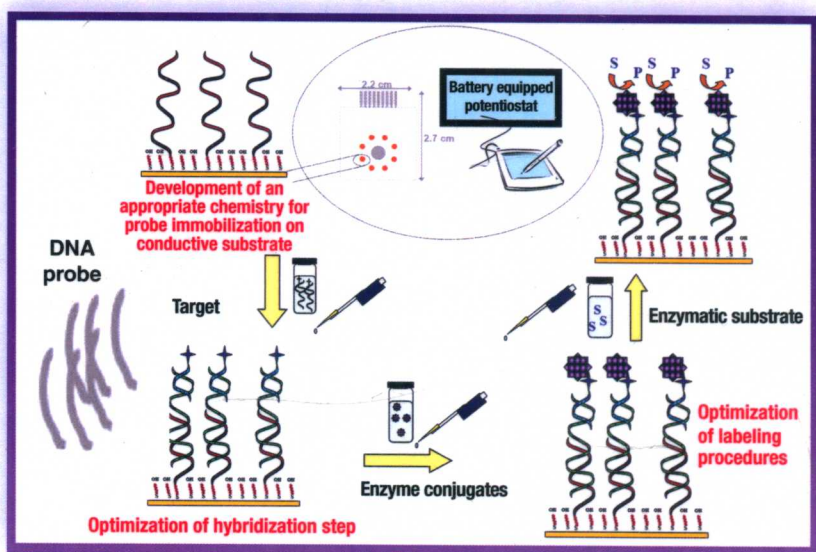


SERIES IN SENSORS

Portable Biosensing of Food Toxicants and Environmental Pollutants



Edited by

Dimitrios P. Nikolelis • Theodoros Varzakas • Arzum Erdem
Georgia-Paraskevi Nikoleli



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Preface

A chemical sensor is a device that transforms chemical information, ranging from the concentration of 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 domain. A biosensor is a self-contained integrated device that is capable of providing specific quantitative analytical information using a biological recognition element (e.g., enzymes, antibodies, natural receptors, and cells), which is retained in direct spatial contact with a transduction element. Recent advances in the technology of artificial receptors have prompted a clear distinction between chemical sensors and biosensors. The latter utilize a transduction element of biological origin; however, since there has not been much development in engineered molecules, both terms are and can be used in the literature for this class of devices. The chemical sensors should be clearly distinguished from an analytical system that incorporates additional separation steps, such as liquid chromatography (LC), or additional hardware and/or sample processing, such as specific reagent introduction, for example, flow injection analysis (FIA). Biosensors have not yet made a large impact in the area of environmental, food, and biomedical applications but clearly offer advantages in comparison to standard analytical methods, such as minimal sample preparation and handling, real-time detection, rapid detection of the analytes of concern, and use of nonskilled personnel. Biosensors have the ability to be repeatedly calibrated; the term "multiple-use biosensor" is limited to devices suitable for monitoring both the increase and the decrease of analyte concentrations. Devices that cannot rapidly be regenerated should be named "single-use biosensors."

This book aims to provide both the basic knowledge regarding biosensor technology at a postgraduate level (MSci or PhD) as well as recent advances in chemical sensor technology and thus can be utilized by researchers. It covers the major areas of biosensors as follows:

1. Transducer schemes, that is,
 - a. Optical photonic crystal waveguide sensors
 - b. Electrochemical sensors
 - c. Piezoelectric sensors
 - d. Surface-enhanced Raman spectroscopy (SERS)-based biosensors

2. Recognition element, that is,
 - a. Enzyme sensors
 - b. Antibody-based biosensors
 - c. Ion-channel switch and lipid film-based biosensors
 - d. Nucleic acid biosensors
 - e. *De novo* DNA synthesis and its biosensor detection
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 - b. Water-soluble vitamin and drug residue determination
 - c. Optical biosensors in food safety and control
 - d. Biosensors in express control of quality assurance of products
 - e. Efficiencies of biosensors in environmental monitoring
 - f. Oligonucleotide and DNA microarrays as versatile tools for rapid diagnostics
 - g. Biosensors for pesticides and foodborne pathogens
 - h. Micro- and nanopatterning for bacteria- and virus-based biosensing applications
 - i. Electrochemical DNA biosensors in food safety determination of phenolic compounds and antioxidant capacity in foods and beverages
 - j. Biosensors in quality of meat products
 - k. Microbial cells and enzymes for assaying the fermentation processes of alcohol production—starch, glucose, ethanol, BOD
 - l. Biosensors in control of biochemical quantities to diagnose diseases

Chapter 1 introduces the basic principles of biosensing that will be useful to newcomers to this technology. Chapter 2 explains how the incorporation of a “receptor” can provide analytically useful information. Chapter 3 describes recent trends in biosensing and how a small-sized device can have portability for *in situ* determination of toxicants. Finally, Chapter 4 provides several examples for the determination of toxicants in food and

environmental samples. The book is organized in such a way as to introduce to both newcomers and experts a roadmap to this technology by providing useful information on recent trends in biosensing devices.

Environmental and food biosensors come in thousands of forms and types based on a wide range of physical and chemical principles with varying types of usable outputs. Typical environmental contaminants monitored include metals, volatile organic compounds, biological contaminants, and radioisotopes. The field applications of sensors are also extremely varied. Among the key trends in the environmental and food sensor business is miniaturization down to the nanoscale, continuous and/or real-time sensing capabilities, wireless networked operation, rapid processing, and increased sensitivity or flexibility. Areas of environmental focus include vehicular emissions, combustion of fossil fuels, agricultural runoff, industrial and mine waste disposal, ocean spills and dumping, and climate change and weather monitoring. Very few examples of portable biosensors which can rapidly detect environmental pollutants directly in the gas phase have been reported in the literature.

Progress in nanosciences has led to a range of new technologies that allows us to drastically improve and even rethink and create industrial processes and products offering new functionalities. Sensors are core elements in any intelligent system for monitoring and controlling natural and industrial environments, and nanotechnology offers new possibilities for sensors, sensing-based systems, and applications. Recent trends in integrated electronics have started a revolution in this field, allowing the shrinking of very complex electronic systems into millimeter-sized squares. This would allow implementing complex and sophisticated instrumentation in cheap and portable devices for rapid detection of harmful and toxic agents.

The specific objective of this book is to tap into the progress made in nanosciences to deploy nanotechnology in affordable, mass-produced sensors and to integrate these into components and systems (including portable ones) for mass market applications in environmental and food monitoring. Sensing includes chemical and microbiological environmental toxicants, such as chlorinated hydrocarbons, heavy metals, polychlorinated dibenzodioxins (commonly called dioxins), nitrogen oxides (NO and NO_2), components of the photochemical smog that is a noxious mixture of air pollutants (SO_2 , aldehydes, nitrogen oxides, peroxyacyl nitrates, etc.), chlorofluorocarbons (CFCs), hydrogen sulfide, water pollution (by the discharge of wastewater from commercial and industrial waste), discharges of untreated domestic sewage, as well as insecticides, pesticides, and herbicides.

The book also aims to make the rapid detection of bioterrorism weapons possible. The objective of this book is also to present advances in the development of portable chemical sensors for the rapid detection of biotoxins in the environment; the scope encompasses the advances of devices that can be used for the rapid real-time detection of toxicants such as microbes,

pathogens, toxins, and nerve gases, for example, *Clostridium botulinum* toxin, *Escherichia coli*, *Klebsiella pneumoniae*, sarin, VX, *Listeria monocytogenes*, *Salmonella*, marine biotoxins (such as palitoxins and spirolides), staphylococcal enterotoxin B, saxitoxin, gonyautoxin (GTX5), *Francisella* spore virus, *Bacillus subtilis*, and ochratoxin.

Topics include sensor design and fabrication (including the use and development of modeling tools), a technology demonstrator, and a positive production capacity feasibility study (including economic assessment) and plans for their commercial implementation.

Systems integration aspects to consider include easy and fast (multi)sensor interrogation and interfacing with monitoring and control functions. Reliability is required within the foreseen operating environment, considering temperature, humidity, and other parameters that affect stability. Initiation (resetting) and calibration require special attention.

The functionality should be demonstrated by integrating the developed sensor element into an existing or prototype system for validating its industrial relevance. The target is to realize the market potential of the results of the existing research, which will lead to improved performance of applications in the fields of environmental monitoring and provide significant benefits to citizens and for the environment.

Topics are focused toward the rapid detection of indoor and outdoor pollutants such as heavy metals, nitrogen oxide dioxins, CO₂, sulfur dioxide, volatile organic compounds (VOCs), low levels of methane, insecticides, pesticides, plant and vegetable hormones, etc.

Although biosensors exhibit double-digit growth rates, they still have to overcome a number of challenges, including the following:

- New research focuses less on fundamental research due to increasing challenges in applications.
- Development of a single biosensor platform with multipurpose diagnostic capability has restricted biosensor application.
- A number of problems prohibit the successful commercialization of biosensors. These problems have to be encountered in their development strategies.
- Competition from nonbiosensor technologies has hindered revenue growth.
- A low rate of technology transfer and lower level of development have deterred the development of new biosensors.
- The fabrication of portable chemical sensors in field uses has seen very little development.

In recent times, the market thrust has shifted to nanosensors' capabilities to detect environmental toxicants and food toxicants rapidly. A recent report from the market research firm In-Stat revealed that the media spotlight on

this application may be premature: Despite the public's anticipation that biosensors with real-time detection will be able to monitor biochemical environmental toxicants, the technology has not matched expectations. Presently, biosensors in environmental monitoring stations nationwide can detect compounds like anthrax—but detection can take 12 to 24 h. The best ones in the market take 20 min.

Gas phase chemical detection is of critical importance for the sensing of highly toxic molecules, such as chemical warfare agents (CWAs) and toxic industrial chemicals (TICs). Beyond the ability to detect CWAs in a laboratory, that is, in the context of large, technical, and relatively expensive apparatus, of significant interest is the detection of CWAs with SERS in the field. The advent of small, portable Raman spectrometers with dimensions close to that of a smartphone, such as the ReporteR spectrometer (Intavec, Inc.) or the First Defender (Thermo Scientific, Inc.), and the development of stand-off SERS detection have begun the transition from the lab to the field.

In this book, portable and handheld nanosensors, for example, dynamic DNA and protein arrays for rapid and accurate detection of environmental pollutants, pathogens, etc., are described. The following challenges are focused on

1. High sensitivity—*detecting* very small amounts of environmental pollutants such as heavy metals, pathogens, toxins, and chemical toxicants in the environment
2. High selectivity—*discriminating* targets from other materials
3. High parallelism—*detecting* multiple pathogens, *minimizing* false positives, and *having* rapid response without sample preparation
4. High transportability—*being* nanosized and transportable yet robust and easy to operate
5. High affordability—*being* made up of inexpensive materials
6. High adaptability—*being* adaptable to new biotoxins and integrated with chemo/biosensors
7. High precision—*allowing* for the detection of single molecules

Therefore, the state of the art of our devices is summarized as follows: Nanosensors will be highly sensitive, selective, rapidly responding, real-time, massively parallel, with no or minimum sample preparation, on a platform suited to portable and handheld nanosensors for the rapid detection of environmental pollutants for in field uses even by non-skilled personnel.

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This book has benefited from contributions by well-known and distinguished researchers worldwide. We would like to acknowledge their valuable contribution and cooperation. It has been an honor for us to edit this book. Two of the editors (D. P. Nikolelis and G.-P. Nikoleli) express their gratitude to the Greek Ministry of Development, General Secretariat of Research and Technology, and the European Commission (in particular the European Regional Development Fund and National Resources) (Contract 12SLO_ET30_1036) for their financial assistance.

Editors

Dimitrios P. Nikolelis earned his PhD from the University of Athens in 1976. He currently serves as a professor of environmental chemistry in the Department of Chemistry, University of Athens. He has coordinated three European projects on environmental biosensors (CIPA96-0231, IC15-CT96-0804, and QLK3-2000-01311) and has served twice as a NATO director in the following advanced research workshops: (1) Biosensors for Direct Monitoring of Environmental Pollutants in Field, Smolenice, Slovakia, in May 1996 and (2) Portable Biosensors for the Rapid Detection of Biochemical Weapons of Terrorism, Lunds, Sweden, in July 2012. Prof. Nikolelis has published over 200 scientific papers in scientific journals and conferences. In addition to this book, he is the editor of two other books on biosensors: *Biosensors for Direct Monitoring of Environmental Pollutants in Field* (Springer, 1996) and *Portable Chemical Sensors—Weapons against Bioterrorism* (Springer, 2012). He is also the editor of the scientific journal *Chemical Sensors*. His research is targeted on the fabrication of portable biosensors for uses in the field, and he has authored a large number of scientific papers on the detection of environmental pollutants such as hydrazines, dioxins, insecticides, and toxins. His current interests include the construction of novel chemical nanosensors that can be used for the rapid detection of environmental pollutants directly in the gas phase. Professor Nikolelis' group has recently utilized graphene and ZnO electrodes to develop chemical nanosensors for species of clinical or food significance such as urea, uric acid, and cholesterol. Work in progress includes evaluation and validation of nanosensors that are based on stabilized lipid films supported on glass fiber filters. This is done by using a dry spot test and optical methods of analysis (i.e., fluorescence) to detect environmental pollutants such as insecticides, plant hormones, toxins, and hydrazines.

Theodoros Varzakas earned his bachelor's degree (honors) in microbiology and biochemistry (1992) and his PhD in food biotechnology and MBA in food management from Reading University, United Kingdom (1998). He worked as a postdoctoral research staff at the same university. He has also worked in large pharmaceutical and multinational food companies in Greece for 5 years and has 14 years of experience in the public sector. Since 2005, he has been serving as assistant professor in the Department of Food Technology, Technological Educational Institute of Kalamata, Greece, specializing in issues of food technology, food processing, food quality, and safety. In 2012, he was elected as associate professor. He has served as a reviewer in many international journals such as the *International Journal of Food Science & Technology*, the *Journal of Food Engineering*, *Waste Management*, *Critical Reviews in Food Science and Nutrition*, the *Italian Journal of Food Science*, the *Journal of Food Processing and Preservation*, the *Journal of Culinary Science*

and Technology, the *Journal of Agricultural and Food Chemistry*, the *Journal of Food Quality*, and *Food Chemistry*. He has also written more than 80 research papers and reviews and has presented more than 80 papers and posters at national and international conferences. He has published two books in Greek, one on genetically modified food and the other on quality control in food. He has also edited a book on sweeteners, which was published in 2012 by CRC Press.

Dr. Varzakas has participated in many European and national research programs as a coordinator or scientific member. He has been a fellow of the Institute of Food Science & Technology since 2007.

Arzum Erdem is a professor at the Analytical Chemistry Department in the Faculty of Pharmacy of Ege University in Turkey. She earned her PhD in analytical chemistry from Ege University in 2000. She was recognized as a highly skilled young scientist in 2001 by the Turkish Academy of Sciences (TUBA) and also received the Junior Science Award in 2006 from The Scientific and Technological Research Council of Turkey (TUBITAK). Dr. Erdem has initiated several international collaborative research projects on the development and applications of electrochemical (bio)sensors based on drug, enzyme, and nucleic acids. Her recent research is centered on the development of novel transducers and chemical and biological recognition systems using different nanomaterials (e.g., magnetic nanoparticles, carbon nanotubes, gold and silver nanoparticles, and nanowires) designed for the electrochemical sensing of nucleic acid (DNA, RNA) hybridization, and also the specific interactions between drug and DNA, or protein and DNA and aptamer–protein, as well as the development of integrated analytical systems for environmental, industry, and biomedical monitoring.

Georgia-Paraskevi Nikoleli earned her BSc from the University of Athens in 2007. She is currently pursuing her PhD in the Laboratory of Inorganic and Analytical Chemistry, School of Chemical Engineering, Chemical Sciences, National Technical University of Athens, Greece. Her research interests include environmental and food analysis and nutrition. Her research is targeted on the fabrication of portable biosensors for in-the-field uses. She has authored a large number of scientific papers on the detection of environmental pollutants such as insecticides, toxins, and dioxins. Her current interests include the construction of novel chemical nanosensors that can be used for the rapid detection of environmental pollutants directly in the gas phase and for real-time monitoring of environmental pollutants in waters. Her other interests include waste recycling.

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