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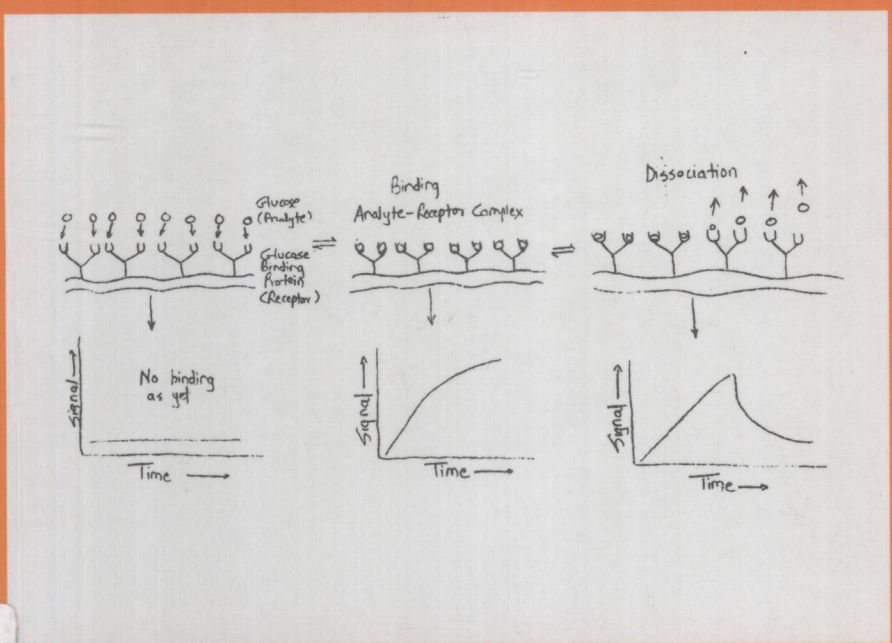

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2005

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Amsterdam – Boston – Heidelberg – London – New York – Oxford – Paris
San Diego – San Francisco – Singapore – Sydney – Tokyo

ELSEVIER B.V.
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Amsterdam, The Netherlands

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The Boulevard
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Oxford OX5 1GB, UK

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84 Theobalds Road
London WC1X 8RR
UK

First edition 2005

Library of Congress Cataloging in Publication Data
A catalog record is available from the Library of Congress.

British Library Cataloguing in Publication Data
A catalogue record is available from the British Library.

ISBN: 0-444-51945-9

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).
Printed in The Netherlands.

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This book is dedicated to my daughters Neeti and Richa

Foreword

The abilities of biological organisms to recognize foreign substances are exquisite and have to some extent been exploited by researchers in the development of biosensors. Using bioreceptors from biological organisms or receptors that have been patterned after biological systems, scientists have developed a new means of chemical analysis that often has the high selectivity of biological recognition systems. These biorecognition elements in combination with various transduction methods have helped to create the rapidly expanding fields of bioanalysis and related technologies known as biosensors. Nowadays, the increasing interest in biosensors is largely a result of the wise applicability of the method, and the considerable selectivity, good sensitivity, and possibility of automation for bioanalysis. Biosensors have been of greater research interest during the past 10 years and this trend is likely to continue for many years with the expanding growth of biomedical technology.

In this excellent book, fundamental principles and biochemical mechanisms on fractals and their relationships with biosensor design and applications are covered in a tutorial manner. Dr. A. Sadana also covers in a comprehensive, logical, and easily readable fashion such topics as single-fractal and dual-fractal analysis, binding and dissociation kinetics as well as important background in fractals, instrumentation, methodologies for a wide variety of application areas of fractals.

The symbols, units, terminology, and organization in this book are consistent, logical and clear throughout. This book should be of considerable use to anyone who wishes to apply fractal analysis to biosensor research, design and applications.

I am proud to have prepared a foreword for such an outstanding book and a fine friend.

Tuan Vo-Dinh
Oak Ridge National Laboratory
July 2005

Preface

Biosensors are finding increasing application in the areas of healthcare, drug design, environmental monitoring, detection of biological, chemical, and toxic agents, biotechnology, aviation, physics, oceanography, and the protection of civilian and engineering infrastructures. This book emphasizes the two areas of expanding biosensor development that include (a) detection of biological and chemical pathogens in the atmosphere, and (b) biomedical applications, especially in healthcare. In both of these cases, one needs to detect analytes at very dilute concentrations.

Modifications in the fractal mathematics is made in the appropriate chapters, and effectively applied to help model the binding and dissociation kinetics of analytes in solution to receptors immobilized on a biosensor surface. It is anticipated that the application of biosensors for the detection of analytes that have medical applications, such as thrombin, prions, glucose, and heat-shock proteins is bound to increase in the future. The early detection of analytes that lead to the onset of disease will considerably assist in its management and control, and hopefully in the alleviation of insidious and intractable diseases. For example, the early detection of markers of diseases such as cancer, systemic lupus erythomatosus (SLE), arthritis, and cardiac-related diseases is invaluable. Biosensors can assist in this effort, and this book emphasizes these viewpoints.

Chapter 1 provides an introduction to biosensors, that includes a definition of a biosensor, advantages and disadvantages of biosensors, and a brief overview of economics of biosensors. Chapter 2 outlines and examines the fractal theory used to model the binding and dissociation kinetics of analytes in solution to receptors on biosensor surfaces. Both simple and complex mechanisms are presented.

Chapter 3 analyzes the kinetics of detection of harmful pathogens on biosensor surfaces. Considering the present geopolitical environment this is an extremely important area of investigation. Large sums of money are being spent in this area of biosensor development by different governmental agencies around the world. Chapter 4 analyzes the binding and dissociation kinetics of heat shock proteins on biosensor surfaces. The levels of these proteins increase when the body is subjected to physiological stress, and they assist other proteins to fold properly. Protein misfolding is involved in quite a few diseases.

Chapter 5 analyzes the binding and dissociation kinetics of prions on biosensor surfaces. These prions are involved in diseases such as mad cow disease (bovine spongiform encephalopathy, BSE) and Alzheimers. The human form of BSE is variant Creutzfeld-Jakob disease (vCJD). Chapter 6 is the heart of the book and presents different examples of the kinetics of binding and dissociation of analytes related to human health on biosensor surfaces. Examples analyzed include the ever prominent glucose, riboflavin, Pb^{++}, prostrate specific antigen (PSA), etc.

Chapter 7 presents the kinetics of binding and dissociation of human heart fatty acid binding protein on biosensor surfaces. This is an early marker of ischemic heart disease. Chapter 8 presents a kinetic analysis of the binding and dissociation of p38α

mitogen-activated protein (MAP) kinase occurring on biosensor surfaces. P38α MAP kinase is a well-characterized target for inflammatory disease therapy.

Chapter 9 presents a kinetic analysis of heparin–protein interaction studies on biosensor surfaces. Heparin binds to growth factors, and influences angiogenesis and other proliferation dependent processes. Chapter 10 analyzes the binding and dissociation kinetics of thrombin on biosensor surfaces. The prevention of clot formation due to thrombin is an important area of investigation.

Chapter 11 analyzes the binding and dissociation kinetics of Interleukin 5 (IL5) on biosensor surfaces. IL5 is a single-chain major cytokine, and exerts its hematopoietic growth factor function by binding to a membrane-bound receptor. Chapter 12 presents a kinetic analysis of the binding and dissociation of different environmental contaminants on biosensor surfaces. The kinetics of some of the pollutants analyzed include benzene, toluene, 2,4 dinitrophenol, and methanol.

Chapter 13 presents the market size and economics for biosensors. This chapter provides an overall balance for the whole book. Understandably, this type of information is very difficult to obtain in the open literature free of charge. Not unexpectedly, industries working in the area of biosensors will guard their information very carefully. This is exacerbated by the fact that this is a small and niche market, and putting it mildly, the competition is fierce.

The author wishes to express his appreciation to Dr. Kai-Fong Lee, Dean, School of Engineering at the University of Mississippi for his continued support and encouragement for research that facilitates the writing of treatises like this.

A lot of credit goes to my students who have patiently helped me develop over the years the fractal theory for biosensor applications. My present student, Mr. Atul M. Doke has helped me considerably in getting this book ready for Elsevier.

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Introduction

1.1 DEFINITION OF BIOSENSORS

A chemical sensor is a device that transforms, for example, a concentration of a chemical into a useful analytical signal (adapted from IUPAC, International Union for Pure and Applied Chemistry definition). Walsh (2003) indicates that a biosensor may be considered as a chemical sensor with three components: (a) a receptor, a transducer, and a separator. The receptor or biological element (for example, enzymes, antigens, antibodies, tissues, whole cells, bacteria, etc.) converts the biochemical binding event to a measurable component. The transducer converts this measurable component to generally a measurable electrical or optical signal. The transducer could, for example, be an acoustical device, a calorimetric device, an optical device, or an electrochemical device. The separator (for example, a membrane) separates the transducer from the bioreceptor.

Walsh (2003) has provided some examples of biosensors that have been commercialized, and include: glucose sensors to help monitor sugar levels in diabetics, lactate biosensors, amperometric sensors for gases, and ion-selective electrode (ISE) for blood gases and electrolytes.

Figure 1.1 shows the components of a biosensor (Biowise, 2001). Simply speaking, there is a biological component and an electronic device. The biological receptor (component) reacts with the analyte of interest (binding and/or dissociation), and produces a biochemical change. This biochemical change is transduced or converted to a measurable signal. The amplifier increases the intensity of the signal enabling easier measurement. Biowise (2001) indicates that these components are housed in a single unit that may either be placed at a strategic location or made more portable.

With the advent of nanotechnology, miniaturization, and improved fabrication techniques, there is more and more emphasis on hand-held devices, especially for the detection of biological hazards and biowarfare agents. According to Check (2004), the United States Department of Homeland Security has a $41.5 million program to develop and evaluate hand-held kits to detect harmful biologicals in a possible terrorist attack situation. These are to be used by emergency workers and by first responders. However, these hand-held detection devices still have problems during use. One way around this is to use these hand-held detectors to rule out a lot of other things, and to use them along with other techniques. Some evaluators of these hand-held detectors indicated that they were too limited in their use. Hopefully, with more analysis and research these hand-held

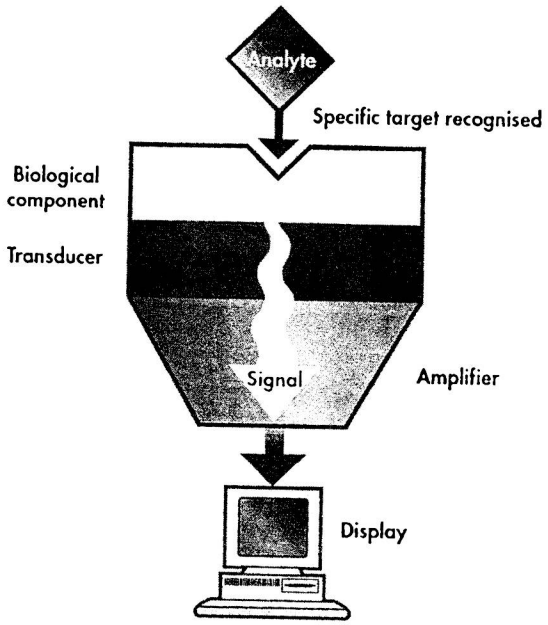

Figure 1.1 Components of a biosensor (Biowise, 2001).

detection devices will become an integral part of the arsenal used by emergency workers and by first responders.

Turner *et al.* (1987) define a biosensor as a 'compact analytical device that incorporates a biological element or a biologically-derived element that is either integrated with or intimately associated with a physicochemical transducer'. These authors further indicate that the signals from the biosensor may be either discrete or continuous. They indicate that the major demand for biosensors is in glucose monitoring for diabetics which comprises 85–90% of the total market for biosensors. This is facilitated by the biosensors providing a convenient, compact, and hygienic method of glucose measurement (Turner, 1996). The three major players involved in glucose monitoring are Abbott, Boehringer Mannheim, and Bayer.

Pharmacia in Sweden has had, and continues to have considerable commercial success with its Biacore surface plasmon resonance (SPR) biosensor. This is based on the SPR principle, and was initially described by Liedberg *et al.* (1983). It is finding increasing application since it monitors reactions in real time. This is an expensive piece of equipment in the $300,000–400,000 range. The software that comes along with it does provide values of the binding and dissociation rate coefficient(s), and affinity values. However, the model used to describe the kinetics assumes (a) the receptors are homogeneously immobilized on the sensor chip surface (no heterogeneity), and no diffusional limitations are assumed to be present if the SPR is run properly. These two assumptions could lead to errors in the estimated values of the binding and dissociation rate coefficients, and affinity values. The fractal analysis presented in this book, and which is used to analyze the binding and dissociation kinetics is an alternative method.

1.2 CURRENT AND FUTURE APPLICATIONS

Traditionally, biosensors have found increasing applications in the biomedical areas. Over the years these areas of applications have expanded to include biotechnology, physics, chemistry, medicine, aviation, food safety, oceanography, and environmental control. Recently, as noted by events occurring world wide, the emphasis has shifted to include biosensor application for the detection of biological and chemical threats, and for homeland security. Other countries, may have a different terminology for homeland security, but the emphasis remains the same: one needs to defend one's borders, and, if one may partially borrow from the motto of the police to preserve and to protect the nation's infrastructure and population.

There has been an increasing emphasis and resource allocation for biosensor research in the recent years, and in the areas of biosensor application. This is made evident, for example, in the Requests for Proposals and Program Solicitations being put out recently by United States Governmental agencies. For example, the National Science Foundation (National Science Foundation Program Solicitation, 2003) in its program solicitation NSF 03-512 entitled Sensors and Sensor Networks (where the proposal deadline was March 06, 2003) indicated the need for the development of sensors to detect biological agents, explosives, and toxic chemicals. Approximately, $34,000,000 was available for competitive research applications. Emphasis was placed on enhancing biosensor performance parameters that included robustness, fewer false alarms, sensitivity, stability, speed of response, regenerability (if possible), and reliability. This document indicated that the availability of wireless and internet communication, and miniaturization and nanotechnology/nanobiotechnology was predicted to place biosensor applications in an increasingly dominant role for sensing and for detection.

This NSF program solicitation was followed by the program solicitation NSF 04-532 entitled 'Sensors and Sensor Networks (Sensors)' (proposals due February 26, 2004) (National Science Foundation Program Solicitation, 2004) that replaced the above mentioned document, and emphasized the advancement of knowledge in materials engineering for biosensor development. Newer concepts and designs were encouraged. Sensors need to be included in engineering systems. Furthermore, it was recommended that sensor data and the analysis of such data should be more included in decision-making processes. The program solicitation emphasized that emerging technologies would impact sensor development significantly, primarily with regard to the decrease in size, weight, and cost. Similar documents, are presumably available in European (United Kingdom, France, Germany, Sweden) and other countries (such as Japan and Australia) to name a few.

The National Science Foundation has come out with a more recent program solicitation document NSF 05-522 entitled 'Sensors and Sensor Networks (Sensors)' (proposals due March 03, 2005) (National Science Foundation Program Solicitation, 2005). The solicitation seeks and if we may quote, 'to advance fundamental knowledge in new technologies for sensors and sensor networks'.

Though, in general academic institutions, can and will place a lot of emphasis on the size and weight of a biosensor, traditionally the economics or the cost of biosensor development and its market cost vis-a-vis the market size is traditionally left to the industry. As expected, one may anticipate that industrial sources would guard their knowledge with regard to biosensor development, especially the economics. Very little,

if any, economic information is available in the open literature. If this information is available in the open literature, then presumably it is sparsely available, and spread out in different sources. One of the goals of this book is to provide under one cover the economic information on biosensors such as market size, cost of development, number of years required to develop and test a prototype, etc. if available in the open literature. The last chapter in the book is devoted to this area.

In order that one may obtain a better perspective of where the current applications of biosensors are (along with the research areas emphasized by the above mentioned National Science Foundation funding possibility documents), we now provide a list of recent areas of biosensor research available in the literature. This is only a partial list. Other recent biosensor examples, where the kinetics of binding (and dissociation) have also been analyzed in detail are presented in later chapters.

Some of the biosensor applications that have recently appeared in the literature include:

(a) *Acoustic Wave Chemical Sensor*: Valentine *et al.* (2004) have very recently developed an acoustic wave chemical sensor. This is based on the microelectromechanical systems (MEMS) approach. Binding of target molecules to a functionalized surface are determined by these types of sensors. These authors indicate that a sensor should be sensitive, easy to use, fast and be reusable. They emphasize that their approach does satisfy all of the above requirements. Besides, since their sensor has a higher surface area to mass ratio than other sensor designs, such as the cantilevers, their approach exhibits potential for increased sensitivity compared to the other sensor designs.

(b) *Sensing Biomolecules and Cells*: Haddock *et al.* (2003) have recently using tapered fibers to develop a rapid, convenient, and accurate sensor for biomolecules and cells. Their sensor uses volumes of cells around 150 μl. They emphasize that the sensing of biomolecules and cells is important in clinical, pharmaceutical, and in cellular applications (Chuang *et al.*, 2001; Cullum *et al.*, 2000; Ferreira *et al.*, 2001). Using their developed biosensor and an analytical grade spectrofluorometer Haddock *et al.* (2003) were available to detect and measure nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), and Chinese Hamster Ovary (CHO) cells at different concentrations. They indicate that their results show that the sensitivity obtained with their tapered fibers is at least an order of magnitude more than that obtained with a cuvette arrangement.

(c) *Drug Screening*: Borch and Roepstorff (2004) have very recently developed a novel strategy to help identify enzyme inhibitors. They indicate that the activities of some medical drugs are based on their inhibitory action on specific enzyme(s). For example, the anticancer drug, Imatinib (Glivec) that inhibits tyrosine kinases (Capdeville *et al.*, 2002), and HIV protease inhibitors that act against the HIV virus (Molla *et al.*, 1998).

The protocol designed by Borch and Roepstorff (2004) is simple. An enzyme is immobilized on a sensor chip. The activity of the enzyme is noted by incubating the enzymes with model substrates and testing by mass spectrometry for the products. Potential enzyme inhibitors are passed over the sensor chip containing the enzyme. The binding kinetics (if any) is noted by SPR. Then, model substrates are passed over the sensor chip again, and mass spectrometric analysis determines if the enzyme activity has been inhibited by the compounds been tested for possible therapeutic usage. Enzyme inhibitors apparently exhibit an increasing potential for use as therapeutic agents, thus

screening procedures, such as those proposed by Borch and Roepstorff (2004) are bound to gain increasing importance in the future.

Skretas and Wood (2004) have recently indicated the need for a variety of drug-screening assays to help test different compounds and protein targets for potential drugs. This needs to be done in a high throughput fashion. These authors have engineered hormone sensitive bacteria for efficient drug screening. Their method is based on ligand binding of *in vivo* sensors. Their *in vivo* sensor was a hormone, and they used it to analyze ligand binding in *Escherichia Coli*. By changing the parameters of their assays and by observing the changes in cell growth these authors were able to report the presence of active compounds. This procedure permitted these authors to help identify drug compounds from a wide range of test molecules.

(d) *Diagnostic Biomarkers*: May *et al.* (2004a,b) very recently indicate that over a million people are diagnosed with cancer each year. It would be extremely beneficial to be able to detect cancer at an early stage. Growth of cancer may be broadly classified into three stages: first (latent phase), second (intermediate) phase, and a third ('blast') phase. In order to improve survivability, it is essential to be able to detect cancer at the earlier stages. May *et al.* (2004a,b) indicate that vascular endothelial growth factor (VEGF) is a potential cancer biomarker. It is present in the normal human blood in very small quantities. These authors indicate that correlations have been obtained between large quantities of VEGF in the serum and in the plasma of cancer patients. They have developed a whole-cell based biosensor for the detection of VEGF *in vivo*. Their biosensor comprises of a monolayer of human umbilical vein endothelial cells (HUVECs) attached to a cellulose triacetate (CTA) membrane on an ISE. These authors were able to optimize the detection limit as a function of exposure time. This increased the sensitivity of their whole-cell based biosensor.

(e) *Pathogen Detection*: Fitch *et al.* (2003) have very recently provided an overall perspective of the detection and identification of chemical and biological agents that may be considered as 'terrorism' threats. They indicate the need for increased sensitivity, greater automation, and fewer false alarms. Furthermore, on a more practical note they indicate the attempts being made to make these systems more cost effective as well as reducing the complexity of these systems in order that they may be more effectively employed in the field. They emphasize the need for *early intervention*.

For example, Inglesby (2000) indicates that plague (caused by *Yersinia pestis*) symptoms occur within 1–6 days after exposure. Fitch *et al.* (2004) indicate that antibiotics are most effective when administered within 24 h of exposure. In order that early intervention may be facilitated in the case of an inadvertent or deliberate (terrorism) exposure to a chemical or a biological agent, Fitch *et al.* (2004) indicate that environmental monitoring systems are in place at major United States cities (Cole, 2003).

Hostadler *et al.* (News, 2004) have developed the triangular identification for genetic evaluation of risk (TIGER) to identify both known and uncharacterized pathogens. This method has the capacity to identify viruses, bacteria, fungi, and parasitic protozoa. The authors claim that TIGER is able to detect mixtures of organisms in the same sample. They anticipate the use of their technique in infectious disease epidemics, biowarfare, food contamination, and human forensics. Using their technique Hostadler and his colleagues were able to identify the SARS virus as a new member of the coronavirus family.

Bae *et al.* (2004) have recently used imaging ellipsometry (IE) to detect *Yersinia enterocolitica*. These authors indicate that this is an optical technique that involves measuring the change of a polarization state of an elliptically polarized beam reflected from thin films (Azzam and Bashara, 1997). Bae *et al.* (2004) point out that the advantage of using the IE for biosensor applications is that it permits label-free detection, it is simple to operate, and it is highly sensitive. Durisin *et al.* (1997) have indicated that *Y. enterocolitica* is a human pathogenic species and causes yersiniosis. This disease is characterized by fever, diarrhea, and abdominal pain. Using their developed immunosensor Bae *et al.* (2004) were able to detect *Y. enterocolitica* concentrations in the range of $10^3 - 10^7$ cfu/ml.

Joshi *et al.* (2004) have recently used a carbon nanotube based biosensor to detect a VX analog and its degradation products. These authors indicate that sarin, soman, and VX are highly toxic nerve agents. They indicate that their degradation products are more stable than the original compounds. Thus, their detection in the atmosphere can be used to (a) prove the existence of the use of these toxic nerve agents, and (b) assist in monitoring the destruction of these harmful compounds. Using electrochemical detection and the use of carbon nanotubes (CNT) these authors were able to (a) detect VX degradation products and (b) with a modification, the detection of the VX analog, Demton-S.

May *et al.* (2004a,b) have very recently developed a whole-cell based biosensor to detect histamine as a model toxin. These authors indicate that histamine resides in seafood, and in patients with severe allergic reactions (Niwa *et al.*, 2000). May *et al.* (2004a,b) emphasize that their biosensor could find applications in homeland security, food and medical areas, and in environmental monitoring. A monolayer of HUVECs was attached to a CTA membrane of an ISE. These authors indicate that histamine alters the permeability of HUVECs. In the absence of toxic agents, and in the presence of potassium (K^+) ions, the monolayer blocks the interface, yielding no response from the ISE. In the presence of toxins, the permeability of the cells is affected, K^+ reaches the ISE, which gives rise to a change in the potential of the ISE.

(f) *Homeland Security*: Viswanathan and Staples (2004) indicate that virtual chemical sensors and odor profiling can be combined to yield effective virtual chemical sensors. They indicate that chemical signatures and electronic odor profiles permits one to quickly recognize and identify the presence of hazardous materials. They emphasize that cargo and port security are very important with regard to preserving homeland security. According to them approximately 20,000 containers enter the United States daily, and screening methods are urgently required that are rapid, and cost-effective. They describe an electronic nose wherein a single solid-state sensor is able to create an unlimited number of chemical sensors. These authors indicate that their method permits them to speciate chemical vapors in less than 10 s with picogram sensitivity using a solid-state surface acoustic wave (SAW) sensor with electronic variable sensitivity. They provided examples to detect chemical and biological compounds from the odors released. These included explosives, contraband drugs, hazardous chemicals, and biologicals.

(g) *Water Safety*: Acha *et al.* (2004) recently indicate that environmental water pollutants such as atrazine (a pesticide) are persistent and can remain in the aquatic environment for years. They indicate that atrazine levels as low as 0.1 ppb (μg/l) are known to cause hermaphroditism in frogs, affect the health of humans, and are responsible for ecological damage. These authors developed a sensitive fiber optic biosensor that