OPTICAL SENSORS for BIOMEDICAL DIAGNOSTICS and ENVIRONMENTAL MONITORING

Banshi Dhar Gupta
Anand Mohan Shrivastav
Sruthi Prasood Usha

CRC Press
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Boca Raton London New York

CRC Press is an imprint of the
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CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Printed on acid-free paper

International Standard Book Number-13: 978-1-4987-8906-6 (Hardback)

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Library of Congress Cataloging-in-Publication Data

Names: Gupta, Banshi Dhar, author. | Shrivastav, Anand Mohan, author. | Usha, Sruthi Prasood, author.
Title: Optical sensors for biomedical diagnostics and environmental monitoring / Banshi Dhar Gupta, Anand Mohan Shrivastav, Sruthi Prasood Usha.
Description: Boca Raton, FL : CRC Press, Taylor & Francis Group, [2017] | Includes bibliographical references and index.
Identifiers: LCCN 2017023196 | ISBN 9781498789066 (hardcover ; alk. paper) | ISBN 1498789064 (hardcover ; alk. paper) | ISBN 9781315156033 (eBook) | ISBN 1315156032 (eBook) | ISBN 9781498789073 (eBook) | ISBN 1498789072 (eBook) | ISBN 97813151652100 (eBook) | ISBN 13151652109 (eBook) | ISBN 97813151642569 (eBook) | ISBN 13151642561 (eBook)
Subjects: LCSH: Optical fiber detectors. | Environmental monitoring. | Plasmons (Physics)
Classification: LCC TA1815 .G87 2017 | DDC 681/.25--dc23
LC record available at <https://lcn.loc.gov/2017023196>

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<http://www.crcpress.com>

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Preface

This book is an effort to bring a comprehensive book on optical sensors for biomedical diagnostics and environmental monitoring for the undergraduate and graduate students apart from researchers working in various related fields. The idea of writing this book came during a group discussion where it was felt that a detailed information on various kinds of sensors realized on this topic incorporating the newly implemented and upcoming techniques should be provided to the community because of their direct relation to the health of the living body like human beings, animals, etc. Various kinds of newly developed techniques are being used for the development of sensors for such applications. Surface plasmon resonance (SPR) is one of such techniques which has been widely used and can be easily understood with the elementary knowledge of electromagnetic theory and hence can be recommended to the students with its possible applications. The literature has also shown the upcoming technique of lossy mode resonance (LMR) for sensing applications. LMR exhibited by the semiconductor metal oxide (SMO) in the visible region and possessing applications in sensing of various gases, chemical and biological analytes also need wide attention. The invention of optical fiber technology has made easy implementation of SPR- and LMR-based sensors with the unique advantages of flexible designs with capability of online monitoring and remote sensing using cost-effective substrate. Nanotechnology is an intense branch that has made its role clear in sensing applications by its irreplaceable performances in many aspects. However, the integration of optical fiber and nanotechnology has fascinated the world of the sensors. Therefore, a comprehensive collective knowledge of this integration and its advantages in the development of sensors is required. The very new field of molecular imprinting is gaining its attraction mainly in the field of biomolecular recognition. This book is the first work to explain in detail the usage of this method mainly in fiber-optic sensors and, in particular, in integrating it with the nanoparticles for recognizing biosensors. With this book, we aim to introduce the graduate students and early researchers to an overview of the plasmonic and LMR-based sensors for biomedical diagnostics and environmental monitoring employing the miracles of the fascinating fields, their working principles, and applications.

This book will benefit the researchers as well as the industrial and public society with an overview of recent advances of optical sensors and their applications for human health and safety. This book starts with the introduction of sensors, need of sensors, and distinct types of sensors applicatory for biomedical and environmental monitoring ([Chapter 1](#)). Biomedical applications of sensors are gaining importance intently and hence a deep attention is given to biosensors for the diagnosis of various diseases important for

healthcare in this chapter. [Chapter 2](#) introduces the basic concepts of resonance and various resonance-based sensing techniques that are currently being used in various research and industrial applications. SPR and its collateral techniques are discussed in detail along with the chronological development of the field. In addition, a new technique, LMR, developing its application in the sensing, is also explored. In [Chapter 3](#), a discussion about the fiber-optic sensors, the essential components of fiber-optic sensors, their functions, and the parameters tailoring sensor performance are presented. The multiple design possibilities such as D-shaped, tapered and U-shaped miniaturized probe, in addition to the uniform straight core probe, are included. This is followed by the discussion on nanostructures and their role as a sensing platform in [Chapter 4](#). The chapter includes various metallic nanostructures useful in sensing and their fabrication methods. A number of sensors utilizing nanostructures are presented for environmental and agricultural monitoring, biological applications, biomarkers, surgical, and clinical diagnostics. In [Chapter 5](#), an analysis on the action of SMO for sensing purpose is presented. The section illustrates the SPR and LMR mechanism of sensing, involving metal oxide, for the monitoring of gases, their upcoming applications in biomolecular detection and diagnosis. [Chapter 6](#) introduces the latest technique of molecular imprinting incorporating the qualitative analysis and amelioration done by the technique in the field of sensing applications. The sensors realized using molecular imprinting for food safety, environmental monitoring, and biomedical usage are discussed in the chapter. The last chapter of this book ([Chapter 7](#)) summarizes all the contents with a note on future trends of research in the field of fiber-optic and other technique-based sensors for environmental monitoring and biomolecular recognition. It is hoped that this book will serve the purpose for which it has been written.

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Acknowledgments

I consider myself very fortunate to have worked with Dr. Chandra Deep Singh, Professor Sunil Khijwania, and Dr. Navneet K. Sharma on fiber-optic evanescent field absorption sensors. My continuous interaction with them has made me wiser in the field of fiber-optic sensors. It was this interaction that resulted in the publication of my first book titled *Fiber Optic Sensors: Principles and Applications* (2006). In 2004, I took an initiative to work on fiber-optic sensors utilizing surface plasmon resonance technique. The research carried out on this topic resulted in 10 PhD theses. I thank Drs. Anuj K. Sharma, Rajan Jha, Rajneesh K. Verma, Sachin K. Srivastava, Sarika Singh, Priya Bhatia, Roli Verma, Satyendra K. Mishra, and Rana Tabassum who have worked with me in this exciting field. A part of their work resulted in my second book titled *Fiber Optic Sensors Based on Plasmonics* (2015). For the last 5 years, my focus has been on the research and development of optical sensors for biomedical diagnostics and environmental monitoring. My present graduate students, apart from Anand Mohan Shrivastav and Sruthi Prasood Usha (also the authors of this book), Ravi Kant, Vivek Semwal, Anisha Pathak, and Sonika Sharma are working in this area. I learnt a lot from these colleagues through interaction on this topic. Indeed, a part of the book has emerged from the work carried out with these graduate students and with some of my old students. I thank all of them for their hard work in the laboratory without which publication of this book was not possible. Finally, special thanks to my wife Uma Gupta for her patience and understanding during the writing of this book.

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Banshi Dhar Gupta earned his MSc in physics in 1975 from Aligarh Muslim University, India and a PhD in physics in 1979 from the Indian Institute of Technology, New Delhi. In 1978 he joined the Indian Institute of Technology, New Delhi, where he is currently a professor of physics. In addition, Professor Gupta has worked at the University of Guelph, Canada between 1982 and 1983, the University of Toronto, Canada in 1985, the Florida State University, Florida, USA in 1988, the University of Strathclyde, UK in 1993, and the University of Birmingham, UK in 2010. In 1992, he was awarded the ICTP Associateship by the International Centre for Theoretical Physics, Trieste, Italy, which he held for 8 consecutive years. In this capacity, he visited ICTP, Italy in 1994 and 1996. Professor Gupta is a recipient of the 1991 Gowri Memorial Award of the Institution of Electronics and Telecommunication Engineers, India. He has published more than 170 research papers in international journals and 100 papers in conferences. Professor Gupta has authored books titled *Fiber Optic Sensors: Principles and Applications* (New India Publishing Agency—NIPA, New Delhi, 2006) and *Fiber Optic Sensors based on Plasmonics* (World Scientific, 2015), and is the coeditor of the *Proceedings of SPIE*, USA, Vol. 3666, 1998 and Vol. 8173, 2010. Professor Gupta has delivered invited talks on plasmonics-based sensors in various international conferences held in the United States, China, Australia, Korea, and India. Professor Gupta is a member of the editorial board of the *Journal of Sensors*. His current areas of interest are plasmonic biosensors, fiber optic sensors, and nanotechnology. He is a regular member of the Optical Society of America and life member of the Optical Society of India.

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1

Introduction to Sensors

Measurements of various physical, chemical, and biological parameters/analytes such as pressure, temperature, magnetic field, pH, pesticides, gases, glucose, urea, cancer, etc. are required for various applications such as environment and health monitoring, process control, and electrical utilities/power plants. The devices used to measure these parameters are called sensors. Tremendous amount of work has been carried out on the development of low-cost, compact, sensitive, and analyte-specific sensors. Their progression over the decades has reached well-established pace due to the rapidly developing technology and science. The sensors are named based on the technique used by them for the measurement of abovementioned parameters. The technique can be optical, electrical, mechanical, electrochemical, or any other. The present book deals with the biomedical diagnostics and environmental monitoring sensors utilizing optical techniques. Below, we shall first define a sensor and then discuss the important features of a sensor such as need of a sensor, its components, performance parameters, and classifications.

1.1 What Is a Sensor?

Sensor is a device which detects and analyzes a parameter continuously by providing an output in a way that is readable by humans or human-controllable instruments in a continuous manner with possibilities of reproducibility and recovery. Further, in a sensor, change in one form of input energy to corresponding single-valued change in another or same form of energy takes place. The output of a sensor is usually electrical or optical in nature. The sensors of the current decade can quantify and qualify almost all the physical, mechanical, chemical, and biological entities. There are many things which are expected from a sensor. First, the sensor should be insensitive to the change in external environmental parameters such as temperature and humidity (for nontemperature and humidity sensors) to provide the invariable results. Second, the allocation of a sensor to analyze a particular parameter in a natural environment should be made considering its ability to withstand harsh atmosphere conditions and changes, the domain in which the sensor holds application, its lifetime, remote activities, and online

compatibility with good recovery. Considering all these aspects, the work reported in the literature so far has concluded that the optical way of communicating changes is better than the electrochemical and calorimetric means. In optical sensors, the method of converting one form of energy to another is optical; however, the system may make use of other fields such as electrical, electronic, mechanical, biological, nanostructural, material, biochemical, chemical, and physical engineering along with optics. Thus, the versatile-integrative behavior of optical sensors covers a broad area of requirements.

The optical-sensing system, as in the case of all other methods, has four basic units. The foremost unit is the light source of the sensor that needs to be selected according to the design of the sensor system and the application to which it is oriented. The second unit is the sensor receptor/recognition element which interacts with the analyte (to be sensed) and changes its own optical properties such as refractive index. The change in the properties of recognition unit is determined by the third unit called as transducer. The transducer is the unit which converts the change in the recognition unit properties into some measurable signal by means of an optical transducing phenomenon. The measurable parameter of the signal can be the intensity, phase, or wavelength of the light source which is detected with the help of detector/analyzer which is the fourth and last unit of a sensor. [Figure 1.1](#) shows the schematic of a generic sensor with all the required units. The working of the whole system depends on the mechanism as well as the principle on which the sensor works and also on its utilization. For a sensing system based on optical method, the variation in one of the properties of light such as amplitude/intensity, phase, polarization, frequency, and wavelength due to the change in the sensing medium is analyzed.

Optical-sensing system may use lens, prism, mirror, grating, microscope objective, waveguide, fiber, etc. and optical detection as well as analyzing units. The detection in optical system can be performed by schemes of luminescence, fluorescence, and absorption. The advantages of optical sensing include immunity to electromagnetic interference, possibilities of point and distributed arrangement with multiplexing, compatibility, and

FIGURE 1.1

Schematic of a generic sensor with the essential units.

electrical isolation (Grattan and Sun 2000). The usage of fiber as the optical substrate competes with all other schemes of optical sensing, as well as sensing based on transducer elements other than optical due to its capability of remote sensing and online monitoring. Hence, in this book, we shall impart the significance of optical sensing, mainly using optical fiber, for the physical, chemical, and biological sensing to cover environmental supervision and biomedical diagnostic applications, which cover the relevant areas of sensor operation in present and future. The upcoming sections discuss the relevance of sensors in detecting various biological, gaseous–chemical measurands with the parameters which quantify the sensor performance. Section 1.5 will discuss the types of classifications of sensors followed by sensor regeneration aspects in Section 1.6. Finally, Section 1.7 contests the framework of the book.

1.2 Need of Sensors

The sensors are one of the most important devices that we use in day-to-day life. As we all know, our nervous system consists of five vital sensing organs: nose, skin, eyes, ears, and tongue. These are used for the sensing of olfaction (smell), tactioception (touch), ophthalamoception (sight), audioception (listening), and gustaoception (taste), respectively. Various types of devices and machines are equipped with sensors that help them in working. Consider a mobile phone, it is a device combined with multisensor technology such as accelerometer and gyroscope that are used for the determination of linear and angular rotations. Various types of temperature and humidity sensors are being used daily in several electronic machines such as refrigerators, air conditioner, and air cooler for the automated working. Light-dependent resistors (LDRs) are used as sensors in the automatic doors and streetlights with an ability to switch their operation. Apart from these, sensors are also used in various fields for the human and environmental safety, biomedical applications, military applications, etc. The aim of sensor development for the environmental monitoring is the safeguard of the living organisms from a number of pathogens and toxic contaminants that are continuously released into the environment, resulting in the pollution of air, soil, and water (Rodriguez-Mozaz et al. 2004). Sensors are also required in various biomedical and clinical applications to detect glucose, urea, vitamins, and nutrients concentrations in human body, and also need to be detected for ensuring their level within the normal range in the body. In order to ensure the safety of food and water, several types of sensors for the detection of impurities such as heavy metal ions (to secure for the use of hard drinking water), pesticides, melamine, etc. are used and further advancements in these sensors are required for highly specific and selective detection. In accordance with

the applications of sensors for biomedical, clinical, environment, and food safety, the sensors can be classified in three broad categories as biosensors, gas sensors, and chemical sensors.

1.2.1 Biosensors

Biosensor is a device which is generally designed and fabricated with a biological sensing element incorporating a transducer. It generates a change in the transducer signal or bio-recognition element characteristics on exposure to an analyte for which it has been designed. The change in signal depends upon the concentration of the analyte. A number of definitions have been given to the biosensor by the people working in the field of biosensing. The general definition is: "Biosensor is a device that utilizes biological components such as enzymes, antigens/antibodies for the determination of the concentration of the biomaterials" (Wang and Wolfbeis 2013). The biomaterials to be detected are usually the materials which are toxic in nature and can harm human body as well as the environment. The invention of first biosensor was by Leland Clark and hence he was renowned as the father of the biosensing concept (Clark and Lyons 1962). He showed detection of glucose using oxygen electrode, where glucose oxidase was entrapped over a Clark oxygen electrode using dialysis membrane. The estimation of glucose concentration was performed by measuring the reduction in the dissolved oxygen concentration. The idea proposed was industrialized by Yellow Springs Instrument Company (Ohio, USA) in 1975 and the glucose sensing analyzer, based on the amperometric detection method, was successfully relaunched. Later, the detection of urea concentration using direct potentiometric method was reported (Katz and Rechnitz 1963). Further development in the sensor was made with the introduction of urea electrode (Guilbault and Montalvo Jr 1969). The biosensor based on microbe technology was pioneered in 1976 (Cammann 1977) and the term "biosensor" was introduced. The technology was further developed with the application of artificial redox mediators (Kulys and Svirnickas 1980) and later, the first ferrocene-mediated amperometric glucose biosensor was proposed (Cass et al. 1984), which was industrialized by Medi Sense Inc. in 1987. As a continuation of the work done by various researchers, IUPAC introduced the definition of biosensors analogous to chemosensors as "Biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immune-systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" (Monosik et al. 2012). Followed by this great achievement, a number of research communities from various fields like very-large-scale integration (VLSI), life science, physics, chemistry, and material science initiated their interest for the development of biosensing devices incorporating more sophisticated, reliable, and miniaturized biosensing capabilities. In 2007, an implanted glucose biosensor (freestyle navigator system) was operated for 5 days and the work on the development of biosensors continued its

advancement exploiting the enzymes, antibodies, and microbes in combination with various types of transducers (Weinstein et al. 2007).

The term “biosensor” signifies a device which is fabricated by the combination of two parts: bio-recognition element and the sensor element. In the biosensors, bio-recognition elements like enzyme and antigen recognize the selective analyte to be detected, while the sensor element is used for the transduction of the change in biomolecule concentration into an electrical/optical signal. Hence, the sensor element is also recognized as the transducer. The bio-recognition element should be highly specific to the analyte, to which it is sensitive for the change in the electrical/optical signal. Biosensors are used for industrial applications, biomedical diagnostics, food safety, and military applications. In the beginning, major application of biosensors was the sensing of glucose concentration in blood due to its plentiful application in biomedical industry. However, nowadays, biosensors are used in a number of commercialized applications such as fabrication of devices for the calibration of foods and beverages concerning the human health, development of the devices for environmental sampling, and noninvasive instruments for clinical analysis (Mello and Kubota 2002, Patel 2002). However, the industrialization of biosensor devices is quite slow due to various technological issues and limitations. For example, it is necessary to prevent the biosensor from contamination and is challenging too at the same time, because of the presence of semiconductor materials along with the biomolecules in biosensing devices.

1.2.2 Gas Sensors

The development of the gas sensors became the topic of discussion after the discovery of the effect of harmful gases on human health. During the nineteenth and early twentieth centuries, coal miners used to take canaries to the coal tunnels for the early detection of life-threatening gases such as carbon monoxide, carbon dioxide, and methane. Canary, a very songful bird, stops singing and eventually dies in the continuous presence of gases. This was used as a signal for the detection of gases (Thompson 2004). The first gas detection and monitoring system was a flame safety lamp invented by Darway in 1815. In the lamp, the height of the oil flame was adjusted to a specific position in fresh air which was kept inside the glass sleeve with a flame arrester. High flame was the indicator of the presence of methane gas, while the low flame was for low oxygen. Later, during 1926–1927, Oliver Johnson introduced a gas sensor based on catalytic combustion method. After this, various types of gas sensors based on methods such as electrochemical and catalytic combustion were commercialized. In the 1970s, further establishment of various types of gas sensors was carried out with different techniques including humidity analysis, semiconductor combustibility, and solid electrolyte oxygen. Later, extensive attempts were made not only for the advancement of these sensors but also for the implementation of several

types of new gas sensors, which were in great demand for various applications such as environmental reservation, energy saving, health safety, air pollution monitoring, etc. Gas sensors are the most important part of environmental monitoring system because of the gases produced from various sources such as home appliances (chimneys to air conditioners) and various industries that expel toxic waste products. Hence, continuous monitoring of environment is required for human safety and unpolluted atmosphere. Due to these reasons, the gas-sensors-producing industries have been developed up to a significant level to meet the requirements of providing a standard and clean atmosphere for better living. However, further improvement in the gas-sensing technology is required for the improvement of the quality of life and sustainability of our society.

1.2.3 Chemical Sensors

Chemical sensor is used for the determination of chemical composition of the surrounding medium which can be either in gaseous phase or in liquid phase. If the target to be detected is in the gaseous phase, then the device prepared for the detection of gases is generally termed as the gas sensor. The sensor recognizes the presence of the target species and transforms it into a physical signal. Thus, a variation in the characteristic physical signal is observed due to the variation in the concentration of target species. Being a broad area, the technique of gas sensing is now considered as a separate field. The first significant work for the development of chemical sensor was reported by Arnold Backman in 1932 with the invention of modern glass electrode for sensing application, which was later commercialized. Later, in 1936, he designed and patented a pH meter based on a pair of glass electrodes. In the patented device, the electrode pair was suspended in the test solution and the potential difference between the electrodes was used to measure the pH value of the test solution. In 1937, the application of solid-state electrodes such as silver halide and fluoride-specific electrodes for chemical sensing was introduced (Kolthoff and Sanders 1937). The chemical sensors utilizing antibodies as the recognition system for the detection of biological samples were developed (Moore and Pressman 1964). Almost 2 years later, the selective and reversible properties of alkali metal ions were reported (Stefanac and Simon 1966). This study was further used for the development of K^+ -selective electrochemical sensor (Frant 1994). An electrochemical serum analyzer for NASA's Space Shuttle was proposed and the first industrialized sodium/potassium analyzer in blood sample was reported, which was named as SS-30.

Followed by the development of chemical sensors based on naturally occurring antibodies with highly specific properties, the researchers started focusing on the fabrication of artificial ligands for the detection of various analytes. The studies for the development of artificial receptors were pioneered by Pederson, Lehn, and Cram who were awarded the

Nobel Prize in 1987 (Pedersen 1967, Cram 1988, Lehn 1988). Various synthetic ligands such as synthetic macrocyclic polyethers, crown compounds, cyclophanes, and macrohetero-bicyclic were developed for the chemical sensor applications by the team. In the same period, an optical chemical sensor was introduced by evaluating the first optical potassium test based on dry reagent chemistry (Keller 2008). The method was commercialized by Ames Division. In 1968, the first fluorosensor for oxygen detection was used for the biomedical applications (Lubbers and Optiz 1975). Lubbers suggested the name of optical sensor as, "optrode" analogous to the "electrode," which was further corrected as "optode." A considerable amount of work was carried out by various research groups in 1980s using optical chemical sensors for food safety and environmental monitoring. Later in 1991, after the remarkable growth of the chemical sensor industry, IUPAC gave the definition of the chemical sensor as, "A chemical sensor is a device that transforms chemical information, ranging from concentration of specific sample component to total composition analysis, into an analytically useful signal" (Hulanicki et al. 1991). Few more definitions of the chemical sensors were reported in a more general way by various authors. A well-known one by Wolfbeis is as, "Chemical sensors are small sized devices comprising a recognition element, a transduction element and a signal processor capable of continuously and reversibly reporting a chemical concentration" (Wolfbeis 1991).

1.3 Sensor Components

As mentioned above, the sensor can be divided into four components: analyte, recognition unit, transducer, and analyzer (Diamond 1998). The working of the sensor is based on the interaction of analyte to be sensed with the recognition unit, which makes a significant change in the characteristics of the input signal that is detected with the help of a transducer, by a detection unit or analyzer. The analyte/sample may be biological (such as glucose, cholesterol, proteins, etc.), chemical (such as pH, metal ions, etc.), and environmental (gases like CO_2 , CH_4 , etc.). Recognition unit is the most important component of the sensor. The analyte to be detected reacts with the recognition unit through a predesigned interaction. Numerous types of natural and artificial receptors have been introduced by researchers for developing the sensing devices. These will be discussed in further sections. Transducer is the next salient component of the sensing unit. It is used for the conversion of one type of signal into other form, thus converting the analyte concentration into corresponding signal. Analyzer or detector is the last but essential part of the sensing unit. The analyzer is required for detecting/measuring several types of signals which correspond to recognition of the analyte. A block

FIGURE 1.2

Block diagram of the sensor components, showing the analyte/s that needs to be interacted with recognition element/s interfaced with the transducer and then the results of interaction are transferred to the detection and analysis unit.

diagram showing the sensor components with possibilities in their selection and path in which the working proceeds is shown in [Figure 1.2](#).

1.3.1 Analyte

Analytes (samples) are various types of atmospheric, chemical, biological elements which need to be detected. The analyte may be either in liquid state or in gaseous form. According to the type of analyte, sensors can also be classified as chemical, gas, or biosensors. Various hazardous gases, atmospheric pollutants, chemical wastes through industries, biological elements present in blood/urine, nutrients and vitamins, pesticides, etc. are the few examples of analytes needed to be sensed. Several types of ambient conditions and physical parameters such as pH, temperature, and pressure also need to be sensed as they play important roles in the industries for the fabrication of various medicines, devices and need to be monitored for the human and environmental safety. Thus, these can also be considered as analytes. Most common gases such as hydrogen, hydrogen sulfide, chlorine, methane, carbon monoxide, water vapor (humidity), ethanol vapor, etc. can be treated as analytes in the gas-sensing unit. The concentration of metal ions in water should be optimized because higher concentration of various metals such as lead, calcium, cadmium, iron, arsenic can result in unhealthy conditions. The vitamins A, B, C, D, E, and K are essential for human body which can

be found in various food stuffs such as egg, milk, fish, etc. except vitamin D, which is obtained from direct sunlight. Each vitamin has its own functionality and its deficiency causes many health-related problems in human body. Thus, their concentration/availability should be sensed in various foodstuffs as well as in blood. In blood, the analysis of various biological elements for clinical applications is possible. Other analytes such as pesticides, minerals, antibodies, and antibiotics are required to be detected for a healthy life. The concentration of analyte is recognized by its interaction with the receptor/recognition unit.

1.3.2 Recognition Unit

Recognition unit, generally termed as the receptor, is the most important part of any sensing device since it decides the interaction nature of the sensor with the analyte and hence the result. This is also called as the “heart of a sensor.” The recognition unit or receptor should be selective and specific, while interacting with the target analyte to be sensed. It should interact with the analyte reversibly, and specifically without any interference from other similar chemical analytes except the target analyte. Generally, in sensors, receptors are immobilized over the surface of the transducer, so that transducer can transform the interaction between analyte and recognition unit into a readable output signal which can be analyzed by the analyzer. For example, in the case of enzymatic biosensors, the enzyme which interacts with a specific analyte is the receptor of the sensor. In the case of few physical sensors like temperature and pressure sensors, no receptor is required as the working of the sensor is based on the physical properties of materials used in the fabrication of the fiber/transducer. A detailed discussion on the types of receptors and their compatibility with the transducer is given in the next section.

1.3.3 Transducer

By definition, transducer is a device which converts one kind of signal into another form. In the case of sensors, transducer is the unit which helps to measure the change in the properties of the receptor due to its interaction with the analyte, by converting the changes in to some readable signals. In optical sensors, the readable signal is the change in the parameters of the output light collected by a detector/analyzer. The phenomenon by which this conversion is performed is the transducing phenomenon which can be optical, electrical, etc. Further, the element which realizes the transducing phenomenon is called as the transducer such as metal films, nanoparticles, fluorophores, etc. Generally, the recognition unit is immobilized over the transducer surface which is sensitive toward the changes over its surface properties. When the analyte interacts with the receptor unit, the optical properties of the receptor unit change. Since the receptor is immobilized over

the transducer surface, the properties of transducer surface change and due to the transducing mechanism (such as surface plasmon resonance [SPR]), the change in the specific parameter (such as minimum transmission at a specific wavelength, resonance wavelength) is obtained at the detection unit. Thus, the light from the source interacts with the optical transducer unit and is collected at the output after undergoing changes in its parameters. The change in the parameter value specifies the interaction and sensitiveness of the transducer surface to the analyte.

1.3.4 Detector/Analyzer

After the working of the transducer, the change in the signal is measured by the detector. These are selected according to the transducer used, for example, thermometer for measuring the temperature, ammeter for the measurement of current, voltmeter for the voltage observation, magnetometer for analyzing the magnetic field, optical power meter for measuring the light intensity, and so on (Gupta 2006). There are various types of optical detectors such as spectrometer, optical power meter, photodetector, charge-coupled device (CCD), fluorometer, etc. Since the present book deals with the optical sensors, we shall now provide a brief introduction of some of the optical detectors used in the optical sensors.

1.3.4.1 Spectrometer

Spectrometer is one of the most broadly used detectors in optical sensors. It is used for the measurement of light intensity for a particular wavelength range. The spectrometer consists of an entrance slit, collimator, dispersing element (prism or grating), focusing lens, exit slit, and a detector. The grating-based spectrometer has resolution better than the prism-based spectrometer. The grating with few hundred lines per mm is placed at a specified blaze angle, which decides the operating wavelength range of the spectrometer. For the detector of the spectrometer, usually a CCD linear array with thousands of pixels is used. These are responsible for the fast processing of the data. The selection of all the components of a spectrometer such as entrance slit, diffraction grating, order shorting filter, and the detector coating strongly affects the performance parameters of the spectrometer such as spectral range, resolution, sensitivity, and stray light.

1.3.4.2 Optical Power Meter

Optical power meter is used for the measurement of the power in an optical signal. It is usually used for the determination of the power of the light guided through fiber-optic cable. It is made up of a calibrated sensor interfaced with an amplifier and display. It is also used for the observation of power loss in the optical signal when light is guided through the fiber. In

the case of sensing applications, it is used for analyzing the output power through the fiber-optic sensor after transducer recognizes the interaction of the analyte and the recognition unit (Gupta 2006).

1.3.4.3 Charge-Coupled Device

A CCD is a light-sensitive integrated circuit which is used to store and display the data for an image. The working of CCDs involves the conversion of each pixel (picture element) in the image to an electrical charge, the intensity of which is related to the color in the color spectrum. These are used as sensors in digital cameras and video cameras for the recording and storage of steady and moving images. The CCD captures light and transforms it to digital data, which is stored by the camera. The quality of an image captured by a CCD depends on the resolution of the sensor. In digital cameras, the resolution is measured in megapixels (or thousands of pixels). Therefore, an 8MP digital camera can capture twice as much information as a 4MP camera. The result is a larger photo with more detail. These are used in the biosensors for the human-noninvasive monitoring under clinical situations.

1.4 Sensor Performance Parameters

In this section, we shall discuss various static and dynamic parameters which are used for the characterization of the sensor to evaluate its performance. On the basis of these parameters, the sensor performance can be enhanced which is greatly required for the advancement in the sensor technology. These are the following parameters:

- *Sensitivity*: Sensitivity is one of the most important parameter of the sensor. It can be measured by calculating the change in the sensor output signal with respect to unit change in analyte concentration which is to be detected. It can also be evaluated from the slope of the calibration curve, which is generally the plot between the measured output signal and analyte concentration.
- *Selectivity/specificity*: Selectivity of the sensor corresponds to whether the sensor responds either to a group of analytes or the sensor responds specifically to a single analyte. The sensor is highly selective, if it responds only to the target analyte to be sensed in the presence of interfering analytes.
- *Operating range*: The analyte concentration range to which the sensor operates is the most important parameter and it decides the sensor's applicability in the industry/hospitals, etc. Usually the sensors'

operating ranges are fixed because an increase in the sensor operating range decreases the sensitivity of the sensor.

- *Linear range*: The linearity range of the sensor defines the sensor's operation with fixed sensitivity, which makes the sensor to be operated easily. This can be extracted from the calibration curve of the sensor by fitting an ideal straight line. In other words, this tells us the analyte concentration range up to which the output signal varies linearly.
- *Limit of detection*: The lowest analyte concentration which can be detected by the sensor is defined as the limit of detection (LOD) of the sensor. The evaluation of the value of LOD depends upon the type of sensor fabrication and the detection system.
- *Limit of quantification*: Limit of quantification (LOQ) is also referred to as the lowest analyte concentration which can be detected by the sensor, considering the standard deviation in the output signal.
- *Resolution*: It is the lowest change in analyte concentration which can be recognized by the sensing device. This is an important parameter for the sensors in flowing streams.
- *Response time*: Response time of a sensor denotes the time taken by the sensor to show a saturated output for a given change in the analyte concentration. Generally, it is defined as the time required by the sensor to respond for the analyte detection.
- *Repeatability and stability*: As named, these terms correspond to the sensor's ability for reproducing its output response after characterizing the sensor up to certain cycles and maintaining its performance for a specific time period. These parameters play the most important role for the sensor to be commercialized.
- *Hysteresis*: It is the main characteristic of the sensor which decides the difference in sensor's response for an increasing and decreasing analyte concentration. It is normally given as the percentage change in the output response after the interaction and removal of the analyte from the recognition unit of the sensor. Zero percentage hysteresis refers to the exactly reversible nature of the sample.
- *Shelf life*: This parameter refers to the time period for which the sensor will operate. If the sensor is not usable for a favorable time period, then it affects the sensor's preference.

1.5 Biosensor Classifications

An independent sensor incorporating a biological material in its active form in the system, to interact with the analyte which is chemical/biological, is

generally considered as a biosensor. The design, selection, and application of biosensors are critical since its interaction with the desired analyte to be sensed is always affected by interferands. The application needs to be performed in external environments, harsh atmospheres, laboratories and health clinics, and even for *in vivo* and *in vitro* measurements in human body. The analytes and the active biological elements may also need to be sterilized in some cases, which consume time, high-cost equipments, man power, and control measurements. However, the possibility of purifying these increases the interaction of sensor with the analyte and provides better analysis.

The necessity of sensors in the area of health, environment, pharmaceutical, agricultural, water monitoring and food quality, hygiene, drug and medicine development, and dialectic cases is increasing. The sensor should be easily available, economical, portable, easy to handle, commercialized into a small module so that even laymen can use it for agricultural applications, food safety, and environmental monitoring. Thus, the processing and the analysis should be performed in a smart way. In the case of biosensors, especially, the analysis should be rugged to make the sensor extremely selective, sensitive, reliable, recoverable, and vigorous. This is where we can find the difference between biosensors and bioanalytical schemes, which require multiple processing and are of onetime use. For the use of biosensor as a tool for diagnosis, in medical field such as cancer/tumor cell determination, the sensor needs to be reproducible with capabilities of multianalyte detection (Tothill 2009).

Biosensor works either in direct or indirect monitoring mode, depending on the kind of reaction or analysis the sensor is intended to perform. In direct monitoring mode, the analysis is done by monitoring the direct products and reactions occurred due to the interaction of analyte (Gauglitz 2005), whereas in the case of indirect monitoring mode, the analysis is performed indirectly through the inhibitors or catalysts consumed for the reactions. The central unit of every biosensor is the bio-recognition element synced with the transducer unit. The functioning of the biosensor depends on its recognition element and transducer. Hence, biosensors are mainly classified on the basis of their recognition element and transducer units. In the following sections, we shall discuss the classification of biosensors and their importance for different applications and measurements by correlating the recognition and transducer modes.

1.5.1 Classification Based on Transducer

The transducer part of the biosensor system is the unit, which provides bidirectional signal conduct. The transducer part determines the signal mode available at the output for analysis and readable by humans. There is a predetermined relationship between the output and the input signal in the biosensor system, and by calibrating the results from the analyzer one can evaluate the measurements and changes. The transducer element in a

biosensor can be chosen according to the design and substrate of the sensor probe. Thus, based on the transducer element, biosensors can be broadly classified into five main types as electrochemical, optical, calorimetric, mass-sensitive, and light-addressable potentiometric transducer (Patel 2002, Reyes et al. 2003).

1.5.1.1 Electrochemical Transducer

Electrochemical types of transducers are the commonly used ones because of their fast response, automation possibility, and compatibility with modern technologies of fabrication, portability, and minimal requirement of power (Thevenot et al. 2001). These sensors are usually used in point-of-care applications such as glucose sensor. In the case of electrochemical sensor, the substrate is the electrode, which is chemically modified. This can be done by modifying the electrode with ionic/conducting/semiconducting material along with a chemical/biofilm (Durst et al. 1997). There are two electrodes, one a working electrode which performs sensing and another a reference electrode dipped in the electrolyte. Electrochemical sensors can be potentiometric, amperometric, conductimetric, and impedimetric.

An amperometric electrochemical sensor setup consists of working electrodes (cathode and anode) and a reference electrode dipped in an electrolyte as shown in [Figure 1.3](#). For a particular potential applied between the electrodes, a chemical reaction occurs due to the oxidation or reduction of electroactive species. The process of oxidation or reduction generates current in correlation with the concentration gradient of the electroactive species/analyte which is measured by an ammeter. For the sensing purpose, the receptor is immobilized over one of the electrodes. When analyte is added into electrolyte, the current starts flowing with the electroactive species toward the electrodes. In the presence of receptor, the analyte binds with the receptor and the conductivity of the receptor unit changes. Since the receptor is immobilized over the electrode, the conductive properties of the transducer surface change. This results in the change in current flowing through the electrode setup, which is analyzed by the ammeter. Thus, by observing the change in current, before and after adding the analyte, in ammeter, one can detect the presence of analyte. Electrodes are generally made up of metals/steel/carbon that are conductive in nature and are modified according to the application. The bio-recognition element and the immobilization methods with matrix can be varied accordingly, which may also use an electron mediator or a selective/discriminative membrane. Amperometric sensors are fast, more sensitive, and exact than other types in electrochemical system. The calibration curve of these sensors is, in general, linear. The first biosensor using amperometric transducer was designed for glucose monitoring based on the consumption of oxygen (Updike and Hicks 1967). This kind of amperometric sensor, which performs analysis based on the consumption of indicator or formation of

FIGURE 1.3

Schematic of a general amperometric transducer setup.

product, comes under first-generation sensors. The sensors utilizing electron mediators and performing redox reactions come under second generation, whereas the reagent-less sensors come under third generation. The third generation uses enzyme or antigen–antibody coupling over electrode which transforms the electrode by the catalytic reaction and generates a catalytic current for the analysis. The amperometric-electrochemical sensor holds applications in food industry for the detection of bacteria, food pathogens, and food adulterations.

A potentiometric transducer measures the potential drop between the reference and working electrode as the signal to be analyzed or the potential generated across an ion-selective/semipermeable membrane which separates two solutions, when no current is flowed (Frant 1994). The potentiometric transducer can be based on field-effect transistor (FET), where an ion-selective membrane replaces the gate of FET to give ion-sensitive FET (ISFET) or enzyme immobilized to give enzyme-sensitized FET (ENFET). Thus, the transducer element can be either an electrode with ion-selective membrane (ion-sensitive electrode) or a membrane which works as bio-recognition element. Potentiometric-electrochemical sensors are commonly used as pH sensors or gas sensors and the potential difference is in proportion to

the logarithm of the gradient in analyte concentration. A disadvantage of the potentiometric-transducer-based sensor is the affinity of the system to the buffer competency.

Another kind of transducer under electrochemical type is conductimetric-electrochemical transducer. In this type, the analysis is carried out by measuring the electrical conductivity which changes during biochemical reactions that release electrons. Usage of this kind of electrochemical sensor is slightly restricted in biosensing applications due to its implementation difficulties especially when the bio-recognition element is an enzyme.

The last class of electrochemical transducer, came up in 1992, is impedimetric which measures the impedance or resistivity of the cell. This is also called as conductometric since the resistance and conductivity are inversely proportional. The main drawback of conductometric-electrochemical sensors is the poor sensitivity of the system. Both conductimetric and conductometric sensors are used for food quality monitoring, air and food sanitation microbiology. Portable impedance-electrochemical biosensor for microorganism detection is available in markets, in which the analysis is performed by measuring the impedance/conductance caused by the metabolic reaction that increases the conductance implying a decrease in impedance.

1.5.1.2 Optical Transducer

The advancement in optical techniques and their implementation using substrate from prism to grating, waveguide, and finally the fiber has made the evolution of optical biosensor as a principal real-time analytical tool. The optical biosensors available are based on a large number of techniques such as reflection, refraction, interference, diffraction, polarization, absorption, fluorescence/phosphorescence, chemi/bioluminescence, scattering, spectroscopy, etc. (Santos and Farahi 2014). High sensitivity, small response time, robustness, simplicity, and small size apart from immunity to electromagnetic interference make the optical sensor easy to handle and install at various locations. If the substrate used for optical sensor is an optical fiber, then the possibility of remote sensing and capability of online detection of analyte become the additional advantages.

The emission of energy in the form of radiation when a molecule/electron excited by a photon of high energy moves from a higher energy to lower stable energy level is termed as photoluminescence. When the excitation of molecules occurs by some chemical reaction, then the process is called as chemiluminescence, whereas in bioluminescence, the biological or biochemical reaction/metabolism occurring in organisms results in the excitation of molecules. Thus, in chemiluminescence, light source is not required. The chemi/bioluminescence depends on the type of molecule, concentration, temperature, and environmental conditions. Luminescence-based optical sensors are used for the detection of carbohydrates, alcohol, etc., where the fermentation process needs to be controlled. A considerably selective

and sensitive sensor can be designed using fluorescence technique where the frequency variation in the emitted electromagnetic radiation is analyzed, implying that the analyte absorbs one wavelength/frequency and emits another (Wang et al. 2002). Fluorescence occurs because of the absorption of the input radiation and the generation of radiation of lower frequency after interaction of the input radiation with the fluorophore. The technique of fluorescence emission is used for the analysis of contaminants in food, pharmaceuticals, etc. The concentration of the analyte is determined directly or indirectly from the duration/intensity of the fluorescence emission. The emission and excitation occur at particular levels of energy and hence there is a special spectrum of fluorescence for each analyte and for each molecule of analyte. This makes the technique selective for a particular sensing application. Fluorescence is a kind of photoluminescence, where the transition occurs between the “excited singlet state of lowest energy and the ground singlet state” by the general definition. Phosphorescence is another kind of photoluminescence in which the electron changes its spin during the transition since it moves in the same level. The mostly used optrodes (optical + electrode) for biological application are based on fluorescence phenomenon. Generally, the device consists of light source, detector, and sensing probe made up fiber. The sensing fiber head is coated with the bio-recognition element. While choosing the technique of fluorescence for sensing or analysis of analyte, the parameters that help in diagnosis are the intensity and lifetime of fluorescence emission. The intensity measures the analyte concentration, whereas the lifetime shows the period for which the electron/fluorophore remains in the excited state. The lifetime is measured by calculating the shift in phase between the emitted and the excitation light since lifetime and phase shift are directly proportional. Fluorescence-based optical biosensor is commonly used as immunosensor- or catalyst-mediated sensor such as enzyme-immobilized ones. The emission of fluorescence is proportional to the analyte concentration in the case of immunosensor, while the catalytic reaction decides the emission in the catalyst-mediated sensor.

Scattering-based optical transducer uses either forward scattering or backward scattering mode, especially in the case of fiber-based system. Both ends of the fiber are operated in the case of forward scattering, while only one end of fiber is operated in backward scattering mode. Although the mode of back scattering provides advantages over forward scattering in terms of high sensitivity and easy operation, the method also holds disadvantages such as large response time, low-intensity signal, etc. This difficulty is usually overcome by integrating the signals received over a number of times. Scattering mechanism can be elastic or inelastic depending on the wavelengths or frequencies of the incident and scattered light. If the incident and scattered light frequencies/wavelengths are same, then it is called elastic scattering, for example, Rayleigh; while the frequencies/wavelengths are different, then it is called inelastic scattering, for example, Raman and Brillouin scattering. Generally, the scattering-based techniques are employed using

interferometric methods. Rayleigh scattering occurs due to the change in the dielectric constant, which is caused by the variation in the composition and density as the light traverses through the optical fiber. Rayleigh scattering-based technique is in use for the environmental monitoring such as identification of explosives. The technique can be used in a better way by enhancing the surface using the Raman-active nanostructures or molecules adsorbed on the prepared surface of a metal, to be called as surface-enhanced Raman scattering. The enhancement is carried out either chemically or electromagnetically. The technique has applications in protein detection and biomedical sensing with advantages of limited response time, trace element identification, which make it useful in the fields of pharmaceuticals, food industry, and environmental monitoring (Han et al. 2009). Similarly, diffraction-based optical transducer-employed sensors are in use. Such sensors, in general, make use of grating structures that offer multiplexing potential and compatibility to work with fiber technology which helps in biosensing applications.

Absorption-based optical sensors, in general, use the amount of light absorbed by the analyte to quantify its variation in concentration. The analysis is performed by calibrating the transmitted light, after absorption by the medium, with the incident input light. The transmitted light intensity from an absorbing medium, ideally considering uniform absorption, is, mathematically, related by the Beer–Lambert law as

$$I = I_0 \exp(-\varepsilon C \Delta l),$$

where I_0 and I are the intensities of the incident and transmitted light, ε is the extinction coefficient of the absorbing analyte, C is the concentration of the analyte that absorbs light, and Δl is the thickness of the analyte medium. The constant, εC , is called the linear attenuation coefficient which can be equated to the absorption coefficient if the scattering of light by the medium is zero. The thickness/depth of the medium and the transmitted light intensity are inversely proportional to each other. The absorption of light by the analyte depends on the wavelength of the incident light, which holds a major application in biosensors. Although the method is cost-effective and simple, its application on biological samples is limited because of limitations of samples/biological analytes to absorb light, which causes a large background signal in the spectrum. Another type of optical-based transducer employs evanescent wave absorption and is generally used in environmental monitoring. This kind of sensor is generally realized on optical fiber with the possibilities of modifications in fiber structure, fiber geometry such as U-shaped, and tapered to enhance the sensor's performance. At the interface of core-cladding/medium where the total internal reflection occurs, evanescent wave is generated by the emanation of electromagnetic energy with a decaying field in the medium. The sensor system is operated

in intensity modulation scheme and the change in the imaginary part of the refractive index of the medium around the core causes the change in the intensity of the transmitted light. A simple evanescent wave-based optical transducer system has the physical/chemical/biological measurable on the unclad region of optical fiber. This unclad region of the sensor probe is called the sensing region. Immunosensors are possible to realize with evanescent wave absorption-based optical sensors. The evanescent wave has an average penetration depth of around 200 nm. A modified type of evanescent wave absorption-based optical transducer uses fluorescence along with it, where the antigen–antibody complex on the interface absorbs evanescent wave and emits the fluorescence. The concentration of analyte can be determined from the emission spectra. Evanescent wave absorption-based optical sensor system does not require any coupling components and provides sensors with better sensitivity compared to sensors using bulk optics.

Another category of optical transducer is based on the refractive index. Although grating, interferometric, Raman scattering, and evanescent wave-based transducers can also work with the change in the refractive index, the most importunate techniques that come under this category are the SPR, localized surface plasmon resonance (LSPR), and lossy mode resonance (LMR). The calibration curve of these kinds of refractive-index-based optical sensors shows the change in the sensor calibration parameter with the change in the refractive index, thereby measuring the analyte concentration indirectly. The technique can be realized on prism, optical fiber, or grating. The phenomenon of SPR accomplished on optical fiber has been a great achievement since 1993 when prism was replaced by the core of the fiber for SPR-based sensor (Jorgenson and Yee 1993). The combination of surface plasmon technology and optical fiber has brought advantages such as miniaturization of the probe with real-time implementation, online monitoring and remote sensing facility, simple, easy-to-handle, and cost-effective probe. This combination has overruled all the existing techniques. The usage of metal nanoparticles/nanostructures instead of metal film has introduced LSPR. The combination with optical fiber brings biosensor with high sensitivity and better performance. The basics and developments of SPR and LSPR phenomena will be discussed in detail in [Chapter 2](#).

A latest sensing technique, employing the optical transducer based on refractive index, is LMR where a metal oxide/suitable polymer/ceramic replaces the metal in SPR technique. The method has been reported to have huge sensitivity even for biosensing. The method requires more studies and also developments to implement it for biomedical applications that would be a huge turnover in the medical field. A combination of SPR/LSPR and LMR has also been explored in the literature. The methods as well as their combinations provide high-performance biosensors with good biocompatibility and long life if implemented using optical fiber substrate.

1.5.1.3 Mass-Sensitive Transducer

In a mass-sensitive transducer employed in biosensor, a bio-layer is kept in contact with a piezoelectric element and the change in mass, density, dielectric, electric, or elastic properties of the bio-layer is measured. The mass-sensitive transducer can be divided into acoustic wave- and cantilever-based transducer. In acoustic type, bulk acoustic wave and surface acoustic wave propagation transducers are employed. Further, the operation of sensor is based on the resonance of the oscillating crystal (usually quartz) at its basic frequency. The oscillator part is the one with the quartz crystal connected to an amplifier. The resonance frequency changes according to the properties of the bio-layer connected. In the case of surface acoustic wave propagation transducer, the bio-recognition layer is placed over the substrate with input and output ports. The wave traverses along the surface of the bio-recognition layer and the properties of wave are affected by the properties of the bio-layer. Using an electrical signal of particular frequency, the piezoelectric crystal is made to vibrate. The interaction of the analyte with the bio-recognition layer changes the mass of the crystal which changes the resonance frequency. The materials that exhibit piezoelectric properties are quartz, tantalite, tourmaline, zinc oxide, etc. Molecular sensing is an important application of piezoelectric sensors with label-free detection possibilities.

The second type of mass-sensitive transducer is based on the microcantilever. The cantilever-based-transducer-type sensor generally measures the change in vibration frequency or bending of cantilever. The interaction of the analyte with the microcantilever surface causes a tilt in the cantilever. The method has advantages of real-time detection, usage of small quantities of analytes and bio-layer elements, good LOD, etc. Microcantilever-based sensors are reported for the implementation of cancer biomarkers.

1.5.1.4 Calorimetric Transducer

The sensor monitors the change in energy, especially, in the form of heat. The bio-recognition layer is immobilized on thermistors or temperature sensors (Hoffmann et al. 2007). The interaction of analyte on the bio-recognition layer results in heat generation, which is sensed by the sensor. The heat absorbed or produced depends on whether the reaction is exothermic or endothermic, and is related to the total number of molecules involved and molar enthalpy. The thermistor is independent of the optical or chemical properties of the analyte. Depending upon how heat is transferred, the calorimeters are divided into three main categories: isothermal, heat conducting, and isoperibol. In isothermal, the temperature of the reaction chamber is maintained constant by Joule heating and the quantity of energy that is necessary for that is measured. In the case of heat conduction calorimeter, there is an isothermal heat sink around the reaction chamber and the heat difference between the two is measured (Maskow et al. 2012). In isoperibol,

the system is the same as that of heat conduction one, but the reaction chamber is thermally insulated. Calorimetric biosensors are mainly used in food industry and pharmaceutical investigations.

1.5.1.5 Light-Addressable Potentiometric Transducer

The light-addressable potentiometric transducer is a combination of optical and potentiometric/electrochemical transducer systems. The method was first time introduced in 1988 (Hafeman et al. 1988). It is a semiconductor device having heterostructure and generates photocurrent when excited by light of enough intensity. The amplitude of the photocurrent generated is proportional to the change in surface potential. Thus, a potential variation caused by an electrochemical means is measured in terms of optical parameter. The advantages of this method include easy fabrication and encapsulation. Since it is an integrated sensor system by itself, it has achieved a level of miniaturization with capability of array sensing.

1.5.2 Classification Based on Bio-Receptors

The bio-recognition element in a biosensor interacts with a specific analyte in a sensor. The bio-recognition elements are broadly based on catalytic reaction, affinity, and hybrid-complexing. In catalytic-reaction-based sensors, the catalytic/chemical reaction occurs when analyte comes in the vicinity of the bio-recognition element. In the case of affinity-based sensor, there are specific interactions between the analyte and the receptor/bio-recognition element. The affinity-based sensors are inherently selective in nature. In hybrid-complexing-based sensors, bio-recognition elements undergo complex reactions and hybridizations while sensing. The general bio-recognition elements include enzymes, nucleic acids/aptamers, antibodies, cells, microbes or microorganisms, membranes, supramolecular structures of cell/cell organelles, tissues, organic molecules, and artificial receptors. [Figure 1.4a](#) shows the bio-recognition elements with examples of their applications toward environmental and biological analytes, while [Figure 1.4b](#) shows the possibilities of transducer elements that are commonly chosen for each bio-recognition element.

Enzymes are the most common class of bio-recognition elements used in sensing. The first biosensor reported utilized glucose oxidase that was entrapped at a Clark oxygen electrode (Clark and Lyons 1962). The enzymatic reactions are specific, which produce predetermined products where the enzyme acts as a catalyst by deciding the rate of generation of intermediate and by-products. Although the enzymatic reactions are certain to occur, the reaction and its rate are influenced by various factors such as enzyme mounting, stability, pH, temperature, and the environmental conditions in which the sensor is supposed to work. Sometimes, coenzymes are also

FIGURE 1.4
(a) The groups of bio-recognition elements with their common applications in biomedical applications and environmental monitoring, (b) transducer elements that are interfaced in common for environmental and biomedical applications for the groups of bio-recognition elements.

used to ensure the stability and activity of the enzyme. There are different categories of enzymes according to the reaction catalyzed such as oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase. The oxidoreductase family of enzymes catalyze the oxidation–reduction reaction, while the transferase catalyzes the transfer of one molecular group to another. The group hydrolases perform the breakage of bonds such as C–O and C–C by hydrolysis reactions. The lyase group of enzymes also perform the breakage of bonds in molecules by using catalytic actions other than hydrolysis and oxidation. Isomerases cause structural changes and molecular rearrangements, while ligases join molecules. The enzymes are immobilized by non-covalent and covalent interactions. Electrostatic adsorption of enzyme has also been reported with good stability. Enzymatic bio-recognition element can be used with optical-, electrochemical-, acoustic-, and calorimetric-type transducers.

Antibodies are a class of bio-affinity-type recognition elements, also called as immunoglobins, used to design biosensors. Antibodies are generally made up of amino acids which are arranged in a particular sequence to form a complex structure. Each antibody is developed to detect a particular antigen and therefore antigen–antibody-based biosensors are highly selective. The antibodies can be monoclonal or polyclonal depending on whether they are made from same or different immune cells. Monoclonal antibodies are expensive and difficult to maintain. The antigen and antibody interact by a lock-and-key model and the interaction force is generally weak in nature such as hydrogen bonding, electrostatic interaction, etc. Hence, the possibility of the reversal of the interaction is also high. Thus, reversible/recoverable biosensors can be designed with antigen–antibody bio-recognition element. To fabricate the sensor, antibodies are immobilized over transducer element surface using methods of amino conjugation, aldehyde, or carboxyl groups after functionalizing the surface. Advantages of antibody–antigen-based sensors include fast response and stability and they also hold disadvantages such as dependency on pH, temperature, environment, and have high cost even for single analyte detection with possibilities of nonregeneration in some cases. The common transducer elements used for such sensors are optical, piezoelectric, electrochemical, and light-addressable potentiometric. For labeling purpose, fluorophores and substrates which are chemically active or complexes are usually used in conjugation with antibody–antigen biosensors.

Microbes are microorganisms that can be used as bio-recognition elements in sensors. The microorganisms are able to metabolize a wide range of chemical substances and complexes and the measurement in the sensor is based on their metabolic activities. Microbial sensors are low cost, stable, and able to carry out complex reactions as compared to the enzymatic and antigen–antibody-based sensors (D'Souza 2001). Their disadvantages are large response time, low sensitivity, and large regeneration time (Karube et al. 1987). The immobilizations of microbes are not performed directly on

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