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– 1 –

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

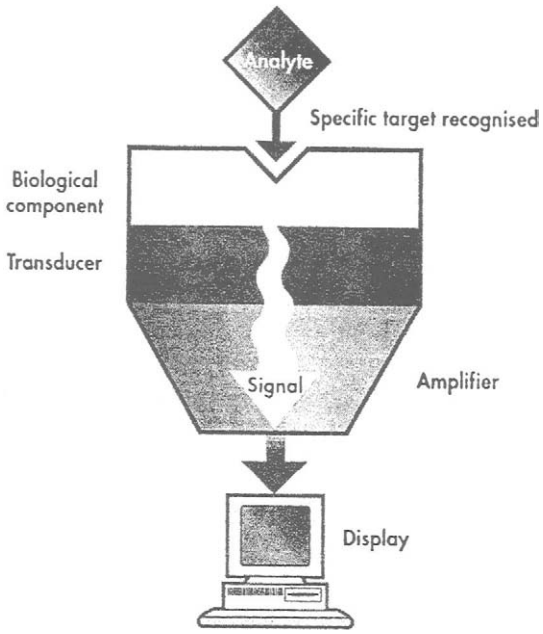

Figure 1.1 Components of a biosensor (Biowise, 2001).

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

contained a two-layer detection element: (a) a cellular layer that contained the detection enzyme, and (b) a pH-sensitive fluorophore. These layers were attached to the distal end of an optical fiber. These authors were able to detect atrazine at sub-ppb concentrations, and their atrazine biosensors had a life time of the order of days.

(h) *Biomimetic Imprinted Polymers*: Bolisay *et al.* (2004) very recently indicate that molecularly imprinting, an emerging technology, has permitted the synthesis of materials with highly specific receptor sites for different analytes (target compounds). These authors have used hydrogels with imprinted cavities to bind to select plant and insect viruses. In spite of the swelling of hydrogels in water these authors indicate that the affinity for the viruses remains high. They indicate that their hydrogel imprinted virus cavities could find application in national security, biologicals production, crop deterioration prevention, and in human and animal health.

Wilson *et al.* (2004) state that molecularly imprinted devices have been used for drug delivery and in chemical detection. They emphasize that molecularly imprinted polymers may be used as a robust substitute for antibodies. For example, they state that conditions in the gastrointestinal (GI) tract would denature antibodies, making them unsuitable as drug delivery devices. They have also formulated lab-on-a-chip microfluidic platforms for the binding and detection of cells.

Lauten and Peppas (2004) very recently indicate that naturally occurring biologicals are not only expensive but are also unstable. Thus, the need to generate synthetic biomaterials that mimic natural recognition properties. These authors have developed the configurational biomimesis process whereby they are able to generate surfaces and polymeric recognition networks that have stereo-specific three-dimensional binding cavities based on a given molecule. They indicate that their technique has the potential to generate synthetic biomaterials with molecular recognition properties that may be applied in the therapeutic and diagnostic areas.

Hilt *et al.* (2004) very recently indicate that biomimetic networks are more robust and cost effective than biological compounds for use as recognition elements as biosensors. These authors have developed methods to integrate biomimetic networks onto silicon substrates. For example, they have micropatterned polymer networks onto silica substrates to recognize D-glucose amongst similar molecules. They have also analyzed the binding and dissociation kinetics, as well as affinities.

(i) *Sol–Gels*: Rayss and Sudolski (2002) indicate that the sol–gel method may be readily employed for transducer immobilization. A glass-like porous structure is created at room temperature, and unstable transducers (typically organic compounds) may be entrapped in a rigid network of silica. Changing the sol–gel composition, gelation conditions, as well as the gel treatment process permits one to tailor-make the properties of the sol–gel matrix (Klein, 1988). Rayss and Sudolski (2002) showed that due to the relationship between the refractive index of a silica film and pH, a sol–gel film deposited on an optical fiber core could be used as a pH-transducing element in a pH biosensing system.

(j) *Food Pathogens*: McLeish (2000) indicates that more than 75 million people become ill every year in the United States due to food poisoning. This is 5-year old data. The food poisoning is due to pathogens such as Salmonella and *E. coli*. Out of these 75 million people about 1–1.5% are hospitalized (~325,000), and about 0.05–0.1% (~5000) of these cases are fatal. This author indicates that Rand, Letcher, and Brown at the University of Rhode Island have developed a fiber optic probe along with immobilized Salmonella

antibodies that bind to the pathogen (Salmonella) cell. The Salmonella antibodies are labeled with a fluorescent dye. Rand indicates that the binding of the pathogen cells to the antibodies takes about an hour (~ 60 min), and the processing of the concentration signal occurs in about $1\frac{1}{2}$ min. The aim was to extend the application of their biosensor to the detection of pathogens in seafood and a (hand-held) scanning system for supermarket checkout.

Taylor *et al.* (2004) have recently used the SPR biosensor to detect food pathogens and toxins in complex media. They used a multichannel SPR biosensor for the quantitative and simultaneous detection of food pathogens and toxins. For example, using their biosensor these authors were able to detect *E. coli* at levels as low as 104 cfu/ml. They emphasize that this level is two orders of magnitude lower than that obtained and reported by standard SPR or ELISA methods.

Pal *et al.* (2004) have very recently developed a membrane-based immunofiltration assay that is able to detect T2 toxin in wheat and poultry feed. The limits of detection are 12.5 and 25 μg/kg, respectively. This is a competitive analysis method wherein the labeled analyte is T-2 toxin-horseradish peroxidase (T-2 toxin-HRP) and the substrate is 4-chloro-1-naphthol (4CN). These authors indicate that the ELISA method is an order of magnitude more sensitive than their membrane-based methods. They state that soil-fungi contaminate food grains in temperate climates. These fungi produce mycotoxins, such as the T2 toxin which causes alimentary toxicity. ELISA is an appropriate way to do this, but it is time consuming and sophisticated equipment is required. Furthermore, laborious and elaborate procedures are required to remove interfering substances from the matrix of the sample prior to detection of the T2 toxin (Sukhadin, 2003; Langseth and Rundberget, 1998; Pascale *et al.*, 2003).

(k) *Microcantilevers*: Bottomley *et al.* (2004) indicate that an emerging class of chemo-mechanical sensors are microcantilevers (Barnes *et al.*, 1994; Thundat *et al.*, 1995; Chen *et al.*, 1995). A differential stress results when there is adsorption of molecules on one side of the microcantilever. This leads to bending of the microcantilever. Bottomley *et al.* (2004) indicate that this cantilever bending can be measured with angstrom resolution using optical resolution, capacitance and piezoelectric measurements. These authors analyzed the influence of nano- and mesoscale particles on the performance of microcantilever sensors. They noted that the direct injection of, for example, biological fluids without the removal of particles (>0.7 μm) may cause problems when cantilevers are used. These authors suggest that the presence of particles in the fluid produces scattering of the laser beam used to measure the cantilever deflection. This may significantly influence the results, thus particle sizes greater than 0.7 μm should be removed from fluids prior to injection to a cantilever.

(l) *Self-assembling arrays*: LaBauer *et al.* (2004) indicate that although protein micro-arrays may be used for high throughput interactions, they are still not widely used. These authors suggest a method, which overcomes the limitations of the currently used methods by making proteins directly on the microarray slide. Their method is the nucleic acid programmable protein array (NAPPA) method. They do indicate that their method, however, still needs refining; for example, a third protein may be necessary to bridge the interaction.

(m) *Nitrogen Monoxide Sensors*: Nitrogen monoxide (NO) is a pollutant that needs to be monitored. Liu *et al.* (2004) indicate that NO also plays a critical role in biochemical

processes (Lewis *et al.*, 1995; Palmer *et al.*, 1987). Liu *et al.* (2004) have very recently designed an electrochemical sensor for the selective detection of NO. These authors immobilized a polyoxometalate (POM) cluster on an electrode through a polyelectrolyte matrix. They suggest that apparently the POM electrocatalyzes the reduction of NO. Liu *et al.* (2004) further suggest that the reduction current is proportional to the NO concentration in the range analyzed from 1 nM to 10 μM.

(n) *Catecholamines*: Stoica *et al.* (2004) have very recently developed a biosensor to detect catecholamines using cellobiose dehydrogenase. Henriksson *et al.* (2000) and Cameron and Aust (2004) indicate that cellobiose dehydrogenase is an extracellular hemoflavooxidoreductase that catalyzes the oxidation of cellobiose, cellodextrins, and a few low molecular saccharides. A cellobiose dehydrogenase-modified electrode was used for the amperometric detection of catecholamines in the flow-injection mode. Stoica *et al.* (2004) indicate the need to detect catecholamines (a biogenic amine) as they are involved in a wide range of neural pathways. These biogenic amines may act as neurotransmitters and as hormones. These authors further indicate that the concentration of these biogenic amines is in the sub-nanomolar range, and very sensitive methods with low detection levels are required. Their sensor was able to detect catecholamines at levels lower than 1 nM.

(o) *Antibody Nanoarrays*: Klenerman *et al.* (2004) have developed a technique that attaches antibodies to a nanoscale surface. This permits the authors to create antibody nanoarrays. A nanosurface was created by using a gallium focus ion beam microscope. Regularly spaced holes were on a thin gold film of thickness 50 nm. A self-assembled monolayer of 3-mercaptopropionic acid permitted the immobilization of IgG antibodies on the array surface via electrostatic interactions. The authors were also able to minimize nonspecific adsorption.

(p) *SPR and Improved SPR*: Inherent diffusional limitations are present in the SPR biosensor. Furthermore, Knoll *et al.* (2004) indicate that an additional limitation is the thin metal layer on the SPR chip surface may quench the fluorescence signal. This is especially true if the fluorophore is near the surface. One way of overcoming this fluorescence signal limitation, these authors suggest, is to keep the protein interaction 'far away' from the surface. They used long molecules as a scaffold on which the target proteins reside. This permitted these authors to obtain complete fluorescence detection.

(q) *Sol–gel Particle Polyurethane Glucose Biosensors*: Shin *et al.* (2004) recently indicate that mild synthesis conditions involved in sol–gel synthesis and the chemical flexibility involved has stimulated research on sol–gels for biosensor applications. They emphasize that sol–gels are porous in nature, and diffusional limitations may be minimized if these sol–gels are used as thin coatings. Sol–gel biosensors have been developed (Chen *et al.*, 2002; Pandey *et al.*, 1999) due to the ambient and aqueous processing conditions required that are favorable for enzymes (which may be used as receptors or as biocatalysts, as required, for example, in glucose biosensors). Shin *et al.* (2004) have developed a nitric-oxide releasing sol–gel particle/polyurethane glucose biosensor. These authors indicate that *in vivo* glucose biosensors still remain a challenge due to the poor incompatibility which leads to scar formation and infection. These authors indicate that nitric oxide is a potent inhibitor of platelet adhesion (Radomski *et al.*, 1987), and an antibacterial agent (Nablo *et al.*, 2001). Nitric oxide has also been identified as an

angiogenic factor (Ziche *et al.*, 1994). Thus, the hybrid sol–gel/polyurethane glucose biosensor that releases nitric oxide is perhaps a step in the right direction.

(r) *Wireless Glucose Biosensor*: Gai *et al.* (2004) have recently developed a wireless and remote query biosensor. They used a pH-sensitive polymer. A ribbon-like mass-sensitive magnetoelastic sensor is used as a transducer. The magnetoelastic ribbon was coated with a pH-sensitive polymer followed by a layer containing glucose oxidase. These authors indicate that the enzymatic oxidation of glucose decreases the pH. The decrease in pH is detected by the pH-sensitive polymer, which shrinks. This results in a reduction in the mass load on the magnetoelastic transducer. This decrease in the mass load leads to an increase in the resonance frequency of the magnetoelastic sensor.

The oxidation of β-D-glucose results in the production of β-D-gluconic acid. The dissociation of gluconic acid produces H^+. This leads to the shrinking of the polymer after the H^+ has diffused to the bulk solution.

(s) *Multianalyte Sensors*: Misiakos *et al.* (2004) have recently developed an optical real-time affinity sensor. This sensor uses a monolithic silicon optoelectronic transducer and a microfluidic module. These authors indicate that some of the features that permit the application of biosensors to a variety of fields include miniaturization, portability, multianalyte potential, and interfacing with electronic functions. Turner (2000) has emphasized that optical detection in biosensors is superior to other sensing approaches since optical transducers are versatile, and a large variety of labels (such as fluorescent tags) could be used. This real-time affinity biosensor developed by Misiakos *et al.* (2004) was able to detect, for example, gold nanoparticle labeled streptavidin at 3.8 pM. Furthermore, these authors demonstrated the multianalyte capabilities of their biosensor by simultaneously monitoring in real time the binding of (a) streptavidin to biotinylated bovine serum albumin, and (b) antimouse IgG to mouse IgG. Streptavidin and antimouse IgG were in solution, whereas the biotinylated bovine serum albumin and antimouse IgG were immobilized on adjacent fibers of the same chip.

1.3 BIOSENSOR ECONOMICS

In this section we briefly provide some economic numbers on the biosensor market, and the estimated growth worldwide. These are estimates, and should be treated as such. More details about the biosensor market, and other factors involved therein are provided in the last chapter of the book.

Biosensor and bioelectronic devices may be characterized into the following areas: agriculture, food analysis, medical analysis, high throughput screening, and nano-biotechnology (Talukder, 2002). According to this author these categories may be sub-divided further into specific applications. Kelzai (2004) emphasizes the four major driving forces for the development of biosensors:

(a) Increasing rate of obesity and the rising rate of diabetes. This necessitates the monitoring of diabetic patients' glucose levels.
(b) The pharmaceutical industry is continuously looking for methods to be able to screen for new drugs. Biosensors are a method to provide these rapid assays required.

(c) The newly emerging war on terror and biowarfare is bound to gain increasing importance worldwide with an increasing investment in standard, unconventional, and innovative biosensors. The need for efficient and accurate handheld devices for the field diagnoses of harmful biologicals is now more and more evident.
(d) Other—environmental, food safety, and in general, improving the quality of life.

Kelzai (2004) indicated that the worldwide market for biosensors in the year 2003 was projected to be $7.3 billion. At a conservative projected growth rate of 10.4% for biosensors, the estimated market is provided in Table 1.1a.

Projections have been made using the 10.4% growth factor for 5 years hence presuming the present day geopolitical worldwide conditions. The growth rate may become more significant (increase more than the assumed 10.4% if the geopolitical conditions demand it), if, presumably, there is another incident of the magnitude of September 11, 2002 that occurred in New York, NY.

Technical advances would also very significantly impact the market size for biosensors. The estimated cost of developing a biosensor is around $20 million (Walsh, 2003). This is an older report, but a 10% increase in cost per year puts the present estimated cost of development around $40 million (once again, using the factor of 72). Needless to say, this type of investment requires important marketing choices (Walsh, 1998), since one really has to look for a 'niche'. It is estimated that around 90% of the market is in the medical area, and testing for diabetes is the major market there. However, and as expected, the more established companies already have diagnostic tests for diabetes (glucose testing), and one might expect fierce competition for market share.

Perhaps, one needs to pick up on another ailment, and develop a biosensor to detect it before it gets to be a full-blown disease. For example, cancer markers, or markers for the onset of ischemic heart disease. As expected, the earlier one is able to detect these types of diseases, the better is the quality of life once treatment starts, and perhaps, there is an improvement in the prognosis of the disease. Testing for the onset of autoimmune diseases, such as arthritis, systemic lupus erythomaosus (SLE), etc. is bound to gain

Table 1.1a

Estimated worldwide market in $ billions for biosensors. Base year 2003. Estimated growth rate per year 10.4% (adapted from Kelzai, 2004)

Year	Estimated market $, billions
2003	7.3
2004	8.06
2005	8.90
2006	9.83
2007	10.85
2008	11.98
2009	13.23
2010	14.60

importance in the future. Incidentally, diabetes is also an autoimmune disease. For SLE, one needs to estimate levels of different analytes (such as creatinine, autoantibodies, etc.) to be able to predict the onset of a 'flare', and thus help better control the disease. This was quite evident at the 11th International Congress of Immunology held in July/August 2001 in Stockholm, Sweden, where quite a few companies displayed the state-of-the-art biosensors for the early detection of quite a few autoimmune diseases. However, this type of a market is speciality type of a market, and the demand for these types of biosensors will be nowhere near that of diabetes testing. Finally, hopefully, as technologies develop the cost of manufacturing these integrated devices will decrease, and their reliability will improve to the extent to make biosensors a competitive product with regard to other existing methods of detection.

As expected, the worldwide estimates for the biosensor market differs when projected by different authors and sources. It is perhaps useful to provide another estimate, from a very reliable source (*www.cranfield.ac.uk/biomark.htm*) even though the estimate may be rather dated (for the year 1996). The estimate was for one billion British pounds. Using an exchange rate of one British pound equal to 1.8269 US $ (Exchange checked on October 21, 2004). This works out to $1.82 billion. Using a 10.4% growth rate in the worldwide biosensor market, Table 1.1b shows the estimated worldwide market going back a few years (this is unusual), and then projecting ahead till the year 2010.

It is of interest to compare the estimated worldwide markets for biosensors from the two different sources (Kelzai, 2004) and *www.cranfield.ac.uk/biomark.* The initial estimate from Cranfield University is 1.0 billion British pounds for the year 1996, and this translates to 1.827 billion $. This translates to 3.656 billion $ for the year 2003, which may be compared to the estimate for 7.3 billion $ for the year 2003 by Kelzai (2004). The two

Table 1.1b

Estimated worldwide market in $ billions for biosensors (Base year 1996) (www.cranfield.ac.uk/biomark.htm). Estimated growth rate per year 10.4% (adapted from Kelzai, 2004)

Year	Estimated market, $ billions
1996	1.827
1997	2.017
1998	2.227
1999	2.459
2000	2.715
2001	3.00
2002	3.312
2003	3.656
2004	4.037
2005	4.446
2006	4.91
2007	5.42
2008	5.985
2009	6.61
2010	7.30

Table 1.2

Estimated worldwide market in $ billions for patient monitoring devices. Base year 2002. Estimated growth rate 10.0% (adapted from McWilliams, 2003)

Year	Estimated market $ billions
2002	6.25
2003	6.88
2004	7.57
2005	8.33
2006	9.16
2007	10.1
2008	11.1
2009	12.2
2010	13.4

estimates differ by only a factor of two, which is remarkable considering the way estimates are made, and these are from two different sources.

McWilliams (2003) has recently analyzed the market for patient monitoring devices. This author defines these as 'products that measure, display, and document physiological information obtained at regular intervals of time from sensors or other devices attached to a patient.' The regular intervals phrase distinguishes patient monitoring devices from diagnostic kits and devices. Some common measurements made by these devices include electrocardiogram (ECG), noninvasive blood pressure, body temperature, and respiration rate. This author mentions that estimates for the monitoring market though expanding do differ widely from different sources. In the year 2002, the estimated worldwide market according to McWilliams (2003) was estimated to be $2.5–10.0 billion. Assuming a 10% increase in the worldwide market for monitoring devices Table 1.2 indicates the estimated worldwide market till the year 2010 for patient monitoring devices. As expected, factors such as technological breakthroughs, economic and regulatory considerations would presumably greatly impact the size and structure of the market. For the base year (2002) we start with the average of $2.5–10.0 billion = $6.25 billion.

If one were to look at the numbers presented in Table 1.1a,b and Table 1.2, one would perhaps come to the conclusion that the estimated worldwide market for the year 2010 is roughly the same order of magnitude for the biosensor market and for the patient monitoring devices. This is of course considering the nature of the estimates made, the assumptions used, and the inherent variability of economic estimates.

1.4 OVERVIEW

The material to be presented in the chapters to follow is now briefly presented. In Chapter 2 we present modeling and theory involved in the binding and in the dissociation phases. The theory behind single-, double-, and triple-fractal analysis is presented. In Chapter 3 we present examples of the binding of different types of pathogens (food, involved in

biological warfare, etc.) to biosensor surfaces, and how their binding and dissociation may be modeled using fractal analysis. The detection of pathogens (especially those that have a 'terrorist' potential) is becoming more and more critical.

In Chapter 4 we analyze the binding and dissociation of heat shock proteins. These become important in the human body, when the body is 'stressed'. They are also important with regard to the proper protein folding process. Continuing along the line of protein folding, Chapter 5 analyzes the binding and dissociation of prions on biosensor surfaces. Prions are 'misfolded' proteins, that lead to intractable diseases, such as Alzheimers, etc. As expected, these prions have generated, and will presumably continue to generate a considerable amount of interest in the research community. In Chapter 6 we analyze the binding and dissociation kinetics of analytes related to human health on biosensor surfaces. Initially, the medical area has been the major driving force in the development of biosensors. Testing for sugar levels (for the onset or presence of diabetes, and in the control of this aliment) has been a predominant market for biosensors. However, presently, more and more sensors are becoming available in the market for determining the levels of analytes, other than sugar levels. Also, in some intractable, persistent, and perhaps difficult to diagnose diseases, one needs to analyze for more than one particular analyte, and quite frequently. For example, systemic lupus erythomatosus (SLE), where one needs to determine levels of creatinine, autoantibodies, etc. Once these analyte levels are out of their 'normal range', then one can *quickly* take corrective action, for example, the 'flare' in SLE diagnosed individuals.

Continuing along in the medical vein, in Chapter 7 we analyze for and present the binding and dissociation kinetics for human heart fatty acid binding protein. This is an early marker for ischemic heart disease. One of the major themes of this book is the medial slant, and the preponderance of examples of fractal analysis of binding and dissociation kinetics of analytes that have medical implications. The basic idea is to help provide fresh physical insights into these analytes of medical relevance. In Chapter 8 we analyze the binding kinetics of p38α mitogen-activated protein kinase occurring on biosensor surfaces.

In Chapter 9 we present a fractal analysis for heparin–protein interactions on biosensor surfaces. Heparin is another compound that has medical significance. Heparin is an anticoagulant, and is used to decrease the clotting ability of blood. It helps prevent the formation of harmful clots in blood vessels. In Chapter 10 we present an analysis of the binding and dissociation of thrombin on biosensor surfaces. Thrombin is not a normal constituent of circulating blood. It is generated by the cleavage of its precursor in plasma, pro-thrombin. After injury to a particular area, blood flow is restricted to that area (vascular constriction). Then, platelets become activated by thrombin, and aggregate at the site of the injury. In Chapter 11 we present the binding and dissociation kinetics of interleukin to biosensor surfaces. Interleukin-2 (IL-2) is a protein that is manufactured in the body. It stimulates the immune system, and has been approved by the Federal Drug Administration (FDA, USA) for the treatment of certain types of cancer.

The analysis of environmental pollution is an important area of ongoing investigation. Chapter 12 presents the fractal analysis of the binding and dissociation kinetics of different environmental contaminants on biosensor surfaces.

Biosensors are gradually becoming more and more important in our daily applications. On the practical side it would be useful to know what is the present market size for

biosensors worldwide, and perhaps also in different geographical locations, for example in different continents, or in sections of the world, such as Europe, the Americas, Far East including Australia, etc. Also, what is the investment required to possibly set-up a biosensor industry, etc. This sort of economic information is difficult to obtain in the open literature, and the industrial sources who presumably have access to this type of data, will needless to say guard it very carefully. Nevertheless, this type of information is very valuable to possess, and the last chapter is an attempt to address this critical need. Besides, the last chapter, provides, and if I may quote a reviewer of this book's proposal, 'a balance to the book'. In Chapter 13 we try to address the market size and economics for biosensors. Information is gleaned from different sources, and placed together (pieced together if I may so) in one chapter. Hopefully, the information is accurate. Predictions of market size, etc. should, needless to say, be viewed with caution. An appropriate caveat, and this is fair to indicate up-front, that besides some consultancy experience, the author has not been involved in a biosensor start-up company. On the other hand, tongue and cheek one might add, that those who have been involved in biosensor start-up companies may not want to reveal their methods or 'secrets'.

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