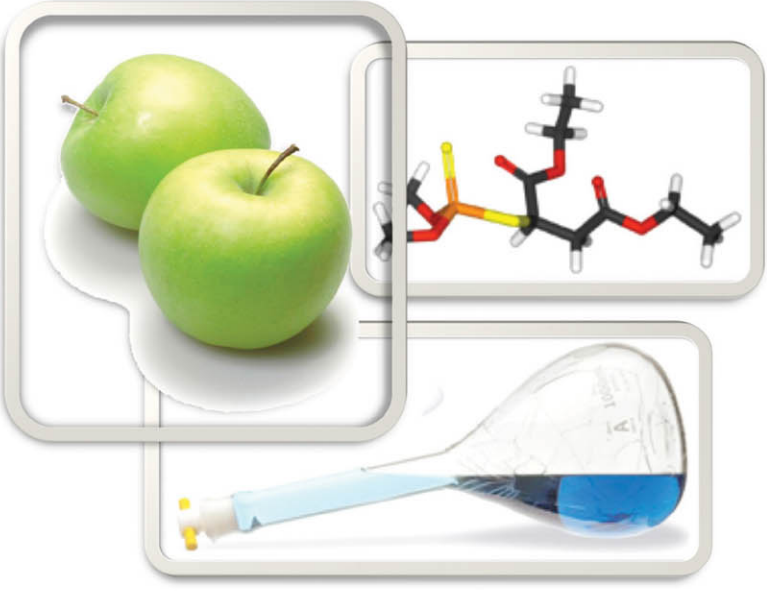
Chemical Analysis of Food
Techniques and Application



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CHAPTER

7

Microfluidic Devices: Biosensors

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OUTLINE

7.1. INTRODUCTION

The detection and monitoring of contaminants, such as chemical compounds, toxins, and pathogens, in food is crucial to assess and avoid risks for human health. Stricter regulations and a greater public awareness of food quality bring requirements to monitor an ever-wider range of analytes, and to do so with greater frequency and accuracy. Despite substantial progress made in the diagnostic field, there is still need for faster, portable, and more accurate diagnostic methods. For the detection of chemical contaminants and toxins, highly sensitive and selective analytical techniques exist, like liquid and gas chromatography combined with mass spectrometry, but they are time consuming and expensive, require specialized equipment and highly trained

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personnel, and therefore do not allow frequent monitoring during many food processes. On the other hand, the detection of pathogenic microorganisms usually requires large cell numbers of a pure cell culture, involving time and labor-consuming enrichment and pre-selection steps. For instance, standard methods for target pathogen diagnosis, including culture, enzyme immunoassay, and polymerase chain reaction (PCR), often take between two and four days. In this sense, biosensors are an alternative over conventional approaches for food analysis offering cost-effective and fast detection, which makes real-time monitoring possible.

Biosensors are defined by IUPAC as integrated receptor–transducer devices, which are able to provide selective quantitative or semi-quantitative analytical information using a biological recognition element (Thèvenot et al., 2001). Biosensors combine a recognition element with a suitable signal transduction method (electrochemical, optical, acoustic, and calorimetric, among others) in such a way that the binding or reaction between the target and the recognition element is translated into a primary signal.

Although a variety of different biosensors have been developed in the past two decades, there is still a need for miniaturized, low-cost, or disposable biosensors capable of rapid detection and accurate identification of a wide range of contaminants, toxins, and pathogens. Recent efforts to minimize the time span between sampling and results include the use of miniaturized devices that do not depend on special infrastructure and sample preparation procedures (Lazcka et al., 2007). The field of miniaturized or microfluidic analysis systems, also called "micro-total analysis systems (μTAS) or lab-on-a-chip (LOC)", has gained increased popularity (Gómez et al., 2001). Initially, the main reason for miniaturization was to enhance analytical performance, but the reduction of size also presented the advantages of reduced consumption of reagents and the ability to integrate separation and monitoring techniques within a single device. The ability of microfluidic systems to conduct measurements from small volumes of complex fluids with efficiency and speed, without the need for a skilled operator, has been regarded as the most powerful application of LOC technologies (Pamme, 2006).

In recent years, there has been great progress in the application of nanomaterials in biosensors. In particular, nanomaterials, such as gold NPs (Au NP), CNT, magnetic NPs (magnetic NP), and quantum dots (QDs), are being actively investigated for their application in biosensors, which have become a new interdisciplinary frontier between biological detection and material science.

During the last few years, several specific reviews on biosensors and biological techniques for food analysis have been published (Cock et al., 2009; Homola, 2008; Vinayaka and Thakur, 2010; Yadav et al., 2002; Yang et al., 2008). The aim of this chapter is to present a general overview of more recent advances in biosensors for food applications with special emphasis in nanosensors, microfluidics, and OC configurations for food-control analysis.

7.2. BIOSENSORS: CLASSES AND FUNDAMENTALS

Biosensors are composed of two main parts: the transduction element and the biological receptor. They can be classified according to the bioreceptor elements involved in the recognition and according to the physicochemical transduction elements. The main biosensor classes are summarized in Fig. 7.1.

7.2.1. Biological Recognition Elements

The main classes of biological elements and interactions that are used for food analysis biosensing are:

- Enzymes
- Antibody/antigens interactions
- Nucleic acids
- Bacteriophages
- Whole cells

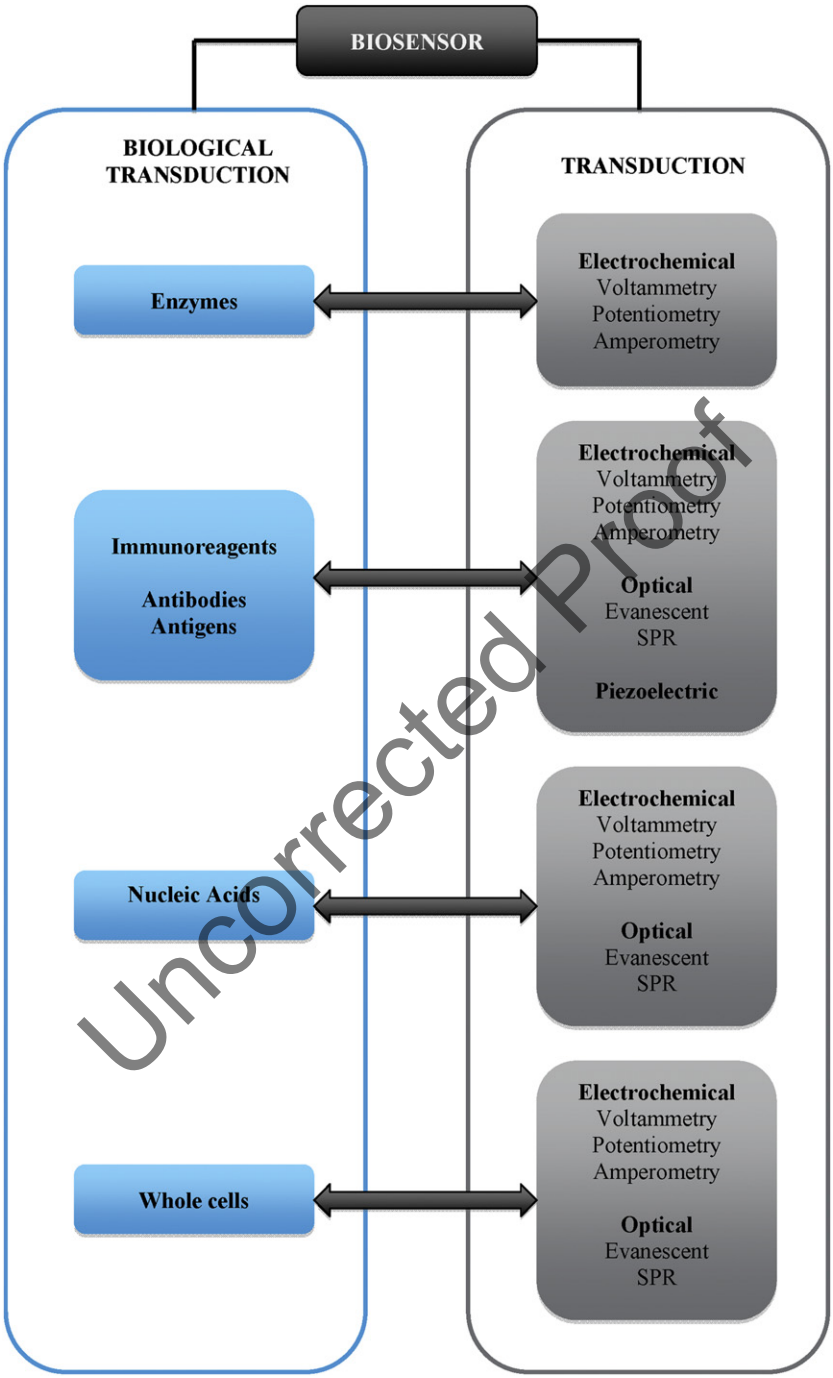

FIGURE 7.1 Summary of the main biosensors applied in food analysis quality control.

Enzymes. The first biosensor described in the literature by Clarck and Lyons (1962) was based on the use of an enzyme, the glucose oxidase, using electrochemical detection. Many examples of applications have been reported over the last 25 years, especially using oxidoreductases (as tyrosinase, peroxidase, and lactase) (Kulys and D'Costa, 1991), and hydrolases (choline estearases) (Andreescu et al., 2005), and in recent years, enzyme electrochemical biosensors appear as suitable alternative analytical tools in food quality and control analysis (Palchetti and Mascini, 2008).

Most of the transduction elements associated with enzyme-based biosensors are electrochemical: amperometric and potentiometric. However, during recent years, several examples of enzyme biosensors have been reported using optical transduction. Optodes, such as fiber optic biosensors, have been demonstrated to be of great interest because they provide some advantages, such as no direct electric connection, easing of miniaturization, possibility of remote sensing, and in situ monitoring. However, the lack of long-term stability of enzyme-based biosensors is one of the main limitations of this type of recognition.

In order to improve the storage stability on enzyme-based biosensors, different immobilizations and electrodes have been assayed, such as carbon paste electrodes (CPEs), solid graphite electrodes, and surface-modified electrodes.

A plethora of applications of enzyme-based electrochemical biosensors have been developed and several reviews have been conducted (Wang, 2008; Pohanka et al., 2009). In addition, as it has been mentioned before, the conjunction of catalytic properties of enzymes has been enhanced using different types of nanomaterials, such as CNT (Pohanka et al., 2009; Serra et al., 2005; Zhao et al., 2008) and metal-NP (Carralero Sanz et al., 2005).

Immunosensors. Antibody–antigen interactions have been exploited in many immunosensors for food analysis, especially in those using electrochemical and optical configurations. If a transduction is achieved using labeled species, the principles are similar to immunoassays. Depending on whether labels are used or not, immunosensors are divided into two categories: labeled type and label-free type.

Labeled formats involve a label to quantify the amount of antibody or analyte bound during an incubation step. Widely used labels involve enzymes (e.g., glucose oxidase, horseradish peroxidase (HRP), β-galactosidase, and alkaline phosphatase), nanoparticles (NPs), and fluorescent or electrochemiluminescent probes (Keay and McNeil, 1998; Seydack, 2005; Wilson, 2005). Commonly, two different formats for labeled immunosensors are available: sandwich assays and competitive assays. A sandwich assay consists of two recognition steps. In the first step, the antibody is immobilized on a transducer surface, allowing it to capture the analyte of interest. In the second step, labeled secondary antibody is added to bind with the previously captured analyte. The immune complexes (immobilized antibody–analyte-labeled antibody) are formed and the signals from labels increase in proportion to the analyte concentration (Sadik and Van Emon, 1996). In competitive assays, the analyte competes with the labeled analyte for a limited number of antibody-binding sites. As the analyte concentration increases, more labeled analyte is displaced, giving a decrease in signal if antibody-bound labeled analyte is detected (Bange et al., 2005). Although the labeled format is usually more sensitive, labeled immunosensors are not capable of real-time monitoring of the antibody–antigen reaction and increase both development and operation costs compared to label-free immunosensors. The amount of target analyte can be inferred from the number of labels that bind to the interface.

Label-free formats detect the binding of target analytes and the antibody on a transducer surface without any labels. There are also two basic types in this format: direct and indirect.

In the first type, the response is directly proportional to the number of analytes present. The vital advantage of these direct immunosensors is the simple, single-stage reagent-free operation. However, such direct immunosensors are often inadequate to generate a highly sensitive signal resulting from antibody–antigen binding interactions and it is still difficult to meet the demand of sensitive detection. The second type, also based on competitive formats, is carried out as a binding inhibition test. The antigen (analyte–protein conjugate) is first immobilized onto the surface of a transducer, and then analyte–antibody mixtures are preincubated in solution. After being injected on the sensor surface, the antibody binding to the immobilized conjugate is inhibited by the presence of target analytes. That is an advanced transducer technology that enables the label-free detection and quantification of the immune complex.

Nucleic acids. Classical nucleic acid biosensors are mainly based on the natural affinity of single-stranded DNA (ssDNA) to its complementary strand. This natural affinity of ssDNA makes the detection of target-specific genes (for example, bacterial specific genes) possible. For this, these classical nucleic acid biosensors measure the hybridization of the ssDNA strand present in the sample to a complementary strand immobilized onto the sensor chip surface.

Nucleic acids also have a natural affinity for intercalating agents, which get inserted into the helical structure of a double-stranded oligonucleotide (dsDNA). These intercalating agents can be measured with a nucleic acid biosensor, by measuring the intercalation of the target in the dsDNA immobilized onto the sensor chip surface.

Recently, aptamer technology enabled the extension of nucleic acid biosensors to virtually any type of analyte, thanks to the unique three-dimensional shape of single-stranded nucleic acid molecules. As aptamer technology is still in its infancy, numerous aptamers have been selected for targets including low-molecular-weight molecules, supramolecular structures, and entire organisms but not all have been developed into biosensors yet. Aptamers are nucleic acid ligands (single-stranded DNA or RNA) that are isolated from oligonucleotide libraries by an in vitro selection process called SELEX (systematic evolution of ligands by exponential enrichment). A library of oligonucleotides containing a portion of randomized sequence is synthesized and incubated with the target. The nonspecific or low-affinity-binding nucleic acid molecules are removed by washing steps and the captured nucleic acid molecules are eluted, precipitated, and amplified by PCR. The double-stranded PCR products are then made single stranded, which is then the input for the next cycle. The whole cycle is repeated until a specific population of high-affinity-binding nucleic acids is obtained. Since they are short and single-stranded oligonucleotides, they are capable of folding into three-dimensional structures due to their self-annealing properties. These DNA/RNA ligands are thought to recognize their target primarily by their structure and not by their sequence. Due to their high-binding affinity, simple synthesis, easy storage, and wide applicability, nucleic acid sensor recognition elements have gained popularity and can substitute the commonly used antibody biosensor recognition elements.

The introduction of nucleic acids into biosensors increases their stability and offers new possibilities. In addition, nucleic acids can be chemically synthesized with high purity and low batch-to-batch variation. Aptamers have emerged as a class of nucleic acid recognition elements, thanks to their high selectivity and affinity toward their targets. Compared to that of antibodies, aptamers too have such high specificity that they can distinguish between chiral molecules and can recognize distinct epitopes of their target molecules, enabling them to differentiate between closely related targets

(Ellington, 1994). They have shown affinities with remarkable dissociation constants ranging from picomolar to nanomolar (Collett et al., 2005). Aptamers can be selected in vitro for target analytes ranging from small molecules to cells. In addition, the use of aptamers avoids ethical problems; the animal-free production of aptamers allows generation of aptamers against toxic or poorly immunogenic molecules as the process does not rely on the induction of an animal immune system, as is the case with antibody generation (Luzi et al., 2003). Contrary to their protein counterparts, aptamers can be selected under nonphysiological conditions or real matrix conditions, which is particularly useful for biosensing environmental and food samples (Torres-Chavolla and Alocilja, 2009). Moreover, the selection process can be directed to recognize specific structural or chemical motifs of the target, an aspect not always possible with antibodies (Tombelli et al., 2007).

Bacteriophages. Bacteriophages are viruses that infect bacteria and use the host bacterial cell as a factory for their own replication. Bacteriophages have the ability to display peptides or proteins on their surface; this technology is called phage display. With phage display, it is possible to screen for peptides or proteins with affinity for all kinds of targets, ranging from small molecules to proteins and even cells. Therefore, phage libraries consist of a high number of different phages (10^8–10^{10}), each of them displaying a different peptide or protein (peptide, cellular proteins [from cDNA libraries] or antibody fragments, like single-chain variable fragments (scFv) and antigen-binding fragment [Fab]) on their surface. Among the large number of phages in these phage libraries, the ones with high affinity and specificity for a target can be isolated in an affinity-selection procedure. These phages with a high affinity and specificity can be used as a target-specific recognition element of a biosensor. Besides the target-specific phages, the peptides or proteins that are identified by phage display as good binders can also be directly used as a recognition element. The peptides or proteins are then chemically synthesized or produced by recombinant expression in bacterial cells.

If phages are used to detect bacteria, it is not always necessary to use phages that display specific binding peptides or proteins because the phage itself can specifically recognize its particular bacterial host strain. The phages identify their host by specific receptor molecules on the outside of the bacterial cell. Once the phages recognize their specific receptors, they bind to the bacterial cells and infect them. The binding between phages and bacterial cells can be so specific that only certain strains of a single species can be infected.

In general, there are different advantages of the use of phages as recognition elements in biosensors for food applications. The first advantage is their high sensitivity and specificity. Phages can very specifically recognize a target bacterial cell and they can display target-specific peptides or proteins on their surface. Phages can be selected out of libraries with a very high diversity. Moreover, the sensitivity and specificity of the selected phages can be increased after the selection procedure by genetic modification. A second important advantage is that phages are fast, cheap, and animal-friendly producible. Another advantage is their stability. Phages are stable in a variety of pH (pH ranging from 3 to 11), using high-temperature conditions, in aqueous solutions, and also they have high solvent resistance. In addition, phages are stable in certain enzymes such as nucleases. The main limitation of phages is that the targets need to be immobilized. For proteins and whole cells this can be performed easily by adsorption on surfaces with high affinity for polar groups, but for small compounds, specific functionalization is necessary to allow for their immobilization.

Whole cell biosensors. Main classes of whole cell biosensors for food analysis are based on

bacteria coupled with electrochemical and optical transduction schemes.

In general, fabrication of a whole cell biosensor requires immobilization of microorganisms on transducers. Since whole cell biosensor response, operational stability, and long-term use are, to some extent, functions of the immobilization strategy used, immobilization technology plays a very important role, and the choice of immobilization technique is critical. Microorganisms can be immobilized on transducer or support matrices by chemical or physical methods (Lei et al., 2006).

Chemical methods of bacteria immobilization include covalent binding and cross-linking (D'Souza, 2001). Cross-linking involves bridging between functional groups on the outer membrane of the cells by multifunctional reagents such as glutaraldehyde and cyanuric chloride, to form a network. This method has found a wide acceptance for immobilization of microorganisms. The cells may be cross-linked directly onto the transducer surface or on a removable support membrane, which can then be placed on the transducer. The ability to replace the membrane with the immobilized cells is an advantage of this approach. However, in both cases, covalent binding and cross-linking are major limitations in cell viability, being suitable when only the intracellular enzymes are involved in the detection (D'Souza, 2001).

Adsorption and entrapment are the two widely used physical methods for microbial immobilization, because they produce a relatively small perturbation of native structure and function of microorganisms (Lei et al., 2006). Physical adsorption is the simplest one. Typically, a microbial suspension is incubated with the electrode or an immobilization matrix, such as alumina and glass bead (Nanduri et al., 2007), followed by rinsing with buffer to remove unabsorbed cells. The microbes are immobilized due to adsorptive interactions such as ionic, polar or hydrogen bonding, and hydrophobic interaction. However, immobilization using adsorption alone generally leads to poor long-term stability because of desorption of microbes.

The immobilization of microorganisms by entrapment can be achieved either by the retention of the cells in close proximity of the transducer surface using dialysis or filter membrane or in chemical/biological polymers/gels such as alginate, carrageenan, agarose, chitosan, collagen, polyacrylamide, polyvinyl alcohol, polyethylene glycol, polyurethane, etc. (Odaci et al., 2008). A major disadvantage of entrapment immobilization is the additional diffusion resistance presented by the entrapment material, which will result in lower sensitivity and detection limit.

7.2.2. Transduction Elements

According to the main transduction elements, biosensors can be classified as:

- **Electrochemical**: Based on voltammetric and potentiometric devices.
- **Optical**: Based on fluorescence/luminescence, reflectometry, or interferometry.
- **Acoustic**: Bulk acoustic wave (BAW) and surface acoustic wave (SAW) propagation transducers are commonly used.
- **Calorimetric transducers** measure the heat of a biochemical reaction at the sensing element. These devices can be classified according to the way heat is transferred.

Electrochemical, optical, and acoustic transduction systems are often applied in food analysis.

Electrochemical transduction. In electrochemical biosensors, the variation of electron fluxes leads to the generation of an electrochemical signal, which is measured by the detector. Two of the most important classes of electrochemical biosensors include the voltammetric and potentiometric biosensors.

Voltammetric sensors investigate de-concentration effects of target species on the current potential characteristics of the reduction or

oxidation of a specific reaction. **Amperometric sensors** are a subclass of the voltammetric sensors. The principle of functioning is based on the application of a fixed potential to an electrochemical cell, resulting in a current due to an oxidation or reduction reaction. The current is then used to quantify the species involved in the reaction. The versatility of amperometric biosensors is also apparent from their direct or indirect measurement capability. Oxidase enzymes have been the most frequently applied biosensors. A number of amperometric biosensors are based on the monitoring of oxygen consumption or hydrogen peroxide generation. Both are electrochemically active; oxygen can be electrochemically reduced, and hydrogen peroxide can be oxidized. The current generated is proportional to the concentration of the enzyme substrate present in a sample. The use of mediators should permit the replacement of oxygen as an electron acceptor and operation at a much lower potential, reducing the effects of other electrochemically active species found in complex matrices.

Potentiometric biosensors examine the potential difference between the working electrode and the reference electrode as it relates to the redox reaction of the species of interest. Potentiometric biosensors are based on the monitoring of the potential produced at a working electrode, with respect to a reference electrode. The potentiometric biosensors monitor the accumulation of charge zero current created by a selective binding at the electrode surface. A limitation of potentiometric biosensors compared with the amperometric counterpart is the extended period of time required for equilibration.

One key step in the development of biosensors, not just under electrochemical configurations, is the immobilization of the biological component at the transducer surface. The immobilization requires both the stabilization of the biomaterial and the proximity and communication between the biomaterial and the transducer. The immobilization methods generally employed are physical adsorption at a solid surface, cross-linking between molecules, covalent binding to a surface, and entrapment within a membrane, surfactant matrix, polymer, or microcapsule. In addition, sol–gel entrapment, Langmuir–Blodgett (LB) deposition, electro-polymerization, self-assembled biomembranes, and bulk modification have been widely used during recent years. In this sense, a great effort of development is being carried out to obtain more robust and more sensitive electrochemical biosensors.

The development of biosensors based on electrochemical transduction is rising again, thanks to the advances that can be offered by nanotechnology, which eliminates some major limitations encountered in the past. Electrochemical biosensors incorporate enzymes with nanomaterials, which combine the recognition and catalytic properties of enzymes with the electronic properties of various nanomaterials; these new materials with synergistic properties originate from the components of the hybrid composites. Therefore, these systems have excellent prospects for interfacing biological recognition events through electronic signal transduction so as to design a new generation of bioelectronic devices with high sensitivity and stability (Li et al., 2009).

New approaches based on nanotechnology are discussed in detail in section 3.

Optical transduction. Optical transducers are based on various technologies of optical phenomena, including adsorption, fluorescence, phosphorescence, polarization, rotation, interference, etc., or nonlinear phenomena, such as second harmonic generation. The choice of a particular optical method depends on the nature of the application and desired sensitivities. In practice, fiber optics can be coupled with all optical techniques, thus increasing their versatility. The optical biosensor formats may involve direct detection of the analyte of interest or indirect detection through optically labeled probes. However, nowadays there is a growing interest in direct detection. Because the class of

optical methods is a very wide field, this chapter concentrates on the currently most common methods for food analysis.

Optical biosensors can be classified according to transduction principles into those using absorption, fluorescence, luminescence, and chemiluminescence.

Absorption. The simplest optical biosensors use the absorption phenomenon to determine changes in the concentration of analytes. The sensor works by sending light through an optical fiber to the biosample. The amount of light absorbed by the analyte is determined by measuring the light coupled out via the same fiber or a second optical fiber. From the physics point of view, absorption is a process in which light energies are absorbed by an atom or a molecule. Based on the Lambert–Beer law, the intensity of transmitted light (I) through a uniform absorption medium can be described by the following equation:

$$I = I_0 \exp^{-\varepsilon C \Delta x},$$

where I_0 denotes the incident light intensity, ε is the extinction coefficient, C represents the concentration of the absorption of analyte, and Δx is the thickness (or length) of the absorption medium. Since absorption is usually wavelength dependent and different species may have different absorption spectra, by measuring the absorption spectra via a fiber optic sensor, different species and concentration levels can be determined. The major advantages of absorption-based sensors are that they are simple, easy to use, and cost-effective.

Fluorescence/luminescence occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy (Lazcka et al., 2007). The common principle of luminescence immunosensors is that an indicator or chemical reagent placed inside or on an immunoreactor is used as a mediator to produce an observable optical signal. Typically, conventional techniques, such as spectrometers, are employed to measure changes in the optical signal. Fluorescence spectroscopy in its numerous variations has become one of the more powerful bioanalytical and diagnostic tools in the past 20 years and – in terms of versatility – seems to be second only to NMR spectroscopy, but with an entirely different field of application. In addition, it must be mentioned that many biomolecules are fluorescent as, for example, the green fluorescent protein (GFP), nucleic acids, flavine nucleotides, and NADH. Recently, a great effort has been carried out in the development of new biosensors using water-soluble QDs (fluorescence semiconductor nanoparticles), with narrow and very specific, stable emission spectra. The intrinsic properties of QDs have been used for the sensitive detection of target analytes in food safety, to develop rapid, sensitive, and specific detection techniques to monitor pesticides, pathogenic bacterial toxins, such as botulin toxin, enterotoxins produced by *Staphylococcus aureus*, *Escherichia coli*, and for the development of oligonucleotide-based microarrays (Vinayaka and Thakur, 2010).

Chemiluminescence and bioluminescence. Chemiluminescence is similar to fluorescence. The difference is that chemiluminescence occurs by exciting molecules with a chemical reaction (usually occurring by the oxidation of certain substances such as oxygen or hydrogen peroxide), whereas fluorescence occurs by exciting molecules via light. Thus, in the case of chemiluminescence, no external source of light is required to initiate the reaction.

Bioluminescence is simply chemiluminescence occurring in living organisms, which represents a biological chemiluminescent reaction process. Many organisms produce bioluminescence for signaling, mating, prey attracting, food hunting, and self-protection. Since bioluminescence is generated via biological reaction processes, detecting it can be achieved by sensing a certain biological process.

Fluorescence and luminescence biosensors are in general based on the use of optrodes. Optrode-based fiber optic biosensors are analytical devices incorporating optical fibers and biological recognition molecules. Optical fibers are small and flexible wires made out of glass or plastic that can transmit light signals, with minimal loss, over long distances. The light signals are generated by a sensing layer, which is usually composed of biorecognition molecules and dyes, coupled to the fiber end. Light is transmitted through the optical fibers to the sensing layer where different optical phenomena such as absorption or luminescence are used to measure the interactions between the analyte and the sensing layer.

Fluorescence-monitoring-based instrumentation relies on equipment well established commercially and achieves a very low limit of detection, even down to single-molecule detection. However, expenditure and costs, and problems with bioactivity are a disadvantage in combination with the problem of photobleaching, which normally does not allow time-resolved monitoring for evaluation of kinetics. In contrast, this type of monitoring can easily be done with direct optical techniques, which at present allow even the use of direct assays in which reagents are no longer necessary; this is an advantage in many routine applications, such as the food industry. The disadvantage is the problem with nonspecific binding and the higher limit of detection compared with fluorescence techniques.

Direct optical detection methods have been reviewed in recent years (Gauglitz and Proll, 2007; Fan et al., 2008) and can be classified according to two principles, microrefractometry and microreflectometry. This means measuring the interaction between a thin biomolecular layer containing recognition sites and a ligand or an analyte in solution monitors the spectroscopy of biomolecules at the surface. The radiation reflectance in general measures changes in the optical thickness, which is the product of the refractive index, n, and the physical thickness, d, of this interaction layer.

The refractive index of the optical thickness is crucial for microrefractometry techniques, because the exponential decay of the evanescent field into the interaction layer causes inhomogeneous signal penetration of this interaction layer. This enables effects in restricted elements of the interaction layer, or close to it and the bulk to be distinguished, but reduces the effects at a distance from the transducer surface. Furthermore, the refractive index is rather temperature dependent, which requires very strict temperature control.

Among techniques based on evanescent field techniques (microrefractometry), surface plasmon resonance (SPR), grating couplers, resonant mirror, Mach–Zehnder interferometer, Young interferometer, and Bragg gratings are the more studied and applied (Homola, 2008; Gauglitz, 2005; Marazuela et al., 2002; Fan et al., 2009; Piliarik et al., 2009). Figure 7.2 summarizes the main classes of evanescent field techniques.

Although each of these methods has its individual strengths and weaknesses, a strong case has been made that optical sensors, in particular, those based on evanescent electromagnetic fields, such as propagating surface plasmon polaritons (SPPs) at planar gold surface, are fast becoming the methods of choice in many affinity biosensing applications. SPP or, more commonly, SPR spectroscopy has been widely used to monitor a broad range of analyte-surface binding interactions. The sensing mechanism of **SPR spectroscopy** is based on the measurement of small changes in refractive index that occur in response to analyte binding at or near the surface of a noble metal (Au, Ag, and Cu) thin film. Biosensors based on SPR spectroscopy possess many desirable characteristics including the following: (1) a refractive index sensitivity on the order of 1 part in 10^5–10^6 corresponding to an area mass sensitivity of approximately 10^{-1} pg/mm^2, (2) multiple

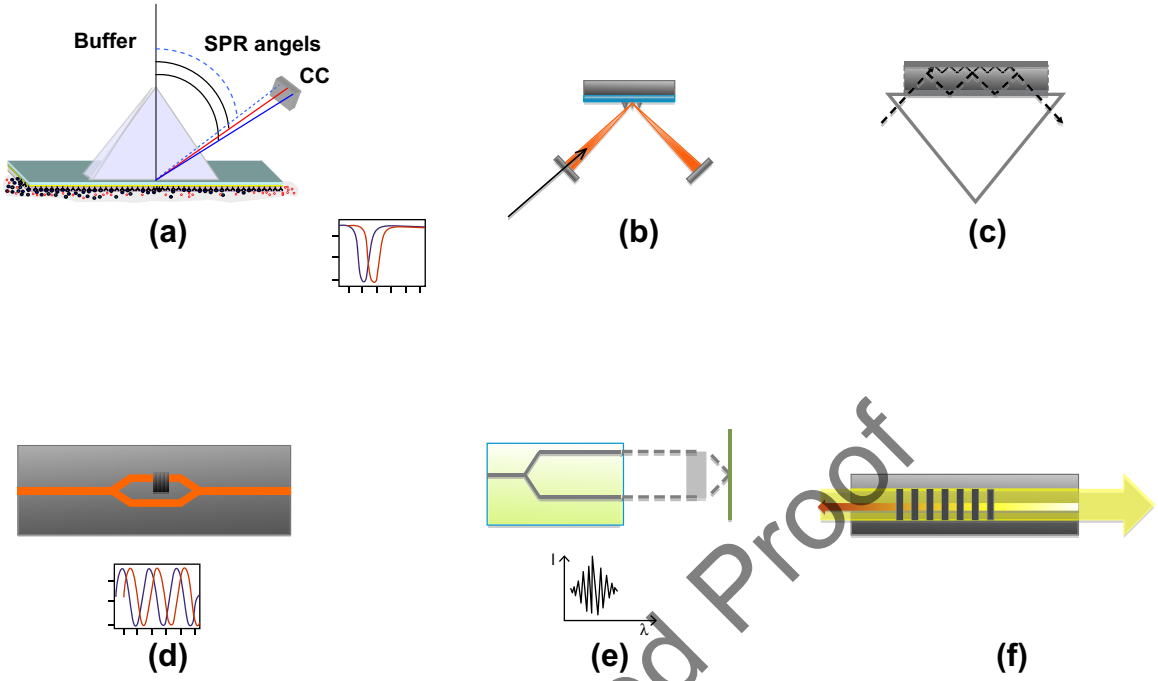

FIGURE 7.2 Schematic diagrams of evanescent field techniques (microrefractometry): (a) Surface plasmon resonance (SPR); (b) Grating coupler; (c) Resonant mirror; (d) March-Zehnder interferometer; (e) Young interferometry; and (f) Bragg grating.

instrumental modes of detection (angle shift, wavelength shift, and imaging), (3) real-time detection on the 10^{-1}–10^{3} s timescale for measurement of binding kinetics, and (4) lateral spatial resolution on the order of 10 μm enabling multiplexing and miniaturization, especially using the SPR imaging mode of detection (Homola et al., 2008). Although SPR spectroscopy is a totally non-selective sensor platform, a high degree of analyte selectivity can be conferred using the specificity of surface-attached ligands and passivity of the sensor surface to nonspecific binding. In addition, it is label free; capable of probing complex mixtures, such as food samples, without prior purification; and benefits from the availability of commercial instrumentation with advanced microfluidic sample handling.

The development of large-scale biosensor arrays composed of highly miniaturized signal transducer elements that enable real-time, parallel monitoring of multiple species is an important driving force in biosensor research. This is particularly significant in high-throughput screening applications where many thousands of ligand–receptor or protein–protein interactions must be rapidly examined.

Recently, several research groups have begun to explore alternative strategies for the development of optical biosensors based on the extraordinary optical properties of noble metal nanoparticles (NPs). Noble metal NPs exhibit a strong UV–vis absorption band that is not present in the spectrum of the bulk metal. This absorption band results when the incident photon frequency is resonant with the collective oscillation of the conduction electrons and is known as the **localized surface plasmon resonance (LSPR)**. LSPR excitation results in wavelength selective absorption with extremely

large molar extinction coefficients of approximately $3.10^{11}\,M^{-1}\,cm^{-1}$ (Jensen et al., 2000), resonant Rayleigh scattering with an efficiency equivalent to that of 10^6 fluorophores, and the enhanced local electromagnetic fields near the surface of the nanoparticle which are responsible for the intense signals observed in all surface-enhanced spectroscopy. It is well established that the peak extinction wavelength, λ max, of the LSPR spectrum is dependent upon the size, shape, and interparticle spacing of the nanoparticle, as well as its dielectric properties and those of the local environment. Consequently, there are at least four different nanoparticle-based sensing mechanisms that enable the transduction of macromolecular or chemical-binding events into optical signals based on changes in the LSPR extinction or scattering intensity, shifts in LSPR λ max, or both. These mechanisms are: (1) resonant Rayleigh scattering from nanoparticle labels in a manner analogous to fluorescent dye labels, (2) nanoparticle aggregation, (3) charge−transfer interactions at nanoparticle surfaces, and (4) local refractive index changes.

It has been demonstrated that nanoscale biosensors can be realized through shifts in the LSPR λ max of triangular silver NPs (Shankaran et al., 2007). These wavelength shifts are caused by adsorbate-induced local refractive index changes in competition with charge−transfer interactions at the nanoparticle surface. Triangular silver NPs have been shown to be unexpectedly sensitive to nanoparticle size, shape, and local dielectric environment (Haes et al., 2006).

Interferometry has also been exploited for biosensor development. When a biological reaction takes place on the waveguide surface, it produces a change in the refractive-index profile within the evanescent field volume; correspondingly, the effective refractive index of the waveguide system is changed. In Mach−Zehnder interferometry (MZI), an optical waveguide is split into two arms and after a certain distance they are recombined. The sensor arm will be exposed to a variation of the refractive index due to a biorecognition reaction such as an immunoreaction in the sensor channel. During this distance, light traveling in the sensing arm will experience a phase shift in comparison with guided light in the reference arm (Prieto et al., 2003a,b).

In contrast, using **microreflectometry**, the signal is nearly independent of temperature because a volume increase of the interaction layer with temperature is compensated by a decrease of the refractive index with temperature. In addition, reflectometry concentrates on measuring changes in the physical thickness of this interaction layer, using an approach that is independent of the layer thickness because exponential decay of the evanescent field is not essential for the signal. In principle, a white incident light passing the interface between different refractive indices, will be reflected in part. These reflected beams superimpose and build a characteristic interference spectrum. The binding of biological receptors, such as an antibody to the surface, changes the thickness of the toggling layer, which causes a change in the reflectance spectrum. Thus, the interaction process between the bioreceptor and the analyte can be detected (Proll et al., 2004). Therefore, this method is called **reflectometric interference spectroscopy (RIfS)**.

Total internal reflection fluorescence (TIRF) has been used with planar and fiber optic waveguides as signal transducers in a number of reported biosensors. In these transducers, light is propagated down a waveguide, which generates an electromagnetic wave (evanescent wave) at the surface of the optically denser medium of the waveguide and the adjacent less optically dense medium. The amplitude of the standing wave decreases exponentially with distance into the lower refractive index material. The fluorescence of a fluorophore excited within the evanescent field can be collected.

At low angles, total internal reflection results when light propagating within a dense medium (e.g., quartz) reaches an interface with a less dense medium (e.g., aqueous solution). Although the light is fully reflected, an evanescent field is generated that extends beyond the interface and into the aqueous solution. Typically, the penetration depth (or the thickness of evanescent field) is in the range of half the wavelength of the light. The evanescent field provides the surface selectivity of TIRF. Only fluorophores adsorbed, adhered, or bound to the surface will be excited and therefore fluoresce. Conversely, fluorophores in bulk solution will not be excited. Therefore, if the surface is made biologically active so that one may 'trap' fluorescently labeled compounds of interest, one can detect analytes within complex sample solutions. Because the excitation light is totally reflected away from the detection, one can easily discriminate the fluorescence signal from the excitation light and achieve high sensitivities and low detection limits. TIRF systems provide measurement of real-time kinetics of a bioanalyte's binding to a surface-immobilized sensor molecule. TIRF is a fast, nondestructive, sensitive, and versatile technique that is well suited for monitoring biomolecular interactions. TIRF allows monitoring of conformational changes, orientation changes, and lateral mobility of biomolecules.

Mass sensitive sensors. Measurement of small changes in mass is a transduction form that has been used for biosensor development. Piezoelectric devices and surface acoustic wave devices can be grouped under this category.

This principle shows great promise for food quality and control, such as the possibility of miniaturization and the high sensitivity and specificity achieved when coupled to the proper bioreceptor; this is one of the most promising approaches.

The vibration of piezoelectric crystals produces an oscillating electric field in which the resonant frequency of the crystal depends on its chemical nature, size, shape, and mass. These crystals can be made to vibrate at a specific frequency of oscillation, which depends on the electric frequency. The frequency of oscillation is therefore dependent on the electrical frequency applied to the crystal as well as the crystal's mass. When the mass increases due to the binding of analytes, the oscillation frequency of the crystal changes and this change can be measured. The general equation of crystal microbalances can be summarized as follows when the change in mass (m) is very small compared to the total mass of the crystal:

$$\text{delta } f = Cf^2 \text{ delta m/A},$$

where f is the vibration frequency of the crystal in the circuit, A is the area of the electrode, and C is a constant determined in part by the crystal material and thickness. Piezoelectric crystals, sometimes referred to as quartz crystal microbalances (QCM), are typically made of quartz and operate at frequencies between 1 and 10 MHz. These devices can operate in liquids with a frequency determination limit of 0.1 Hz; the detection limit of mass bound to the electrode surface is about 10^{-10} to 10^{-11} g.

Acoustic wave devices made of piezoelectric materials are the most common sensors, which bend when a voltage is applied to the crystal. Acoustic wave sensors are operated by applying an oscillating voltage at the resonant frequency of the crystal, and measuring the change in resonant frequency when the target analyte interacts with the sensing surface.

Limitations for this transduction method involve format and calibration requirements, which are time consuming.

7.3. NANOBIOSENSORS, MICROFLUIDICS, AND LAB-ON-A-CHIP

New trends in biosensing technology, by introducing advanced materials and devices, the nanotechnology-based platforms combining

fluid handling, molecular recognition elements, and reporter molecules have the potential to achieve improved selectivity and sensitivity and will enable better biomolecule research tools. The main purpose of this section is to explore the recent developments in nanosensors along with their integration with these three supporting areas: molecular recognition elements, reporter elements, and microfluidics, to create novel nanotechnology-based sensing platforms for food quality and control.

Nanosensors have critical structural dimensions of less than 100 nm. Transduction mechanisms are typically classified into two categories: label based and label free.

- Label-based transduction mechanisms typically rely on the presence of an added labeling molecule or structure consisting of discrete, inorganic NPs that can be transduced using optics, magnetics, and electronics, among others.
- Label-free nanosensors are those based on microcantilevers, nanowires, and resonators (Gupta et al., 2006; Wang et al., 2005).

In spite of the advances in nanotransduction elements during last years, commercial applications have been limited, primarily by limitations in packaging and interfacing.

7.3.1. Label-Based Methods

Some biomolecules due to their small size cannot be recognized directly. To track these biomolecules and their activity, the molecules can be labeled by conjugation with a detectable agent, commonly a fluorophore or an enzyme. These agents for labeling proteins, nucleic acids, and other molecular probes are called tags. These tags have unique detectable properties such as radioactivity, chromogenicity, fluorescence, or magnetism. Additionally, electrical and electrochemical principles, based on the properties of labeled probes, have been developed to establish corresponding detection methods through a target-binding technique. Having a uniquely detectable property, most tags can be functionalized to link to a specific molecular probe. In a related approach, instead of having a detectable group directly attached to a probe molecule, a recognition reagent having strong affinity for a secondary probe can be used to detect the target molecules. A variety of interaction pairs, such as biotin–avidin, hapten–antibody, and DNA–RNA hybrids, are already in use. This two-stage detection scheme can be utilized when a primary-labeled probe is not available. Among a range of options, current biomolecule detection methods have mainly employed fluorescent labels, QDs, or heavy atom complex nanoparticle labels. Chromogenic labels are also available, but they have been replaced with fluorescent labels, which give larger quantum emission yield upon excitation resulting in better detectability. Bioluminescence, which generates detectable light as a result of biochemical reactions, is another popular technique for biodetection. Other detection methods are Au nanoparticle and magnetic nanoparticle labels. These techniques are emerging as better substitutes in terms of simplicity, sensitivity, specificity, and reliability.

Fluorescent molecules contain fluorophores capable of being excited, via absorption of light energy at a specific wavelength, and subsequently emitting at a longer wavelength. Common organic fluorophores are derivatives of fluorescein, rhodamine, coumarin, and cyanine. Despite their considerable advantages in biomolecular imaging, there are some limitations, such as photobleaching (Benchaib et al., 1996), pH-sensitivity (Nakamura et al., 1991), and loss of fluorescence when they are conjugated to biomolecules (Valdes-Aguilera and Neckers, 1989). Combined with nanotechnology, fluorescent core–shell nanoparticle labels offer favorable characteristics, because many dye molecules are encapsulated in nanosized particles that also shield them from photobleaching (Hun and Zhang, 2007). A biarsenical derivative

of fluorescein has been designed to investigate protein–protein interactions via label transfer chemistry, in which a tag is transferred from one protein to partner proteins (Liu et al., 2007; Fu et al., 2007). Nanodiamonds can also serve as fluorescent labels in biosensing.

Carbon nanotubes. Electrochemical sensing approaches have exploited the use of CNTs as electrode materials owing to their unique structures and properties to provide strong electrocatalytic activity with minimal surface fouling. Nanofabrication and device integration technologies have emerged along with significant advances in the synthesis, purification, conjugation, and biofunctionalization of CNTs. Such combined efforts have contributed toward the rapid development of CNT-based sensors for a plethora of important analytes with improved detection sensitivity and selectivity. The use of CNTs opens an opportunity for the direct electron transfer between the enzyme and the active electrode area. Of particular interest are also excellent electrocatalytic activities of CNTs on the redox reaction of hydrogen peroxide and nicotinamide adenine dinucleotide, two major by-products of enzymatic reactions. This excellent electrocatalysis holds a promising future for the simple design and implementation of on-site biosensors for oxidases and dehydrogenases with enhanced selectivity. To date, the use of an anti-interference layer or an artificial electron mediator is critically needed to circumvent unwanted endogenous electroactive species. Such interfering species are effectively suppressed by using CNT-based electrodes since the oxidation of NADH, thiols, hydrogen peroxide, etc., by CNTs can be performed at low potentials. Nevertheless, the major future challenges for the development of CNT-EC sensors include miniaturization, optimization, and simplification of the procedure for fabricating CNT-based electrodes with minimal nonspecific binding, high sensitivity, and rapid response followed by their extensive validation using "real world" samples. A high resistance to electrode fouling and selectivity are the two key pending issues for the application of CNT-based biosensors in food quality and control.

Quantum dots (QDs) have provided a great breakthrough in many biological labeling applications (Jamieson et al., 2007; Tansil and Gao, 2006; Sanvicens et al., 2009; Vinayaka et al., 2010) with their typical core size of 1–10 nm and outstanding fluorescence compared to typical organic fluorophores. QD fluorescence comes about as a result of quantum effects from the three-dimensional spatial confinement of the QDs' core semiconductor atoms. This confinement limits the core atoms' electron excitation states with the end result being that discrete fluorescence emission is produced with broad excitation. This unique type of fluorescence produces a fluorophore that is bright, photostable, has sharp fluorescence peak, and has colors that are controllable by varying size and composition of the core atoms. These properties have in turn enabled several novel uses of QDs in biodetection methods. Water-soluble QDs (Larson et al., 2003) have also been developed to improve biocompatibility, allowing long-term multicolor imaging of live cells and fluorescence correlation spectroscopy (Yeh et al., 2006). Encapsulation within polymeric or lipid-based layers (Campolongo et al., 2010) and coating with a short chain of peptides (Jaiswal et al., 2004) have also helped to disguise QDs as similar-sized biomolecules like proteins or nucleic acids. QDs have also been used in combination with fluorescence energy transfer (FRET) for such applications as signal amplification during DNA sensing and detection of molecular orientation, size, and binding (Ho et al., 2006; Merkosi et al., 2005).

Au (gold) NPs have attracted the attention of many researchers, due to their versatility with a variety of detection approaches, such as optical absorption (Du et al., 2010; Merkosi, 2010; Prabhakar and Mukherji, 2010; Wang et al., 2010), fluorescence (Maxwell et al., 2002), Raman scattering (Vo-Dinh et al., 2006), electrical

conductivity, and an electrochemical redox property. Moreover, Au NPs generate highly efficient multiphoton absorption-induced luminescence without significant blinking, suggesting they are viable alternatives to fluorophores or semiconductor NPs for biological labeling (Farrer et al., 2005). Other advantages are easy preparation and conjugation to biomolecules, very low toxicity, and operation at a safe wavelength of the laser light used to visualize the particles.

Magnetic particles, which respond to an external magnetic field, have been used extensively for separation and preconcentration purposes in optical (Kumar and Chen, 2008; Goulart et al., 2010) and electrochemical biosensors (Hsing et al., 2007). Their unique properties allow magnetic particle-conjugated molecules to be quickly agglomerated or resuspended in the medium according to the external magnetic force, thus making them suitable for purifying biologically active compounds, such as nucleic acids, proteins, and cells. They are stable and safe over time, inexpensive, and the analysis procedure of the magnetic tag generated signal is rapid.

7.3.2. Label-Free Detection Methods

Label-free methods have emerged as a potential way to avoid possible structural and functional alterations of target molecules while providing acceptable sensitivity and selectivity. With recent advances in micro- and nanotechnologies, label-free biosensors have achieved attogram sensitivity and tremendous high-throughput analysis capabilities. Here, we describe the current state of the art in label-free detection techniques, including SPR, surface-enhanced Raman scattering (SERS), micro/nanocantilevers, nanowires, and nanopores.

7.3.3. Micro/Nanofluidics Integrated with Nanobiosensors

One of the most relevant characteristics of analytical microsystems is the omnipresence of laminar flow (Reynold's number is typically very low), in which viscous forces dominate over inertia. This means that turbulence is often unattainable and that molecule transportation only occurs through diffusion, which has direct consequences on the designs of this type of microsystem. *Microfluidics* is the science and technology of systems that process or manipulate small amounts of fluidics (10^{-9} to 10^{-18} L), using channels measuring from tens to hundreds of micrometers. For this reason, the term *microfluidic*s better covers the research and emphasizes the strong impact miniaturization and integration have on the fluidics and chemical engineering of analytical microsystems.

The micro- and nanotechnologies coupled with deep knowledge of organic and inorganic interfaces guarantee an exceptional sensitivity and specificity of the sensor, while the lab-on-a-chip platform reduces assay time and limits sampling and/or simple preparation, providing compact and portable objects. Therefore, the development of innovative biosensors can overcome the evident limits of current technologies, such as time consuming, expense, difficult automation, low sensitivity, accuracy, and precision for quantitative methods.

The micro-total analysis system (μ-TAS) concept, later called "lab-on-a-chip," was developed from the modification of the total analysis system (TAS) approach by downsizing and integrating its multiple steps (injection, reaction, separation, and detection) onto a single device, yielding a sensor-like system with a fast response time, low sample consumption, on-site operation, and high stability.

Food analysis is a challenging issue for microfluidic analytical systems and lab-on-a-chip devices due to the complexity of food matrices. However, the first successful applications of microfluidic biosensors have been developed. Transduction elements in general coupled to these microfluidic devices are SPR and microcantilevers.

7.4. APPLICATION OF NEW BIOSENSING TECHNOLOGIES FOR FOOD SAFETY AND CONTROL

Significant developments based on microfluidic biosensors for food safety technologies have been made in three main fields:

- Pesticide residues
- Veterinary drugs and growth promoting agents
- Pathogenic bacteria and natural toxins

In the next section a summary of these developments has been presented.

7.4.1. Pesticide Residues

The main application of enzyme biosensors in food analysis has been the determination of pesticide residues in fruits and vegetables. The inhibition of choline esterases has been widely reported in electrochemical biosensors for the detection of organophosphorus and carbamate insecticides. Acetylcholinesterase (AChE) especially has been widely used (Gogol et al., 2000; Zhang et al., 2001; Schulze et al., 2003; Crew et al., 2004). However, the main limitations of these technologies are the need for intermediates to increase sensitivity, the lack of long-term stability of some enzymes, and the lack of specificity. During recent years, much development work has been carried out to overcome these limitations, particularly by the use of nanotechnology. The immobilization of biological receptors on electrochemical sensor surfaces is a key point for the final performance of the sensor. For example, a simple method to immobilize AChE on polypyrrole (PPy) and polyaniline (PANI) copolymer doped with multiwalled carbon nanotubes (MWCNTs) was proposed. Due to the biocompatible microenvironment provided by the copolymer network, the obtained composite was devised for AChE attachment, resulting in a stable AChE biosensor for screening of organophosphate (OP) exposure. MWCNTs promoted electron-transfer reactions at a lower potential and catalyzed the electro-oxidation of thiocholine, thus increasing detection sensitivity. Based on the inhibition of OPs on the AChE activity, using malathion as a model compound, the inhibition of malathion was proportional to its concentration, with a detection limit of 1.0 ng/mL. The developed biosensor exhibited good reproducibility and acceptable stability, thus providing a new promising tool for the analysis of enzyme inhibitors (Wu et al., 2006). In another study, a sensitive amperometric AChE biosensor was fabricated based on mesocellular silica foam (MSF), which functioned as both an enzyme immobilization matrix and a solid-phase extraction (SPE) material for the preconcentration of target molecules. The hydrophilic interface, the good mechanical/chemical stability, and the suitable pore dimension of MSF provided the entrapped AChE with a good environment to maintain its bioactivity in basic conditions. The AChE immobilized in MSF showed improved catalytic ability for the hydrolysis of acetylthiocholine, as evidenced by the increased oxidation current of thiocholine, the enzymatic catalytic hydrolysis production of acetylthiocholine. In addition, the MSF with large surface area showed a modest adsorption capacity for monocrotophos, a model organophosphate used in this study, via the hydrogen bond or physical adsorption interaction.

The combination of the SPE and the good enzyme immobilization ability in MSF significantly promoted the sensitivity of the biosensor, and the limit of detection has lowered to 0.05 ng/mL. The biosensor exhibited accuracy, good reproducibility, and acceptable stability when used for garlic sample analysis (Wu et al., 2006). The strategy may provide a new method to fabricate highly sensitive biosensors for the detection of ultra-trace organophosphorus pesticide infield. In a recent work, Tang et al. (2011) have reported the development of three-electrode biosensors with higher stability than

in previous studies of the same group. In this new approach, recombinant *Drosophila melanogaster* acetylcholinesterase (R-DmAChE), MWCNTs, and Prussian blue have been combined. A new disposable screen-printed electrode was developed for rapid detection of organophosphate and carbamate pesticides. After optimization, 10 μg MWCNT and 5 μL enzyme immobilization solution consisting of 0.2% glutaraldehyde, 0.1% Nafion®, 0.2% bovine serum albumin, 0.1 g/L MWCNT, and 1.5 mU R-DmAChE were fixed on each of the R-Dm AChE/MWCNT SPEs. The LOD of this biosensor was 0.5 μg/L for pesticide standards of dichlorvos and carbofuran. The performance of this biosensor was tested for vegetable and water samples at various spiked levels, and good stability and sensitivity were found. In another recent work (Crew et al., 2011) presented a biosensor array based on six AChE enzymes for use in a novel automated instrument incorporating a neural network program. Electrochemical analysis was carried out using chronoamperometry and the measurement was taken 10 s after applying a potential of 0 V vs. Ag/AgCl. The total analysis time for the complete assay was less than 6 min. The array was used to produce calibration data with six organophosphate pesticides in the concentration range of 10^{-5}–10^{-9} M to train a neural network. The output of the neural network was subsequently evaluated using different sample matrices. The biosensor system successfully identified and quantified all samples where an OP was present in water, food, and vegetable extracts containing different OPs. There were no false positives or false negatives observed during the evaluation of the analytical system. The biosensor arrays and automated instrument were evaluated in situ in field experiments where the instrument was successfully applied to the analysis of a range of environmental samples. It is envisaged that the analytical system could provide a rapid detection system for the early warning of contamination in water and food (Crew et al., 2011).

There is great potential in the applications of immunosensors for rapid detection of pesticide residues in food using different transduction formats (Jiang et al., 2008), such as electrochemical, optical, piezoelectric, and nanomechanics. Using labeled formats, enzymes, such as glucose oxidase (Dzantiev et al., 2004), HRP (Yulaev et al., 2001), β-galactosidase, and alkaline phosphatase, and more recently NPs (Cummins et al., 2006) have been widely used.

Label-free formats have also been reported using direct and indirect formats. SPR has an inherent advantage over other types of biosensors in its versatility and capability of monitoring binding interactions without the need for labeling the biomolecules. It is versatile owing to its outstanding attributes of miniaturization, reliable portable instrumentation, and automation. Monitoring of the pesticide chlorpyrifos in water samples was performed using SPR immunosensors (Mauriz et al., 2006a). The chlorpyrifos derivative was immobilized onto the gold-coated sensing surface and competed with free chlorpyrifos for binding to the Ab and, as a result, increasing concentrations of chlorpyrifos will reduce the SPR signal. Other examples of single and multi-analyte assays for simultaneous detection of different pesticides by SPR were reported by the same research group (Mauriz et al., 2006a,b,c). Another sensitive and reusable SPR-based immunosensor was developed for the determination of 2,4-D (Gobi et al., 2005). The SPR sensor was capable of detecting part per billion levels of 2,4-D in 20 min and the regeneration ability enabled the achievement of as many as 20 measurement cycles. In another example, a fluoro-immunosensor based on cadmium telluride QDs nanoparticle (CdTe QD) to detect residues of 2,4-D was developed. The detection of 2,4-D was carried out using competitive binding between conjugated 2,4-D-ALP-CdTe and free 2,4-D with immobilized anti 2,4-D antibodies in an immunoreactor column. It was possible to detect 2,4-D up to 250 pg/mL (Vinayaka et al., 2009).

Piezoelectric immunosensors are alternatives to the conventional immunoassay tools and some examples have been reported. For example, March et al. (2009) have reported a quartz crystal microbalance (QCM) immunosensor for the determination of the insecticide carbaryl and 3,5,6-trichloro-2-pyridinol (TCP), the main metabolite of the insecticide chlorpyrifos and of the herbicide triclopyr. The detection was based on a competitive conjugate-immobilized immunoassay format using monoclonal antibodies. Hapten conjugates were covalently immobilized, via thioctic acid self-assembled monolayer (SAM), onto the gold electrode sensitive surface of the quartz crystal. This covalent immobilization allowed the reusability of the modified electrode surface for at least 150 assays without significant loss of sensitivity. The piezoimmunosensor showed detection limits of 11 and 7 μg/L for carbaryl and TCP. The sensitivity attained (I_{50} value) was around 30 μg/L for both compounds. The good sensitivity, specificity, and reusability achieved, together with the short response time, allowed the application of this immunosensor to the determination of carbaryl and TCP in fruits and vegetables at European regulatory levels, with high precision and accuracy. Another example was reported for the determination of triazophos. In this case, a piezoelectric immunosensor based on a competitive format was developed. The assay exhibited a working range of 5–5000 ng/mL. In this case, cross-reactivity was exhibited with parathion and chlorpyrifos (Huang et al., 2010).

Table 7.1 summarizes different examples of biosensors for pesticide residue analysis in food.

7.4.2. Veterinary Drugs and Growth-Promoting Agents

Animals produced for food may be exposed, legally or illegally, to a wide range of chemicals (e.g., therapeutics, prophylactics, and growth promoters). It is imperative to monitor samples from these animals for levels of chemical residues that could pose a threat to human health. One of the most prominent groups is the antimicrobial agents used to treat infectious diseases; these fall into three main subgroups:

- antibiotics (i.e., natural substances produced by certain groups of microorganisms),
- chemotherapeutic agents, which are chemically synthesized, and
- hybrid antimicrobials, which are semisynthetic antibiotics produced by chemically, modifying a natural microbial compound to achieve the desired antibiotic properties.

In terms of modern usage, all antimicrobial agents used in the treatment of infectious diseases, whether active against bacteria, fungi, or protozoa, are referred to as antibiotics.

In addition, it is well documented that the use of antibiotics can enhance growth rates, improve feed efficiency, and generally improve animal health, but these compounds pose a potential threat to human and animal health through the development of antibiotic-resistant bacteria. For decades, antibiotics have been used abundantly worldwide in animal production, so antibiotic resistance may spread to other microbial populations, as reflected by the emergence of infectious diseases that have become resistant to standard antimicrobial treatments (Sapkota et al., 2007). Monitoring of antimicrobial drug residues in foods relies greatly on the availability of adequate analytical techniques. There is a current need for high-throughput screening methods with a broad-spectrum detection range.

Aminoglycosides are basic, very hydrophilic, thermally labile compounds, which are particularly active against aerobic gram-negative bacilli. Different SPR-immunosensors have been proposed for their detection in food matrices. Direct assays using monoclonal antibodies were developed for the analysis of gentamicin (Haasnoot and Verheijen, 2001), dihydrostreptomycin, and streptomycin (Haasnoot et al., 2002) in milk.

TABLE 7.1 Summary of Recent Biosensors Developed for Pesticide Analysis in Food

Analyte	Transduction characteristics and biological receptor	Matrix	Limits of detection	References
Carbaryl	Amperometry AChE in carbon paste composite. Carbon paste electrode/ cobalt phthalocyanine	Tomato	3.2 ng/mL	(Caetano et al., 2008)
Carbaryl and 3,5,6-trichloro-2-pyridinol	QCM Immunosensor	Fruits and vegetables	11 and 7 μg/L for carbaryl and TCP, respectively	(March et al., 2009)
Carbaryl and Parathion	AchE based amperometric	Spiked food and water	91−98.0% in recovery	(Pedrosa et al., 2008)
Carbofuran, carbaryl, and benfuracarb	SPR Immunosensor antibody used for the immunoassay was specific for GST and the antigens of carbofuran, carbaryl, and benfuracarb	Food	2 ng/L carbamate pesticides	(Yang and Kang, 2008)
Carbofuran, carbaryl, methylparaoxon, and dichlorvos	Amperometric - AChE enzymes—AChE from electric eel, and genetically engineered (B394) and wild-type (B1) AChE from *Drosophila melanogaster*	Apple samples	With the B394 enzyme for dichlorvos and methylparaoxon (9.6×10^{-11} and 2.7×10^{-9} mol/L, respectively), the B1 enzyme for carbofuran (4.5×10^{-9} mol/L), and both the B1 enzyme and the AChE from electric eel for carbaryl (1.6×10^{-7} mol/L)	(Valdés-Ramírez et al., 2008)
Imidacloprid and thiacloprid	QCM-MIM sensor	Celery juice	10 μM	(Bi and Yang, 2009)
Malathion	AChE on polypyrrole (PPy) and polyaniline (PANI) copolymer doped with MWCNTs	No real samples	1 ng/mL	(Wu et al., 2006)
Monocrotophos	Voltammetry QD AuNPs on GCAbsortion of AChE on CdTe-AuNPs-CM/GCE	Garlic samples	0.3 ng/mL	(Du et al., 2008)
Organophosphate pesticides	Amperometry 6 different AChE. Array system using screen printed electrodes	Vegetable extracts	Assay concentration range of 10^{-5} M to 10^{-9} M	(Crew et al., 2011)
Phosphorothionate pesticides	AChE after oxidation of analytes	Orange juice	Chlorpyrifos 5 μg/L	(Roepcke et al., 2010)

Acetylcholinesterase (AChE); Quartz crystal microbalance (QCM); Glutathione-S transferase (GST); Molecular imprinted monolayer (MIM); Polypyrrole (PPy); Polyaniline (PANI); Multiwalled carbon nanotubes (MWCNTs)

The antibodies were immobilized on the biosensor chip and binding of the studied aminoglycosides was measured directly. However, to obtain sufficiently high responses, highly purified antibodies and high immobilization levels were required. Although feasible, the direct format proved hard to optimize and the competitive inhibition format was found to be more flexible and robust. The same research group developed such types of assays for detecting simultaneously in milk, the five relevant aminoglycosides (gentamicin, dihydrostreptomycin, streptomycin, kanamycin, and neomycin) below their MRLs (Haasnoot et al., 2003a). Gentamicin, streptomycin, kanamycin, and neomycin derivatives were immobilized on the surface of a chip in four flow cells (serially connected), and a mixture of selected specific antibodies was used. After optimization, the limits of detection (LODs) were 15–60 ng/mL, which is far below the MRLs set for these compounds (100–1500 ng/mL). Ferguson et al. (2002) reported another immunosensor inhibition assay for the detection of streptomycin and dihydrostreptomycin residues in whole bovine milk, honey, porcine kidney, and porcine muscle. Rebe Raz et al. (2008) described a competitive immunoassay for gentamicin and neomycin developed in a microarray format and using SPR imaging. Its sensitivity was found to be in the range of the MRLs established for milk. The report describes the transfer of the assay from conventional SPR biosensors to the imaging microarray platform and compares the two methods.

Quinolones and (fluoro)quinolones are broad-spectrum antibiotics against both gram-negative and gram-positive pathogens. A specific immunosensor for flumequine in broiler serum and muscle was developed by Haasnoot et al. (2007). The assay offered simplified sample preparation and suitable measurement ranges (15–800 ng/mL in serum and 24–4000 ng/g in muscle). In another work, an immunoassay-directed identification of (fluoro)quinolone in chicken muscle by LC-electrospray ionization (ESI) quadrupole time-of-flight (TOF)-MS has been presented (Marchesini et al., 2007). A dual SPR-biosensor immunoassay was developed, coupling a multi-(fluoro)quinolone immunosensor for the detection of norfloxacin, ciprofloxacin, enrofloxacin, difloxacin, sarafloxacin, and flumequine. The assay allowed the detection of the studied analytes at levels below their MRLs, after a simple preparation of the chicken-muscle sample. The samples found to be noncompliant in the screen were concentrated and fractionated by gradient LC. The effluent was divided between two identical 96-well plates: one was re-screened with the dual SPR system to generate an immunogram, and the positions of the immunoactive wells were used in the second 96-well plate for identification by high-resolution LC-TOF-MS. The system could also be used to discover unknown chemicals of similar structure showing activity in the dual biosensor immunoassay. Further developments have led to on-line nanoscale coupling of an SPR-biosensor-based screening assay (a competitive immunoassay) for enrofloxacin and its main metabolite, ciprofloxacin, with nano-LC-ESI-TOF-MS for identification (Marchesini et al., 2008). Huet et al. (2008) developed an optical SPR-biosensor assay for several (fluoro)quinolones in egg, fish, and poultry meat. In this assay, the reference molecule norfloxacin was 0.1–100 μg/kg in fish and 0.1–10 μg/kg in egg or poultry meat. Cross-reactivity determinations showed that this SPR-based assay can detect 13 of the most widely used (fluoro)quinolones at levels below their established MRLs. This qualitative screening test was fully validated according to the European Decision 2002/657/EC (Huet et al., 2008).

β-Lactams are a wide group of antibiotics, the most important being penicillins and cephalosporins. This group of antibiotics is the most frequently used in veterinary medicine to treat bacterial infections in dairy cows. Gustavsson et al. (2002a,b) developed different biosensors using a novel approach to detect penicillin G

in milk. This approach was based on the use of a β-lactam receptor, a carboxypeptidase. One assay format used a receptor protein as the detection molecule. The other two exploited the enzymatic activity of the β-lactam receptor, one assay measuring the amount of remaining enzyme substrate (tri-peptide assay) and the other detecting the amount of enzymatic product formed (di-peptide assay). Basically, the carboxypeptidase hydrolyzes a tri-peptide into a di-peptide, and this reaction is inhibited by β-lactams. After incubation of milk with the β-lactam receptor protein, antibodies directed against the tri- or di-peptide are added and the sample is injected over the sensor chip surface bearing immobilized tri-peptide or di-peptide. Antibody that is not inhibited by free peptide in the sample can bind to the surface. Assays where penicillin G was replaced with other commonly used β-lactams allowed detection of the active form of the β-lactam ring structure, as specified by European legislation. Several β-lactams, at or below their respective MRLs, were detected in milk (i.e., penicillin G, amoxicillin, ampicillin, oxacillin, cefalexin, cephapirin, and ceftiofur), and cloxacillin was detected at 60 ng/g (2 × MRL) in the tri-peptide assay but not detected in the di-peptide assay (Gustavsson et al., 2004). The performances of the three biosensor assays were compared with those of various commercial screening tests (i.e., a microbial inhibition test, an immunoassay, a receptor assay, and a receptor/enzymatic assay). Good agreement was found for 195 analyzed milk samples (Gustavsson and Sternesjo, 2004). In another study, an SPR-based biosensor was developed for detecting residues of penicillins and cephalosporins in milk. The assay was based on inhibition of the binding of digoxigenin-labeled ampicillin (DIG-AMPI) to the penicillin-binding protein 2× (PBP 2×), a soluble derivative of *Streptococcus pneumoniae* method involves the incubation the samples with PBP 2× to allow its binding to any β-lactams present, adding DIG-AMPI to interact with the remaining free PBP 2×, and then injecting the solution into the instrument. The DIG-AMPI/PBP 2×-complexes formed are captured by an anti-digoxigenin antibody immobilized on the sensor surface. If the sample contains β-lactams, fewer DIG-AMPI/PBP 2× complexes are formed and fewer bind to the sensor surface, and the response generated is lower because of the difference in molecular mass between DIG-AMPI/PBP 2× complexes and free DIG-AMPI. Four penicillins (penicillin G, ampicillin, amoxicillin, and cloxacillin) and two cephalosporins (cefalexin and cefoperazone) were detected in spiked raw milk samples at concentrations below their respective MRLs, but the authors observed that nonspecific binding of matrix components to the sensor chip affected the performance of the assay (Cacciatore et al., 2004). A hybrid biosensor combining classical microbial screening for antibacterial with electrochemical detection and reading was recently used for a novel application (i.e., the detection of β-lactam residues in milk). In this system, *Bacillus stearothermophilus* var. *calidolactis* is used as a test microorganism and the quantity of CO_2 produced is measured electrochemically and depends on how well the microorganism grows. The presence of microbial inhibitors in the milk sample inhibits growth of the test strain and thus decreases the CO_2 production rate. This variation is recorded with respect to a control milk sample (Ferrini et al., 2008).

Macrolides are widely used in veterinary medicine to treat bacterial diseases (e.g., enteric infections). Caldow et al. (2005) developed an SPR-biosensor assay for tylosin in honey. The specificity of the screening procedure was determined against some structurally related compounds and against compounds that may be administered concurrently. Tilmicosin, erythromycin, bacitracin, and lincomycin showed negligible cross-reactivity. Spiramycin produced a measurable response on the biosensor (cross-reactivity of about 60%). In a reported cell-based

microbial biosensor, natural or synthetic macrolides elicit luminescence by promoting the expression of *lux* genes (i.e., the synthesis of luciferase). The test does not depend on the biological activity of the macrolides present. The feasibility of using this system to detect natural products was demonstrated on the basis of its ability to detect pikromycin isolated from a *Streptomyces* species.

Tetracyclines form another usual antibiotic group for veterinary medicine. An ASPR biosensor was developed for tetracyclines, in which the sensor chip was coated with streptavidin linked to a biotinylated DNA fragment corresponding to the tetracycline-responsive operator (tetracycline operator and TetO). At the beginning of each measuring cycle, the tetracycline repressor protein (tetracycline repressor and TetR) is injected over the surface and the TetO−TetR complex is formed. If tetracycline is present in the sample, it will bind to TetR, inducing a conformational change accompanied by a reduction of the affinity constant of TetR−TetO binding. If this happens, the signal decreases proportionally to the tetracycline concentration in the sample. The assay was found to detect seven of the most commonly used tetracyclines in raw milk and honey samples. The estimated LODs were 15 μg/L for raw milk and 25 μg/kg for honey (Möller et al., 2007).

Sulfonamides constitute a large group of synthetic antibacterial compounds widely used in farm-animal feeds and fish cultures as veterinary drugs for prophylactic and therapeutic purposes. They also act as growth-promoting substances. Early publications describing the biosensor detection of sulfonamides were only concerned with two residues (i.e., sulfadiazine and sulfamethazine) (Crooks et al., 1998; Gaudin and Pavy, 1999; Situ et al., 2002). The group of Haasnoot developed a rapid biosensor immunoassay for detecting eight sulfonamides in chicken serum. They used a monoclonal antibody exhibiting 50−149% cross-reactivity toward eight sulfonamides and lower cross-reactivity toward six others (Haasnoot et al., 2003a,b). This same antibody was used in a study on chicken serum, where its performance was compared with those of a sulfonamide-binding protein and a mutant antibody (M.3.4). The M.3.4 antibody proved to be the most sensitive toward most of the sulfonamides tested (Bienenmann-Ploum et al., 2005). Another SPR-based assay described can detect at least 19 sulfonamides in porcine muscle (McGrath et al., 2005). According to the researchers, the risk of false positives is reduced because the method does not recognize the acetylated metabolites of the drugs. Another publication (Gaudin et al., 2007) describes an SPR method, fully validated according to the European Decision 2002/657/EC, for detecting eight sulfonamides in milk and porcine muscle. An electrochemical immunosensing technique based on magnetic sensors has also been used for the detection of several sulfonamides in milk. Advantages of using magnetic beads include elimination of the matrix effect and reduced nonspecific adsorption (Zacco et al., 2007). The recently developed wavelength-interrogated optical sensor (WIOS), which comprises a grating and a waveguide, and measures refractive-index changes near the surface of the wave guiding layer, has been used by Adrian et al. (2009a) to detect several sulfonamide residues in milk (Adrian et al., 2009b). With this system, it is possible to distinguish milk samples contaminated with sulfonamides at or above the established MRL (100 ng/mL). The system is suitable for automated on-site measurements, but further research is needed to allow future use by unskilled personnel.

Fenicols is a family of broad-spectrum antibiotics including thiamphenicol, florfenicol, and chloramphenicol. Chloramphenicol and chloramphenicol glucuronide residues in various food matrices were detected by Ashwin et al. (2005). They used the commercial SPR platform Biacore Q and direct detection format. They detected chloramphenicol in extracts from

honey, prawns, and dairy products and chloramphenicol glucuronide in extracts of porcine kidney at concentrations below 0.2 μg/kg. Detection of chloramphenicol and chloramphenicol glucuronide using an SPR sensor and inhibition assay was performed by Ferguson et al. (2005). They used the commercial SPR sensor Biacore Q and a chip with immobilized chloramphenicol derivative (Qflex Kit Chloramphenicol, Biacore). A known concentration of drug-specific antibody was mixed with the sample and injected over the surface of a sensor chip on which a chloramphenicol derivative was immobilized. Chloramphenicol and chloramphenicol glucuronide in extracts from food matrices were detected at levels down to 0.005 μg/kg (poultry), 0.02 μg/kg (honey), 0.04 μg/kg (prawn), and 0.04 μg/kg (milk). Dumont et al. (2006) demonstrated an SPR sensor for the detection of fenicol antibiotic residues in shrimps. They used the commercial SPR sensor Biacore Q and inhibition detection format. Analyte molecules were immobilized on carboxymethylated dextran using amine coupling chemistry. Chloramphenicol, florefenicol, florefenicol amine, and thiamphenicol were detected in extracts from shrimps at levels down to 1, 0.2, 250, and 0.5 ng/mL, respectively.

β-Agonists are β_2-adrenoceptor agonists, which have been used in veterinary medicine as broncodilators and agents of uterine relaxation. However, they have become better known as illegal drugs used as growth promoters in farm animals. A rapid and sensitive optical biosensor assay was developed to detect clenbuterol residues in bovine urine. The method involved a simple extraction procedure using tert-butyl methyl ether followed by analysis on the biosensor with results obtained against a buffer calibration curve. The limit of detection was determined as 0.27 ng/mL using 20 EU reference blank urine samples. The antibody used in the biosensor test exhibited high cross-reactivity with at least seven other β-agonists allowing detection of these compounds at less than 1 ng/mL in bovine urine (Haughey et al., 2001). A posterior work by Traynor et al. (2003) developed an SPR immunosensor to detect β-agonists in liver tissues. The assay requires a long sample preparation including a photolytic digestion of tissues and posterior purification with solid phase extraction. The sensor surface was regenerated using 0.1 M NaOH. The assay was able to detect mabuterol down to 0.02 ng/g, clenbuterol at 0.11 ng/g, and salbutamol at 0.19 ng/g. A wide range of other β-agonists were also detected at concentrations below 1.5 ng/g. Wang et al. (2009) demonstrated the feasibility of detecting clenbuterol residue in pig urine using CdSe/CdS QDs as fluorescent label based magnetic core/shell Fe_3O_4/Au NPs as solid carriers. The detection of clenbuterol is carried out by a fluoroimmunoassay-based biosensor using competitive binding between conjugated clenbuterol antigen-CdSe/CdS QDs and free clenbuterol with immobilized clenbuterol antibodies on magnetic core/shell Fe_3O_4/Au NPs. This assay method allowed the clenbuterol determination in a linear working range of 0.5−20,000 pg/mL.

Biosensors for veterinary drug residues and growth promoters in food are summarized in Table 7.2.

7.4.3. Pathogenic Bacteria and Natural Toxins

Food-borne pathogens are a growing concern for human illness and death. There is increasing demand to ensure safe food supply. There is continuous development of methods for the rapid and reliable detection of food-borne pathogens. Improvements in the field of immunology, molecular biology, automation, and computer technology have a positive effect on the development of faster, more sensitive, and more convenient methods in food microbiology. Further, development of on-line methods is important for rapid monitoring of food safety.

TABLE 7.2 Biosensors for Drug Residue and Growth Promoter Residue Detection

Analyte	Matrix	Biosensor type	Limit of detection	Reference
ANTIBACTERIALS (AMINOGLYCOSIDES)				
Gentamicin, dihydrostreptomycin, streptomycin, kanamycin, and neomycin	Milk	SPR-Immunosensor	Lower than MRLs	(Haasnoot et al., 2003a,b)
Neomycin, gentamicin, kanamycin, and streptomycin	Milk	Multiplexed detection imaging surface plasmon resonance (iSPR) − Immunosensor	10 ng/L in buffer and in 10×-diluted milk	(Raz et al., 2009)
ANTIBACTERIALS (FLUORO(QUINOLONES))				
Enrofloxacin	Chicken muscle	SPR-MS		(Marchesini et al., 2008)
Enrofloxacin	Milk	A DNA-based SPR for enrofloxacin was developed	3 μg/mL	(Cao et al., 2007)
Flumequine	Broiler serum and muscle	BIA immunosensor	200 ng/g	(Haasnoot et al., 2007)
Fluoroquinolones	Milk	Evanescent waveguide optical biosensors	Lower than MRL	(Adrian et al., 2009a,c)
Fluoroquinolones	Egg, fish, and poultry	SPR-Immunosensor	0.5, 1, and 1.5 ng g^{-1} for poultry meat, egg, and fish, respectively	(Huet et al., 2009)
Fluoroquinolones	Milk	Multiplexed detection imaging surface plasmon resonance (iSPR) − Immunosensor	Lower than MRLs	(Raz et al., 2009)
ANTIBACTERIALS (β-LACTAMS)				
β-lactams	Milk	Hybrid biosensor. Measurements of CO_2 production by *Bacillus stearothermophilus* var. Calidolactis growth	Detection	(Ferrini et al., 2008)
β-lactams	Milk	SPR immunosensor	2 μg/kg	(Gustavsson and Sternesjo, 2004)
β-lactams	Milk	SPR	1.2 μg/L	(Sternesjo and Gustavsson, 2006)

(*Continued*)

TABLE 7.2 Biosensors for Drug Residue and Growth Promoter Residue Detection (*Cont'd*)

Analyte	Matrix	Biosensor type	Limit of detection	Reference
ANTIBACTERIALS (SULFONAMIDES)				
Sulfonamides	Milk	WIOS immunosensor	0.5 μg/L	(Adrian et al., 2009a,c)
8 Sulfonamides	Chicken serum	SPR	Between 7 and 20 ng/mL	(Haasnoot et al., 2003b)
3 Sulfonamides	Fish	Immunoassay	Lower than MRLs	(Chafer-Pericas et al., 2010)
Sulfonamides	Milk and porcine muscle	SPR Immunoassay	40 μg/L in milk and 60 μg/kg in porcine, bovine, and poultry muscles	(Gaudin et al., 2007)
FENICOLS				
Thiamphenicol, florefenicol, florefenicol amine, and chloramphenicol	Shrimps	Hybrid biosensor. Measurements of CO_2 production by *Bacillus stearothermophilus* var. Calidolactis growth	0.2–250 μg/kg	(Dumont et al., 2006)
Chloramphicol and its glucuronide	Prawn, honey, dairy products and porcine kidney	SPR immunosensor	0.1 μg/kg	(Ashwin et al., 2005)
Chloramphicol and its glucuronide	poultry muscle, honey, prawn and cows' milk	SPR immunosensor	0.005–0.04 μg/kg	(Ferguson et al., 2005)
β-AGONISTS				
Clenbuterol	Bovine urine	Immunosensor	0.27 ng/mL	(Haughey et al., 2001)
13 β-agonists	Liver tissues	SPR immunosensor	Mabuterol: 0.02 ng/g Clenbuterol: 0.11 ng/g Salbutamol: 0.19 ng/g Other 1.5 ng/g	(Traynor et al., 2003)
Clenbuterol	Pig urine	Fluoroinmmunoassay biosensor	0.5–20000 pg/mL	(Wang et al., 2009)

One of the most challenging problems is sample preparation. More research is needed on techniques for separating microorganisms from the food matrix and for concentrating them before detection.

In addition, rapid detection of live bacterial pathogens is important in monitoring food safety and water. Selective plating and culturing is the current standard due to its high selectivity and sensitivity. However, the time-to-results depends strongly on the growth rate of the pathogen. For the slow growers, confirmation of positive detection could take as long as 16 days. PCR in combination with the plating method can reduce time-to-results considerably. However, significant time is still needed to enrich and grow the target microorganism, especially when it is present at low concentrations. PCR techniques without an enriching step do not distinguish between viable and nonviable cells because DNA is a stable molecule and is present in both dead and live cells. Similarly, antibody-based biosensors suffer from the deficiency of not distinguishing viable from nonviable cells. Since only the viable cells are virulent, it is important to discern them in a sample. In situations where time-to-results is long, timely corrective decisions cannot be made.

During the last decade, much effort has been expended to develop rapid and robust biosensors requiring minimal sample preparation and good enrichment detection of food-borne pathogens.

Electrochemical, optical (SPR), and piezoelectric-based immunosensors are the most common approaches used to detect microorganisms in food and water (Ricci et al., 2007). In this section, more recent approaches for common food pathogens are revised and discussed.

Escherichia coli has a notorious reputation for causing food poisoning. It mainly contaminates poultry, vegetable, and dairy products, which constitute a large fragment of staple (stable) diets.

Gfeller et al. (2005) presented an oscillating cantilever for the detection of active *E. coli* in less than 1 h. Detection is through the measurement of the change of resonance frequency of the cantilever array, which is a result of an increase in mass caused by adsorption of the pathogen on the cantilever. The reference cantilever was used to exclude any undesired environmental changes. By altering the nutritive layer and gas phase in which the detection takes place, the use of the sensor can be extended for the detection of different microorganisms. A highly sensitive electrochemical immunoassay has been described by Zhang et al. (2009), for the rapid detection of *E. coli* in surface water. Anodic stripping voltammetry based on Cu@Au NPs as antibody labels was used for the detection of *E. coli*. The Cu@Au bimetallic NPs offer high stability, good biocompatibility, and fine voltammetric activity for anti-*E. coli* antibody. The assay has a sensitivity to detect 30 cfu/mL which can further be increased to 3 cfu/mL by incorporating a pre-enrichment step where the sample is passed through a 0.45 μm pore-size filter (Zhang et al., 2009). An electrochemical biosensor based on a thermostable reporter enzyme, esterase 2 (EST2) from *Alicyclobacillus acidocaldarius*, was used for specific detection of bacteria by one-step rRNA/DNA hybridization between a bacterium-specific capture oligodeoxynucleotide (ODN), bacterial 16S rRNA, and a uniform EST2-ODN reporter conjugate. The detection limit was 500 colony forming units (cfu) *E. coli*. Besides high sensitivity, the application of electrochemical biochips allows discrimination of two gram-negative and two gram-positive bacteria demonstrating the specificity and the potential for parallel detection of microorganisms. The feasibility of identification of food-borne bacteria was studied with meat juice contaminated with *E. coli* (Pöhlmann et al., 2009).

Functional mannose SAMs in combination with lectin concanavalin A (Con A) were also used as molecular recognition elements for the

detection of *E. coli* W1485 using a QCM as a transducer. The multivalent binding of Con A to the *E. coli* surface O-antigen favors the strong adhesion of *E. coli* to the mannose-modified QCM surface by forming bridges between these two. As a result, the contact area between cell and QCM surface that increases leads to rigid and strong attachment. Therefore, it enhances the binding between *E. coli* and the mannose deprecating in an improvement of the sensitivity and specificity of the biosensor with an experimental detection limit of a few hundred bacterial cells (Shen et al., 2007). A magnetostrictive microcantilever (MSMC), as a high-performance biosensor platform, was introduced recently. By using physical absorption, an antibody against *E. coli* immobilized onto the surface of the MSMC to form a biosensor. The real-time and in situ detection of *E. coli* in water was reported. The detection limit was 10^5 cfu/mL (Fu et al., 2010). In a recent study, a mass-change sensitive cantilever biosensor and a probe, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), was reported to determine the cell viability in a short time. A poly-L-lysine coated sensor immobilized with live *Escherichia coli* JM101 (a surrogate for a pathogenic target) at various concentrations was exposed to BCECF-AM in a flow arrangement. A log−linear relationship between the sensor surface cell concentration and frequency response was obtained in the range of 1000−4000 cells/mm^2 and as low as ~2000 viable *E. coli* cells were rapidly detected in less than 1 h (Xu and Mutharasan, 2011).

Another deadly strain of *E. coli* responsible for causing global disease outbreaks is ***E. coli* O157:H7**. Only a few hundred cells are sufficient to cause the infection and hence very sensitive methods are required for the detection. Poitras and Tufenkji (2009) have developed a biosensor based on QCM with dissipation monitoring. The biological recognition element used was polyclonal antibodies immobilized on the gold-coated quartz crystal using a SAM of cysteamine. The biosensor was found to have a wide detection range from 3×10^5 to 1×10^9 cells/mL. Another study presented the sensitivity and specificity of a polyethylene glycol-terminated alkanethiol mixed SAM on SPR immunosensor to detect *E. coli* O157:H7. This approach was presented by Subramanian et al. (2006). Purified monoclonal or polyclonal antibodies against *E. coli* O157:H7 were immobilized on an activated sensor chip and direct and sandwich assays were carried out to detect *E. coli* O157:H7. The effect of protein G-based detection and of concentrations of primary and secondary antibodies in sandwich assays were investigated. The sensor could detect as low as 10^3 cfu/mL of *E. coli* O157:H7 in a sandwich assay, with high specificity against *Salmonella enteritidis*. The detection limits using direct assay and protein G were 10^6 cfu/mL and 10^4 cfu/mL, respectively (Subramanian et al., 2006). Another example presented by Huang et al. (2011) was a biosensor based on long-range surface plasmon-enhanced fluorescence spectroscopy (LRSP-FS). The resonant excitation of LRSP modes provides an enhanced intensity of the electromagnetic field, which is directly translated to increased strength of the fluorescence signal measured upon the capture of target analyte at the sensor surface. LRSPs originate from a coupling of surface plasmons across a thin metallic film embedded in dielectrics with similar refractive indices. With respect to regular surface plasmon-enhanced fluorescence spectroscopy, the excitation of LRSPs offers the advantage of a larger enhancement of the evanescent field intensity and a micrometer probing depth that is comparable to the size of target bacterial pathogens. The potential of the developed sensor platform is demonstrated in an experiment in which the detection of *E. coli* O157:H7 was carried out using sandwich immunoassays. The limit of detection was below 10 cfu/mL and detection time lasted 40 min (Huang et al., 2011).

In a different study, *E. coli* O157:H7 cells were isolated via immunomagnetic separation (IMS) and labeled with biofunctionalized electroactive polyaniline (immuno-PANI). Labeled cell complexes are deposited onto a disposable screen-printed carbon electrode (SPCE) sensor and pulled to the electrode surface by an external magnetic field, to amplify the electrochemical signal generated by the polyaniline. Cyclic voltammetry was used to detect polyaniline and signal magnitude indicates the presence or absence of *E. coli* O157:H7. As few as 7 cfu of *E. coli* O157:H7 (corresponding to an original concentration of 70 cfu/mL) were successfully detected on the SPCE sensor. The assay requires 70 min from sampling to detection, giving it a major advantage over standard culture methods in applications requiring high-throughput screening of samples and rapid results (Setterington and Alocilja, 2011).

***Salmonella* serovars** are associated with 26% of all food-borne diarrhea that leads to hospitalization (Joshi et al., 2009). The detection of *Salmonella* has been a major focus of impedance microbiology. The impedance microbiological methods are perhaps the most successful of all the recently introduced rapid methods in automation. Several commercial analytical instruments are based on the principles of classic impedance microbiology. These systems include Bactometer (Bio Merieux, Nuertingen, Germany), the Malthus systems (Malthus Instruments Ltd., Crawley, UK), rapid automated bacterial impedance technique (RABIT) (Don Whitley Scientific Ltd., Shipley, UK), and Bac-Trac (Sy-Lab, Purkersdorf, Austria).

The development of practical biosensors using nanomaterials is promising in eliminating the need for expensive or complicated instruments and allowing the rapid detection of food-borne pathogens on a portable or handheld device. The detection of pathogens can be improved in conventional pathogenic biosensors by using immuno-NPs. For example, the sensitivity of the impedimetric biosensor for *S. enteritidis* cells was improved from 10^6 to 10^4 cfu/mL at 100 Hz of input frequency by incorporating anti-*Salmonella* antibody-conjugated NPs (Kim et al., 2007).

SPR biosensor technology in food analysis continues to increase the number of publications on detection of pathogens such as *Salmonella*. *Salmonella enteritidis* was detected using an SPR sensor with wavelength modulation by Koubova et al. (2001). In that work, a double layer of antibodies was physically sorbed on a bare gold surface and cross-linked with gluteraldehyde. Direct detection of heat-killed, ethanol-soaked *S. enteritidis* at a concentration as low as 10^6 cfu/mL was demonstrated. Bokken et al. detected *Salmonella* groups A, B, D, and E using the commercial SPR sensor Biacore 3000 (Bokken et al., 2003). Antibodies were immobilized in a carboxymethylated dextran layer via amine coupling chemistry, and detection of *Salmonella* serotypes was performed using the sandwich format. *Salmonella* serotypes were detectable at a concentration of 1.7×10^5 cfu/mL even in the presence of other bacteria at 10^8 cfu/mL levels. *Salmonella typhimurium* was detected using the commercial SPR sensor and monoclonal antibodies immobilized via protein G attached to alkanethiolate SAMs on the sensor surface. The LOD was 10^2 cfu/mL (Oh et al., 2004). The detection of *Salmonella paratyphi* was achieved by the same group (Oh et al., 2004) using the SPR instrument Multiskops and a similar method for the attachment of monoclonal antibodies via protein G. Detection of *S. paratyphi* was shown down to concentrations of 10^2 cfu/mL (Oh et al., 2004). Taylor et al. reported SPR-based detection of *Salmonella choleraesuis* serotype typhimurium in apple juice using a custom-built multichannel SPR sensor with wavelength modulation and sandwich detection format (Taylor et al., 2006). Biotinylated polyclonal antibodies against *Salmonella* were immobilized via streptavidin attached to a mixed SAM of oligo (ethylene glycol) alkanethiolate and biotinylated

alkanethiolate. The LOD for *S. choleraesuis* was 4.4×10^4 cfu/mL in buffer and about 10^4 cfu/mL in apple juice with an adjusted pH of 7.4 (Taylor et al., 2006). Waswa et al. detected *S. enterica* serovar Enteritidis in milk using the commercial SPR sensor Biacore 2000 (Waswa et al., 2006). In this work, the antibody was immobilized by first attaching protein A using a carboxymethylated dextran layer and amine coupling chemistry and subsequent attachment of the antibody to protein A (Waswa et al., 2006). The LOD for *Salmonella* in pasteurized milk was determined to be 23 cfu/mL (Waswa et al., 2006). Mazumdar et al. also reported the detection of *Salmonella* in milk (Mazumdar et al., 2007). They used the commercial SPR sensor plasmonic and sandwich detection format. Polyclonal capture antibody was immobilized by self-assembly on the hydrophobic-sensing surface formed by alkylsilanes. Milk spiked with *S. typhimurium* cells, killed by thimerosal (1%, w/w), was incubated with the sensing surface for 15 min and then switched with a solution containing the second antibody. The LOD for *S. typhimurium* cells in milk was at 10^5 cells/mL (Mazumdar et al., 2007). In another configuration, colloidal gold NPs (AuNPs) were directly assembled onto a surface of SPR Au chip via 2-aminoethanethiol for the enhancement of sensitivity as a label-free detection system. A novel fusion protein was constructed by genetically fusing gold-binding polypeptides (GBP) to protein A as a cross-linker for effective immobilization of antibodies. The resulting GBP−protein A protein was directly self-immobilized onto both bare and AuNPs-assembled SPR chip surfaces via the GBP portion, followed by the oriented binding of human immunoglobulin G (hIgG) onto the protein A domain targeting the Fc region of antibodies and anti-hIgG in series. Furthermore, anti-*Salmonella* antibodies were immobilized onto both GBP−protein A layered chips for detection of *Salmonella typhimurium*. SPR analyses indicated the signal increases for successive binding of hIgG and anti-hIgG onto the GBP−protein A layered AuNPs-assembled chip were higher (about 92 and 30%, respectively) than that onto the identically treated bare chip. This signal enhancement in the AuNPs-assembled chip also caused a 10-fold increased sensitivity in detection of *S. typhimurium* compared to the bare one. These results demonstrate that the direct assembly of AuNPs onto an SPR chip could enhance the signal in biomolecular interaction events, and the GBP−protein A protein could be a valuable cross-linker for simple and oriented immobilization of antibodies onto Au chip surfaces without any surface chemical modification (Ko et al., 2009). Mazumdar et al. (2010) reported an SPR-based sandwich immunoassay for serotyping of *Salmonella*. The *Salmonella* were captured on an SPR chip using polyclonal capture antibody. SPR sensorgrams were obtained for the immunoreactions of the somatic (O) and flagellar (H) surface antigens, of the captured bacteria, to their respective antibodies. The sensorgram data were compiled to determine the antigenic formula in accordance with the Kauffmann-White scheme. *Salmonella enteritidis* was completely serotyped using this SPR-based method. In addition, *Salmonella* belonging to serogroups B, C, and D were successfully assigned to their respective serogroups. Before serotyping, the bacteria are grown to a concentration of 1×10^{10} m/L. This SPR-based serotyping provides quantitative data and, thus, eliminates the possibility of false detections as encountered in the conventional slide agglutination test (SAT). This method was also proved to work with rough strains (Mazumdar et al., 2010). Recently, Zordan et al. (2011) reported a hybrid optical device that has the capability to perform real-time SPR and epi-fluorescence imaging. The design of a microfluidic biochip consisted of a two-dimensional array of functionalized gold spots. The spots on the array were functionalized with capture peptides that specifically bind *E. coli* O157:H7 or *Salmonella enterica*. This array

was enclosed by a PDMS microfluidic flow cell. A magnetically pre-concentrated sample is injected into the biochip, and whole pathogens will bind to the capture array. This optical device was used to detect the presence and identity of captured pathogens using SPR imaging. In this configuration, the detection occurs in a label-free manner, and does not require the culture of bacterial samples. Molecular imaging can also be performed using the epi-fluorescence capabilities of the device to determine pathogen state, or to validate the identity of the captured pathogens using fluorescently labeled antibodies. Real-time screening of a sample for the presence of *E. coli* O157:H7 and *Salmonella enterica* was demonstrated. Additionally, the mechanical properties of the microfluidic flow cell were assessed (Zordan et al., 2011).

A biosensor transducer composed by Au NPs and magnetic NPs has been used to detect *Salmonella enterica* serovar *Enteritidis*, which is one of the most frequently reported causes of food-borne illness. A highly amplified bio-barcode DNA assay for the rapid detection of the insertion element gene of *Salmonella enteritidis* was reported. The Au-NPs were coated with the target-specific DNA probe, which can recognize the target gene, and fluorescein-labeled barcode DNA in a 1:100 probe-to-barcode ratio. The magnetic NPs were coated with the 2nd target-specific DNA probe. After mixing the NPs with the 1st target DNA, the sandwich structure was formed. A magnetic field is applied to separate the sandwich from the unreacted materials. Then the bio-barcode DNA is released from the Au-NPs. The released barcode DNA is measured by fluorescence (Zhang et al., 2009).

Campylobacter jejuni is a species of curved, helical shaped, nonspore forming, gram-negative microaerophilic bacteria, commonly found in animal feces. It is one of the most common causes of human gastroenteritis in the world. Wei et al. (2007) presented the development of an SPR biosensor for the rapid identification of *C. jejuni* in broiler samples. Antigen–antibody interactions were studied using enzyme-linked immunosorbent assay (ELISA) and a commercially available SPR biosensor platform (Spreeta™). The SPR biosensor showed a good sensitivity with commercial antibodies against *C. jejuni* at 10^3 cfu/mL and a low cross-reactivity with *Salmonella* serotype typhimurium. The sensitivity of the SPR was similar when testing spiked broiler meat samples. However, research is still needed to reduce the high background observed when sampling meat products (Wei et al., 2007). A flow-injection assay of the pathogenic enterobacteria using novel lectin-based QCM biosensor has been proposed by Sarafina et al. (2008). The biosensing part of the analytical device contained the lectins immobilized on the gold surface of a quartz crystal electrode, which served as a transducer. The immobilization of lectins was carried out using amine coupling on the surface of the crystal modified with 11-mercaproundecanoic acid. The biosensor makes it possible to identify the presence of different bacteria using the lectins immobilized on the surface of QCM crystal, which bind specifically to the certain oligosaccharides present on the cell wall of the bacteria injected. The proposed biosensor is able to detect 10^3 cells. The flow-injection assay of the bacterial cells takes about 30 min.

Listeria monocytogenes is the causative agent of listeriosis and one of the most virulent food-borne pathogens. Twenty percent of clinical infections result in death. In the United States, it is responsible for approximately 2500 illnesses, of which 500 die annually. Different systems have been developed for the detection of *Listeria*. A biosensor for the detection of *Listeria* in milk has been described using a QCM displacement assay (Minunni et al., 1996). The antibody specific for binding *Listeria* was immobilized on the gold coating of the quartz crystal plate using different methods and the antibody–antigen binding was monitored real-time using a liquid

flow cell. The detection range was from 2.5×10^5 to 2.5×10^7 cells/crystal with a detection time of 15 min (Minunni et al., 1996). Banada et al. (Banada et al., 2009) used light scattering sensors for the detection of target bacteria viz. *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* in vegetable and meat samples spiked with these bacteria. The forward scattering was able to detect the presence of contaminants accurately based on the distinct colony/scatter signature. The detection limit of this system was a single cell per 25 g portion of test specimen. The method was able to recognize colonies of target bacteria in the presence of natural background microflora in clinical specimens (Banada et al., 2009).

Natural toxins. Natural toxins are chemicals that are naturally produced by living organisms. These toxins are not harmful to the organisms themselves but they may be toxic to other creatures, including humans, when eaten.

Some plants have the capacity to naturally produce compounds that are toxic to humans when consumed. For example, under certain conditions, different types of algae can produce compounds that are toxic to humans but not to shellfish that eat this algae. Also, micotoxins occurring in food commodities are secondary metabolites of a range of filamentous fungi, which can contaminate food or food crops throughout the food chain. A limited number are considered to play an important part in food safety and for these a range of analytical methods have been developed. Fungal toxins of concern are generally produced by species within the genera *Fusarium*, *Aspergillus*, and *Penicillium*, which frequently occur in crops, in the field, or during storage of major food agricultural crops, including cereals, groundnuts, and various fruits. Besides the deleterious effect of the fungi themselves on agricultural productivity, the fungal toxins have a range of detrimental health effects in humans, including carcinogenesis, immune suppression, teratogenicity, and growth retardation. Similarly, mycotoxin-contaminated animal feeds can lead to animal toxicoses and the possible carry-over of mycotoxins or their metabolites into the human food chain.

Electrochemiluminescence biosensors have been developed for the detection of biotoxins such as staphylococcal enterotoxin B (Bruno and Kiel, 2002) and for *E. coli* mRNA (Baeumner et al., 2003). Nanotechnology has contributed significantly to the field of colorimetric biosensors.

The conjugation of nucleic acids with NPs has led to highly sensitive and selective biosensors. Whole cells such as *Bacillus thuringiensis* (Ikanovic et al., 2007) have been recognized via optical transduction. Ikanovic et al. (2007) used the zinc sulfide capped, cadmium selenide QD for the detection of *Bacillus* species at 655 nm and had an advantage over organic fluorophores in the range of wavelengths that may be employed for excitation and a narrow emission spectrum. Another advantage was that no photobleaching of the QD was observed as it does with organic fluorophores. This method developed for binding of aptamer-QD to the *Bacillus thuringiensis* spores can be applied to a wide range of harmful biological agents, such as *Bacillus anthracis*. Kalogianni et al. (2006) reported the first DNA biosensor in a dry-reagent dipstick configuration for visual detection and confirmation of genetically modified organism-related sequences by hybridization within minutes. The sensor is disposable and does not require special instrumentation. The target sequences are amplified by the PCR and hybridized with probes bearing an oligo(dA) tail. The biotinylated product is applied to the sensor, followed by immersion in the appropriate buffer. Migration of the buffer rehydrates gold NPs conjugated to oligo(dT) strands, which hybridize with the oligo(dA) tails. The hybrids are captured by immobilized streptavidin at the test zone of the sensor, giving a characteristic red line due to the accumulation of the NPs. The sensor was applied to

real samples from various sources. The botulinum neurotoxin was detected using a biosensor based on voltammetry (Wei and Ho, 2009).

A sensor for the lethal bacterial enzyme, botulinum neurotoxin type A (BoNT/A), SAMs was reported. SAMs consisting of an immobilized synthetic peptide that mimicked the toxin's in vivo SNAP-25 protein substrate were formed on Au and interfaced with arrayed microfluidic channels. Channel design provided facile fluid manipulation, sample incubation, analyte concentration, and fluorescence detection all within a single microfluidic channel, thus avoiding sample transfer and loss. Fluorescence detection was achieved down to 20 pg/mL ALC and 3 pg/mL BoNT/A in 3 h. Toxin sensing was also accomplished in vegetable soup, demonstrating practicality of the method. The modular design of this microfluidic SAM platform allows for extension to sensing other toxins that operate via enzymatic cleavage, such as the remaining BoNT serotypes B–G, anthrax, and tetanus toxin (Frisk et al., 2009). Several SPR immunosensors (Shankaran et al., 2007) have been also developed for the detection of staphylococcal enterotoxins (Nedelkov et al., 2003; Medina, 2005), domoic acid (Stevens et al., 2007; Gobi et al., 2007), and aflatoxin B1 (Shankaran et al., 2007).

Table 7.3 presents several examples of biosensor approaches for rapid pathogens and natural toxin detection in food.

Marine biotoxins are produced by natural marine phytoplankton. Marine algal toxins are responsible for more than 60,000 intoxications per year, with an overall mortality of about 1.5%. They can accumulate in aquatic animals intended for human consumption, like filter-feeding mollusks, and are thermo-resistant; thus, normal cooking, freezing, or smoking cannot destroy them.

The most common groups of marine biotoxins are diarrheic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurologic shellfish poisoning (NSP), azaspiracid shellfish poisoning (AZP), ciguatera fish poisoning (CFP), palitoxins, and spirolides.

The more classical approach to assess the presence of marine biotoxins in seafood is the in vivo mouse bioassay, based on the administration of suspicious extracted shellfish to mice, evaluation of the lethal dose, and toxicity calculation according to reference dose response curves, established with reference material. It provides an indication about the overall toxicity of the sample, as it is not able to differentiate among individual toxins. This is a laborious and time-consuming procedure; the accuracy is poor, it is nonspecific, and generally not acceptably robust. Moreover, the mouse bioassay suffers from ethical implications and it is in conflict with EU Directive 86/609 on the Protection of Laboratory Animals.

During recent years, numerous in vitro assays, instrumental approaches, and biosensors have been developed to obtain rapid and reliable alternatives (Marchesini et al., 2009; Vilariño et al., 2009, 2010; Huet et al., 2010). Substantial research in using immunosensors for marine-biotoxin analysis has been conducted with QCM, electrochemical and SPR biosensors, the latter appearing extremely promising (Campas et al., 2008; Ricci et al., 2007; Kantiani et al., 2010). A review of bioaffinity-detection systems summarizes the principles of these methodologies and other upcoming surface-based technologies (Wark et al., 2010).

SPR is widely recognized as being the leading technology for label-free toxin detection, and methods have now been developed and undergone single-laboratory validation for domoic acid, okadaic acid, DTXs, and PSP toxins, with results for naturally contaminated shellfish samples in good agreement with the current regulatory methods for these toxins (Traynor et al., 2006; Stewart et al., 2009a,b; Campbell

TABLE 7.3 Biosensors for Pathogen and Natural Toxin Detection

Microorganism	Nanomaterial	Recognition	Detection	Reference
Campylobacter jejuni	SAMs	Antigen−Antibody	SPR	(Wei et al., 2007)
Campylobacter jejuni	SAMs	Antigen−Antibody	QCM	(Safina et al., 2008)
E. coli O157:H7	SAMs of cysteamine	Antigen−Antibody	QCM	(Poitras and Tufenkji, 2009)
E. coli O157:H7	SAMs	Antigen−Antibody	SPR	(Subramanian et al., 2006)
E. coli O157:H7	SAMs	Antigen−Antibody	LRSP-FS)	(Huang et al., 2011)
E. coli O157:H7	Magnetic NPs	Antigen−Antibody	IMS+Plating	(Varshney et al., 2005)
E. coli O157:H7		Phage with luxI gene insert	Bioluminescence	(Ripp et al., 2008)
Escherichia coli	Gold nanowire array	Antigen−Antibody	Electrochemical impedance spectroscopy	(Basu et al., 2004)
Escherichia coli	Polymeric NPs	Adhesin receptor	TEM	(Edgar et al., 2006)
Escherichia coli	Cu@Au NPs	Antigen−Antibody	Anodic stripping voltammetry	(Zhang et al., 2009)
Escherichia coli		Enzyme esterase 2	Electrochemical	(Pöhlmann et al., 2009)
Escherichia coli		Antigen−Antibody	Amperometric	(Abu-Rabeah et al., 2009)
Escherichia coli		Antigen−Antibody	Magnetostrictive microcantilever	(Fu et al., 2010)
Escherichia coli		Lambda phage with luxI gene insert	Bioluminescence	(Birmele et al., 2008)
Escherichia coli		Lytic phage. Amine coupling of phages with carboxylic groups at a carbon surface	Impedimetric	(Shabani et al., 2008)
Escherichia coli		Lambda phage with a luxI based acyl homoserine lactone	Bioluminescence	(Ripp et al., 2006)
Listeria monocytogenes	SAMs	Antigen−Antibody	QCM	(Minunni et al., 1996)
Listeria monocytogenes		cFv phages with affinity for ActA (=a virulence factor that is expressed on the cell surface of *L. monocytogenes*)	SPR	(Nanduri et al., 2007)
Salmonella enteritidis		Antigen−Antibody	SPR with wavelength modulation	(Koubova et al., 2001)

TABLE 7.3 Biosensors for Pathogen and Natural Toxin Detection (*cont'd*)

Microorganism	Nanomaterial	Recognition	Detection	Reference
Salmonella enteritidis		Antigen–Antibody	Impedimetric sensor	(Kim et al., 2007)
Salmonella typhimurium	SAMs	Antigen–Antibody	SPR	(Oh et al., 2004)
Salmonella typhimurium	Colloidal Au-NPs	Antigen–Antibody	SPR	(Ko et al., 2009)
Salmonella typhimurium		Peptide displaying phage	QCM	(Olsen et al., 2006)
Salmonella typhimurium and *Bacillus anthracis*		Peptide displaying phage	Magnetoelastic	(Huang et al., 2009)

Self-Assembling Monolayers (SAMs); Nanoparticles (NPs); Surface Plasmon Resonance (SPR); Quanrt Crystal Microbalance (QCM); Long-Range Surface Plasmon-Enhanced Fluorescence Spectroscopy (LRSP-FS); Immunomagnetic Separation (IMS)

et al., 2010). An SPR method for TTX has also been developed but not yet validated (Taylor et al., 2008). One of the advantages of SPR is that it can be linked to MS, so that samples screened by SPR and found to contain toxin can be identified and quantified using MS (Marchesini et al., 2009).

A recent concern raised by monitoring laboratories is that in order to perform this task with immunological assays, four separate tests rather than one would be required, which would result in more time-consuming and costly analysis. Research programs are therefore now focusing on multiplex formats for achieving a single test for marine biotoxins.

7.5. COMMERCIAL INSTRUMENTATION AND FUTURE PERSPECTIVES

The continued provision of safe food, free from harmful microorganisms, toxins, chemical contaminants, and other hazardous substances that pose potential risks to human health, remains a huge challenge. In the context of food-safety analysis, it is important that analytical-technology development is moving toward high-throughput multiplexed platforms but yet retaining the high accuracy and specificity required for such analysis.

However, the current system offers valuable qualitative information only, rather than quantitative information, and is less sensitive than the standard flow-cell instrument. Another challenge in food analysis is the complexity of the food matrixes that have to be addressed not only at the sample preparation step but also at the biosensor architecture level, where biomolecular interactions take place.

Modification of surface chemistry and the development of fluidic and buffering systems may play important roles in addressing nonspecific binding problems associated with particular sample matrices. Clearly, instrumentation that can deliver on high-throughput analysis is desirable but it should also provide the high accuracy and specificity for analysis of concentration in complex food matrices.

A number of instruments for food analysis are already commercially available. However, the commercial success of biosensors is limited to a small number of applications, where the market size justified more research, validation, and development investment. These commercial devices are focused on few

applications, such as the determination of saccharides or the detection of bacterial toxins or pathogens.

There are several companies manufacturing SPR instruments for studying biomolecular interactions. Each company produces different SPR systems equipped with a variety of options usable for specific applications. Some of these companies are Biacore, Windsor Scientific, Quantech, Texas, NTT, and Moritex (formerly, Nippon Laser and Electronics). SPR instruments from Biacore have been widely used by sensor researchers around the world. However, other options have much less commercial success. The future commercial status and general acceptance of this technology will depend on the performance characteristics, sample throughput, associated costs, validation and acceptance by regulatory authorities. In addition, high cost has hindered the commercial development of biosensors developed by research laboratories and may have served as prototype models for industry. Incorporation of bioinformatic tools may become not only desirable but also a prerequisite of the system if it is to be suitable for multiplexing analysis.

Some of other important keys in the future of biosensor development is to allow for more stability of biological components; more robust assays; higher repeatability between different batches of production when disposable elements are involved, and the integration of new technologies coupled to biosensors, such as the PCR, and hyphenated approaches.

On the other hand, there are at least two other developments that are expected to have significant impact, the LOC and nanotechnology. Some of the components in LOC technology have already been released on the market (GeneChip® from Affimatrix). Moreover, it has been suggested that nanoscale sensors and ultra miniaturized sensors could lead to the next generation of biotechnology-based industries.

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