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CONTAMINANT SENSORS: NANOTECHNOLOGY-BASED CONTAMINANT SENSORS

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1 Introduction

Food diagnostics is an emerging field that applies "modern" methods of detection of microbes, bacteria, chemicals, biotoxins, heavy metals, and prions in all steps of the food chain from raw materials to end products. Nanobiosensor technology has been a major driver used for the analysis of foods. Throughout the world, food production, preparation, and distribution have become increasingly complex, and raw materials are often sourced globally. Changes in food processing techniques, food distribution, and the emergence of new food pathogens have changed the epidemiology of foodborne diseases. Foodborne microorganisms are continuously changing due to their inherent ability to evolve and their amazing capacity to adapt to different forms of stress (Bisen et al., 2012, 2014b). New primary production technologies and food manufacturing practices are introduced all the time; food consumption patterns and the demographic structure of many countries continue to change. Approximately one-third of all food manufactured in the world is lost due to spoilage (Gustavsson et al., 2011).

1.1 Why Contaminant Sensing is Required in the Food Industry

Food diagnostics is a relatively new and emerging area fuelled in large part by the ever-increasing demand for food safety.

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In addition, nanosensor-based approaches are also essential in the areas of food authentication, detection of foodborne pathogenic microorganisms, and screening for food allergens and adulteration (Debnath et al., 2010). The contaminant can spread rapidly with the quick and efficient distribution systems at multiple locations limiting the reaction time (Hall, 2002). New, flexible tools are required for evaluating and managing new food safety challenges with the use of food safety management tools, most important HACCP (hazard analysis critical control point), and the consequent application of hygienic measures, based on good manufacturing/hygienic practice (GMP/GHP) (Debnath et al., 2010; Bisen et al., 2012, 2014b). However, the lack of reliable data is often limiting the usefulness of this approach and therefore data collection is one of the priorities for future food safety strategies. The safety and quality of food can be tested in source laboratories but the cost and time involved limits the utility (Bisen et al., 2012, 2014b). Also, this type of analysis cannot account for mishandling during transportation or storage, after the product has left the source. Therefore, there is increasing demand for real-time sensors that can sense the quality and safety of the foods on site (Warriner et al., 2014). An outline of the types of contaminants that can enter food at different stages is given in Fig. 14.1.

1.1.1 Ensuring Safe Storage and Transportation within the Sell-By Period

Packaged food products are usually marked with expiry dates that indicate the preferred use by period. However, these periods are determined in standardized conditions assuming that the recommended conditions of storage and transportation have been maintained. In some cases, these conditions might be compromised in the absence of direct physical examination, which will lead to false indications about the suitability of food for consumption. The packing material in some cases may get damaged and

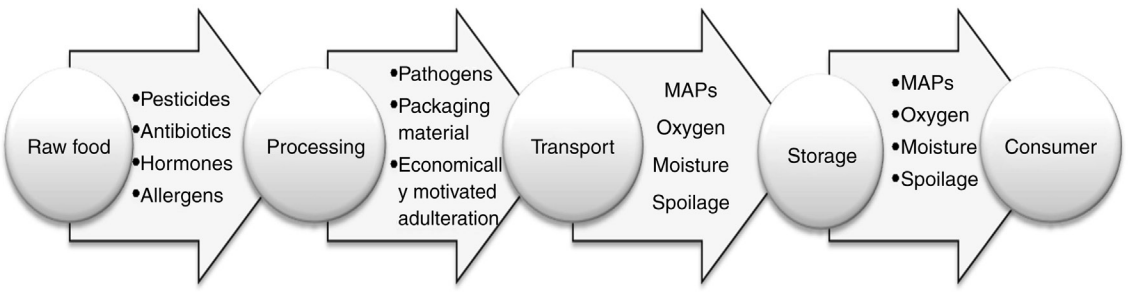

Figure 14.1. Types of food contaminants at various stages from source to consumer.

spoil the food, leading to inadvertent leakage of oxygen or moisture, restricting the use of batch analysis at the source (Luechinger et al., 2007). The majority of foodborne disease outbreaks result from such unintentional contamination of a product as a result of inappropriate processing, handling, and/or packaging. Intentional contamination of our food supply with biological or chemical agents also is a significant threat to national security. Bioterrorist attacks on our food supply could be accomplished with selected bacterial pathogens or toxins. A convenient method would be to use noninvasive sensing mechanism on each and every packet, which would directly inform the consumer about the compromised quality. The contaminants that can be sensed include gaseous indicators of leakage (oxygen, moisture) or spoilage (amines, volatile organic compounds, etc.) and the biological agents. A key step in establishing an effective food-safety program in a forward-deployed theater is to have adequate laboratory diagnostics that can identify and characterize rapidly any agent that could cause the quality of food.

1.1.2 Reducing Potential Health Risks

Food is a rich source of nutrients that attracts microbiological growth. The natural micro flora of food or beverage can consist of three main components: those associated with the raw material, those acquired during processing, and those surviving preservation and storage. They can be further subdivided into harmless organisms, producing either desirable or undesirable flavor changes in the food, and pathogens, forming dangerous enterotoxins (Bisen, 2014b). During processing, foods are subjected to highly complex and rapidly changing environments in which the microorganism may evade inactivation and detection in spite of hygienic manufacturing practices (Hall, 2002). Further, microbial sensing technologies are continuously subject to challenges in the form of novel combinations of types of food and pathogenic microorganisms. In recent times, there has been an increase in the demand for minimally processed food, which further raises expectations of pathogen detection methods as the pathogen elimination points are greatly reduced. Traditional method of pathogen detection requires an enrichment step to bring the target in the detectable range of biosensor. This step increases the time required for analysis and sophisticated containment facilities are required to carry out such analysis. Moreover, not all pathogens are cultivable in similar laboratory conditions. Further detection methods based on traditional culture based batch analysis do not ensure complete food safety (Swaminathan and Feng, 1994). Traditional methods also suffer from the drawbacks of being time consuming and labor

intensive (Baeumner, 2003). The costs incurred on production companies upon potential product recalls due to microbial contamination have increased the interest in on-site pathogen testing (Nugen and Baeumner, 2008). In addition, there has been an increased awareness among people about the possible inadvertent or deliberate contamination of food products, therefore decentralized sensing has become very crucial (Ravichandran, 2010; Vaseashta, 2006). Unique problems encountered during contaminant sensing in food matrices include light scattering, sample opacity, and numerous other interferences (Singh et al., 2009).

1.1.3 Detection of Banned Dyes and Adulterants

In addition to the pathogenic contamination, which might inadvertently enter food, certain dyes, and adulterants are deliberately added in order to gain economic benefits. Food adulteration has caused serious illnesses among infants, adults, and animals (Kumar et al., 2015; Xin and Stone, 2008; Trivedi et al., 2009; Kobayashi et al., 2010). To counteract such incidences of "economically motivated adulteration" (EMA), there is a requirement of rigorous monitoring of food products. Owing to the health issues related to their consumption, many countries have banned the use of these dyes in foods. However, cases have been reported in which Sudan I dye in concentrations as high as 4000 mg/kg has been found in hot chilli products (ASTA, 2005; Botek et al., 2007). This incident led to prohibition of the import of hot chilli and its products by member states of European Commission in the absence of an accompanying analytical report validating absence of Sudan I and IV Sudan, Red 7B and Rhodamine B, curcuma, curry, sumac, and palm oil (RASFF, 2004, 2005; EFSA, 2005).

1.1.4 Detection of Pharmacological Residues Such as Antibiotics and Hormones

Antibiotics and hormones are routinely used in livestock for economic benefits by increasing meat supply and milk production. There is a growing concern regarding the presence of antibiotic/antibiotic residues in food products due to the evolution of MDR pathogenic strains in humans (Craig et al., 2013). Similarly, unmonitored use of hormones may lead to their residual levels in finished products, which might have unfavorable effects on consumers.

1.1.5 Detection of Pesticides

Pesticides are routinely used in agriculture to reduce crop losses due to microbiological or insect pests. However, lack of

awareness among farmers leads to indiscriminate use of pesticides, which might or might not be required for a particular crop. Also, if pesticides are used above a prescribed limit, they may enter the crop plant and cause health concern. Therefore, pesticide levels in the food products needs to be monitored. There are three potential sources of pesticide residues in food grains, arising from (1) application of pesticides to protect the growing crop, (2) contamination of the environment by highly stable pesticides previously applied for other purposes, and (3) application of insecticides to protect the harvested crop during storage and handling.

1.1.6 Detection of Contamination Caused by Packaging Material

Plastic packaging, containers, and cling films often have instructions on how to use them safely to keep chemical migration to a minimum. More than 30 types of plastics have been used as packaging materials including polyethylene, polypropylene, polycarbonates, and polyvinyl chlorides. In certain cases, the material such as melamine used in packaging might enter the food and adversely affect the quality of food.

1.1.7 Detection of Allergens

Certain people are allergic to some specific food items and need to avoid them in order to prevent allergies. For common allergens, such as gluten, gliadin, and so forth, foods free of these allergens are specially processed for the allergenic population. As these components can trigger allergic reactions even in minute quantities, extensive testing of food is required to ensure that the processed food is free of allergen.

2 Biosensors

A biosensor is a device that can report the presence or activity of analytes using a biomolecular component providing specificity to the sensor by binding or interacting with the analyte and is able to cause a detectable change in mass, fluorescence, electric charge, or refractive index, and a transducer element, able to transform this interaction into a suitable electronic signal (Fritz, 2008; Bisen, 2014a). They are coupled together in one of the four possible ways, such as membrane entrapment, physical adsorption, and covalent bonding. The ideal characteristics of an efficient sensing system include speed, sensitivity, accuracy, real-time detection with feasible cost among others (Fig. 14.2).

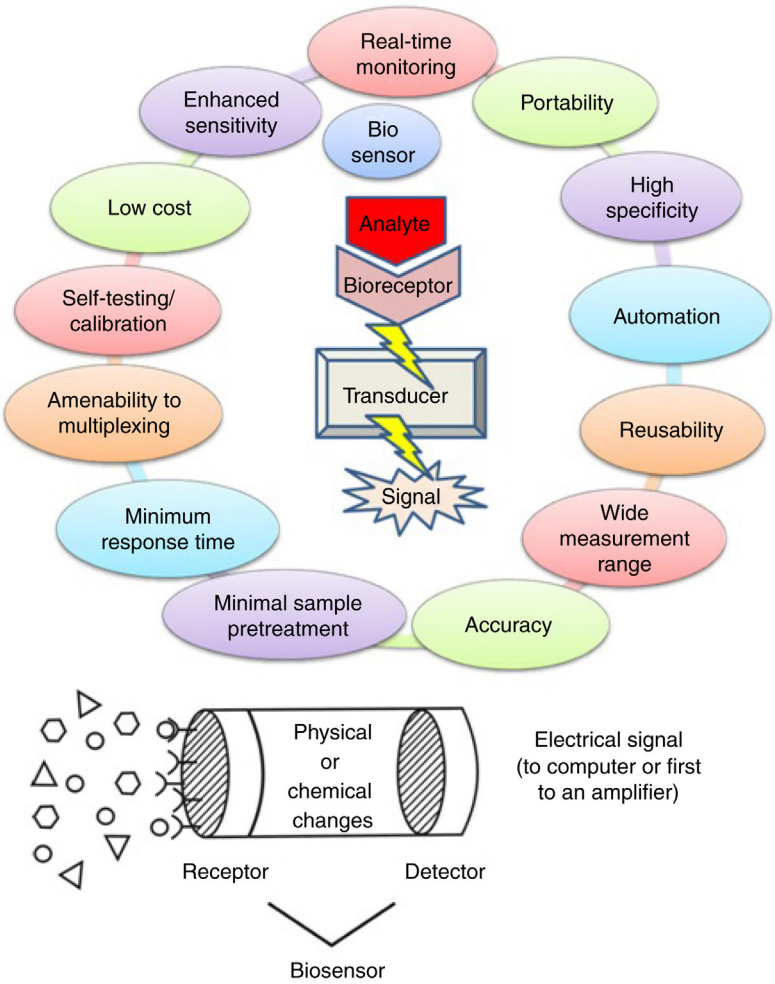

Figure 14.2. Utility and specific properties of biosensors.

Most of the biosensor formats, which were initially developed for health sector, are now beginning to be exploited in the food industry to assess safety and quality of food products (Warriner et al., 2014; Bisen, 2014a). However, only a few of them have been commercialized for food industry due to difference in sample sizes, types of matrices, and so forth. The "bio" and "sensor" elements can be coupled together in one of four possible ways:membrane entrapment, physical adsorption, covalent bonding, and cross-linking. Thus, a typical biosensor consists of mainly three parts: (1) biological material, (2) transducer, and (3) signal processors (Fig. 14.2).

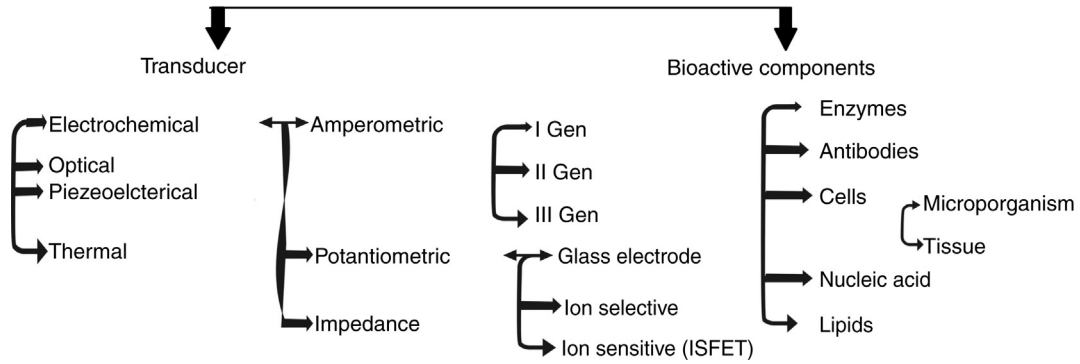

Figure 14.3. Types of transducers and bioactive components.

2.1 Classification of Biosensors

Biosensors may be classified according to components or immobilization techniques used such as transducers and the bioactive compounds. The bioelement is very specific to the analyte to which it is sensitive. Depending on the transducing mechanism used, the biosensors can be of many types such as: (1) resonant biosensors, (2) optical detection biosensors, (3) thermal detection biosensors, (4) ion-sensitive FET (ISFET) biosensors, and (5) electrochemical biosensors (Bisen, 2014a). The electrochemical biosensors based on the parameter measured can be further classified as (1) conductometric, (2) amperometric, and (3) potentiometric. Therefore, biosensors can be divided into different types based on the type of detection (Fig. 14.3).

2.2 Properties of Biosensors

Some of the notable properties of a good biosensor are specificity, linearity, response time, simplicity, continuous monitoring ability, reproducibility, portability, and cost effectiveness (Table 14.1).

2.3 Biosensor Components

2.3.1 Bioactive Components

The biological part of the biosensor specifically reacts with the analyte of interest, sparking a signal that is detectable by the attached transducer. The bioactive components may be a purified enzyme, antibodies, cells, nucleic acids, and/or lipids.

Table 14.1 Various Biosensor Transducers, Principles, and Applications

Transducer System	Principle	Applications
Enzyme electrode	Amperometric	Enzyme substrate and immunological system
Conductometer	Conductance	Enzyme substrate
Piezoelectric crystal	Mass change	Volatile gases and vapors
Thermistor	Calorimetric	Enzyme, prganelle, whole cell, or tissue sensors for substrate, products, gases, pollutants, antibiotics, vitamins, and so forth
Optoelectronic/wave guide and fiber optic device	Optical pH	Enzyme substrates and immunological systems
Ion-sensitive electrode (ISE)	Potentiometric	Ions in biological media, enzyme electrodes, enzyme immunosensors
Field effect transistor (FET)	Potentiometric	Ions, gases, enzyme substrates, and immunological analytes.

2.3.1.1 Sensing DevicesOptical

The biosensor is based on optical diffraction or electrochemo luminescence properties and the output transduced signal measured is light. Optical diffraction-based devices use a silicon wafer coated with a protein via covalent bonds. The resulting signal can be measured or can be further amplified before measuring for improved sensitivity and allowing multiple analytes to be detected by using different monitoring wavelengths. Use of immobilized luciferase greatly reduces the cost of analyses.

2.3.1.1.1 Calorimetric Biosensors Many enzyme catalyzed reactions are exothermic, generating heat and the temperature changes. In these cases, one can carry out measurements in temperature-controlled small packed bed columns with immobilized enzymes and determine the temperature at the entry and exit of these columns by incorporating suitable thermistors (Fig. 14.4).

Calorimetric transducing devices measure the heat released upon a biochemical reaction occurring at the sensor surface. On the basis of the type of heat transfer, they can be categorized as follows:

1. *Isothermal calorimeters*: maintain isothermal conditions and the amount of energy required for cooling or heating is measured.

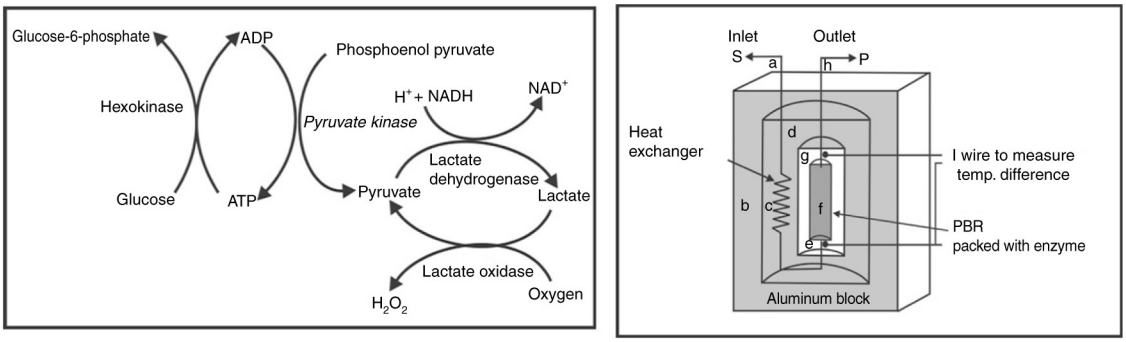

Figure 14.4. Coupling of many heat-increasing enzymes in exothermic reaction and schematic diagram of a calorimetric biosensor.

2. *Heat conduction calorimeters*: measure the difference in temperature between the highly conducting reaction vessel and the surrounding heat sink.
3. *Isoperibol calorimeter*: measures the temperature difference between the thermally insulated (adiabatic) reaction vessel and the surrounding isothermal jacket.

In spite of the progress in the field of biosensors, the demand for development of improved biosensors that are more sensitive in terms of concentration and volume of analyte and which can simultaneously sense broader range of analytes continues to increase (Fritz, 2008). These sensors are characterized by a reaction between analyte and biosensor component to produce a color change (Su et al., 2013). Nanoparticles can also be modified by an ionic ally to couple desired protein. Another approach is aggregation of colloidal nanoparticles with subsequent colorimetric changes, which can be perceived even by an untrained person (Lim et al., 2012).

2.3.1.1.2 Fluorescence Fluorescence analysis using organic dyes has been used traditionally to detect pathogens. They are based on excitation of analyte using suitable laser light followed by detection of emitted fluorescence where fluorescent nanoparticles are used to enhance the signal while providing photostability. Quantum dots labeled with binding molecules can also be used as a fluorescent tracer (Penn et al., 2003).

2.3.1.1.3 Surface Plasmon Resonance Surface Plasmon resonance transducers measure minute changes in the angle of reflectance of plasmon waves on dielectric interface. When a plane polarized light passes through a prism whose one side is coated with a metal, at an appropriate angle, total internal reflection is observed, which induces formation of a charge wave at the

interface (called Plasmon waves) moving up to a few microns. The incident angle of the total internal reflection is measured using a photodetector. The angle of reflectance changes when the refractive index of the surface changes, for example, in response to binding of analyte (Warriner et al., 2014). SPR offers advantages such as being label free and rapid (Cho et al., 2014). The disadvantages include slow diffusion driven mass transfer, low sensitivity due to small refractive index, and insufficient depth of layer influenced by SPR (Wang et al., 2010). To this end antibody nanoparticle conjugates have been used to enhance the signal in sandwich assays (Wang et al., 2010).

2.3.1.1.4 Antigen antibody The binding between an antigen and its corresponding antibody is very specific. This property of antibody is exploited while designing biosensors based on antibodies. Antibodies are usually covalently bonded on the surface of the transducer by conjugation of amino, aldehyde, carboxyl, or sulfhydryl groups (Shaikh and Patil, 2012). The binding reaction between the antibody and antigen can be monitored as a time-dependent change of fluorescence signal, which is proportional to the reaction ratio of antibody to analyte (Bisen, 2014a). Though antibodies have similar limitations with enzymes, immunosensors offer advantages of rapid and on site measurements over traditional immunoassays (Shaikh and Patil, 2012). Immunosensors usually make use of optical or acoustic transducers (Fig. 14.5).

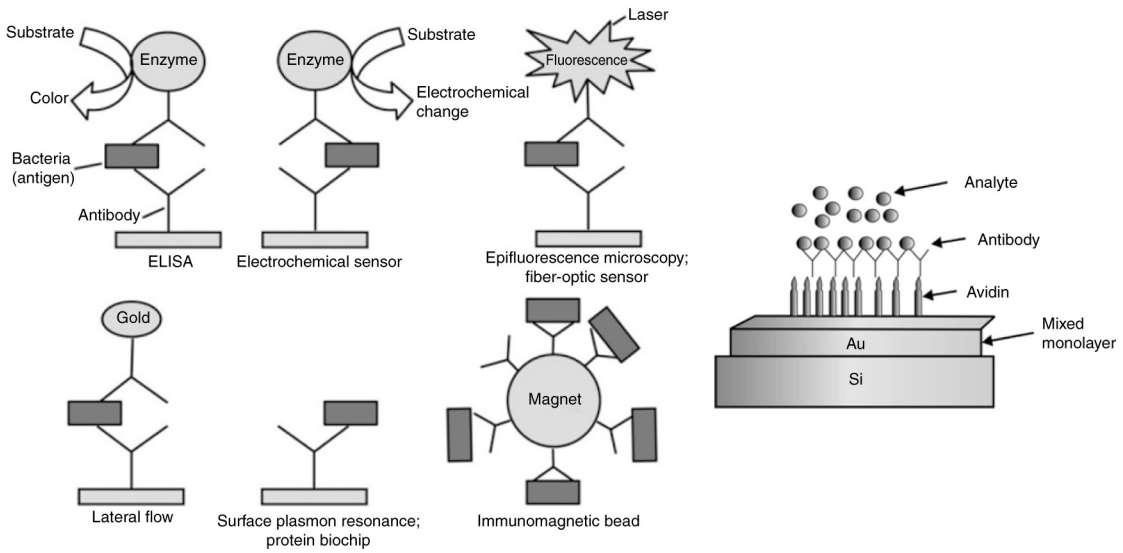

Figure 14.5. Immunosensor design.

2.3.1.2 Acoustic/Piezoelectric

In this mode, sensing molecules are attached to a piezoelectric surface (a mass to frequency transducer) in which interactions between the analyte and the sensing molecules set up mechanical vibrations that can be translated into an electrical signal proportional to the analyte, such as quartz crystal.

Acoustic transducers detect a change in mass density, viscoelastic, electric, or dielectric properties of chemically interactive membrane placed in contact with a piezoelectric material. Following are two commonly used acoustic transducers:

2.3.1.2.1 Bulk acoustic wave sensor (BAW) BAW measures the change in resonance frequency of a resonator (eg, quartz crystal resonator in quartz crystal microbalance), which may be due to mass or viscoelastic changes at the sensor surface. Nonspecific binding effects might interfere with the detection.

2.3.1.2.2 Surface acoustic wave sensor (SAW) SAW measures the change in surface acoustic waves (not the bulk), which may be due to surface effects such as mass, viscosity, pressure, magnetic fields, strain, temperature, and irradiation with UV rays (Arugula and Simonian, 2014).

2.3.1.3 Resonant

An acoustic wave transducer is coupled with an antibody (bioelement) in this mode. The mass of the membrane changes when the analyte molecule (or antigen) gets attached to the membrane. The resulting change in the mass subsequently changes the resonant frequency of the transducer, which is then measured.

2.3.1.4 Thermal-detection

They are constructed by combining immobilized enzyme molecules with temperature sensors, which are based on biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. The use of sophisticated and expensive instrumentation is the major drawback of this technique.

2.3.1.5 Ion-Sensitive

The ISFET can be constructed by covering the sensor electrode with a polymer layer. This type of biosensor is also called an enzyme field effect transistor (ENFET) and is primarily used for pH detection (Fig. 14.6). The technology is based on semiconductor device with ion-sensitive surface, which interacts with the ions and the potential change is subsequently measured.

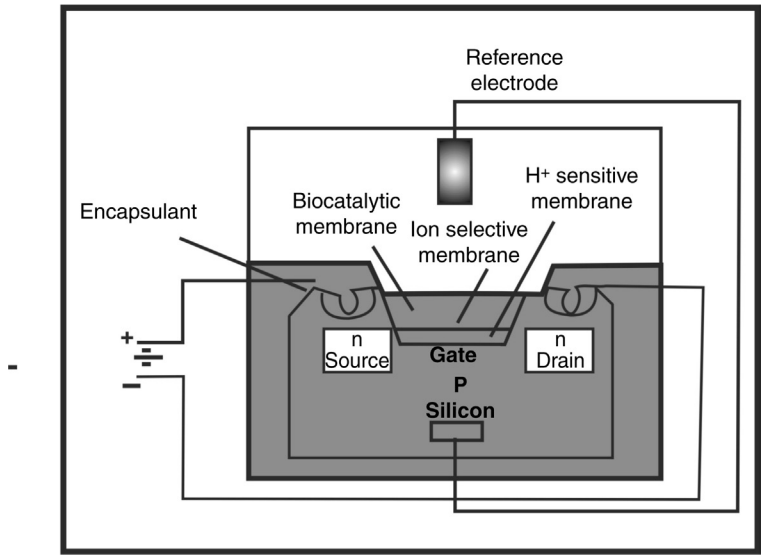

Figure 14.6. Schematic diagram of the section across the width of an ENFET.

2.3.1.6 Electrochemical

The chemical reactions produce or consume ions or electrons that can be sensed and used as measuring parameter in this class of biosensors. The sensing molecule reacts specifically with compounds to be detected, sparking an electrical signal proportional to the concentration of the analyte (Fig. 14.7).

High sensitivity, selectivity, and ability to operate in turbid solutions are advantages of electrochemical biosensors. Electrochemical biosensors are mainly used for the detection of hybridized

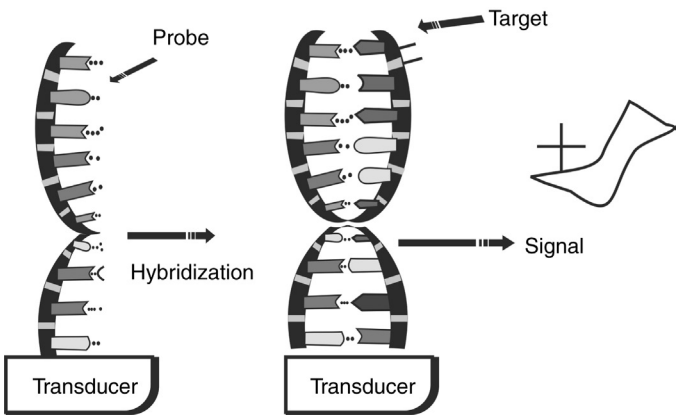

Figure 14.7. Electrochemical DNA biosensor.

DNA, DNA-binding drugs, glucose concentration, and so forth. Electrochemical biosensors can be classified based on measuring electrical parameters as: (1) conductometric, (2) amperometric, and (3) potentiometric.

2.3.1.6.1 Conductometric The measured parameter is the electrical conductance/resistance of the solution. This change is measured and calibrated to a proper scale but having relatively low sensitivity.

2.3.1.6.2 *Amperometric* (measurement of the current resulting from a redox reaction) Amperometric detection is based on measuring the oxidation or reduction of an electroactive compound at a working electrode (sensor) (Fig. 14.8). Enzyme-electropolymer-based amperometric biosensors has also been developed as an innovative platform for time-temperature integrators (Reyes-De-Corcuera et al., 2005)

In vivo sensing: Amperometric biosensors have been also applied for in vivo sensing since their size may be reduced. By the appropriate casting of membranes onto the biosensors tip, high selectivity and biocompatibility may be achieved (Fig. 14.8).

2.3.1.6.3 Potentiometric The measured parameter in this type of sensor is oxidation or reduction potential of an electrochemical reaction. The voltage at which these reactions occur indicates a particular reaction and particular species (Fig. 14.9).

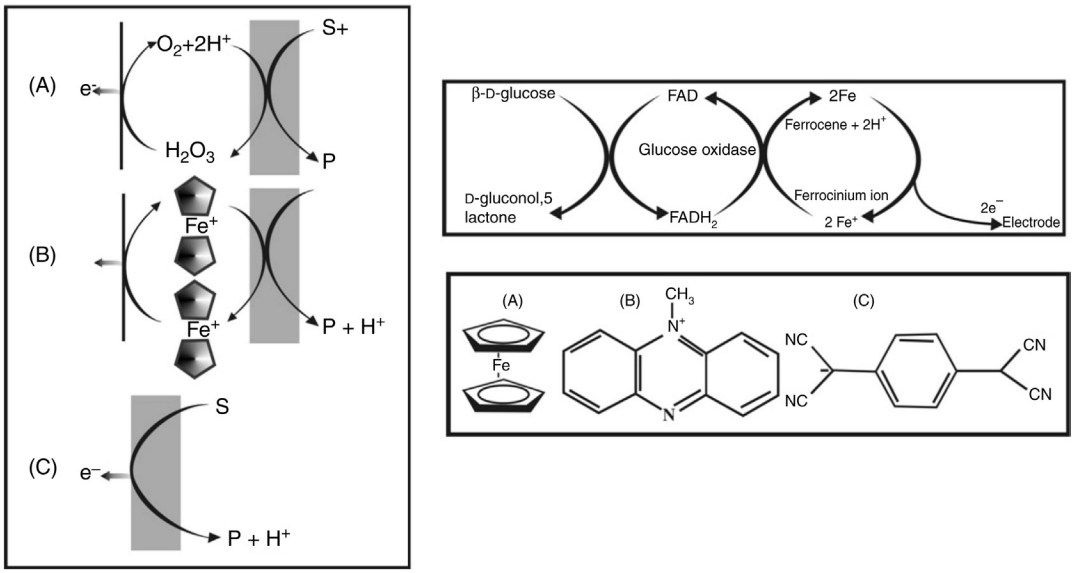

Figure 14.8. Amperometric biosensors for flavor-oxidase enzymes illustrating the three generations in the development of biosensors.

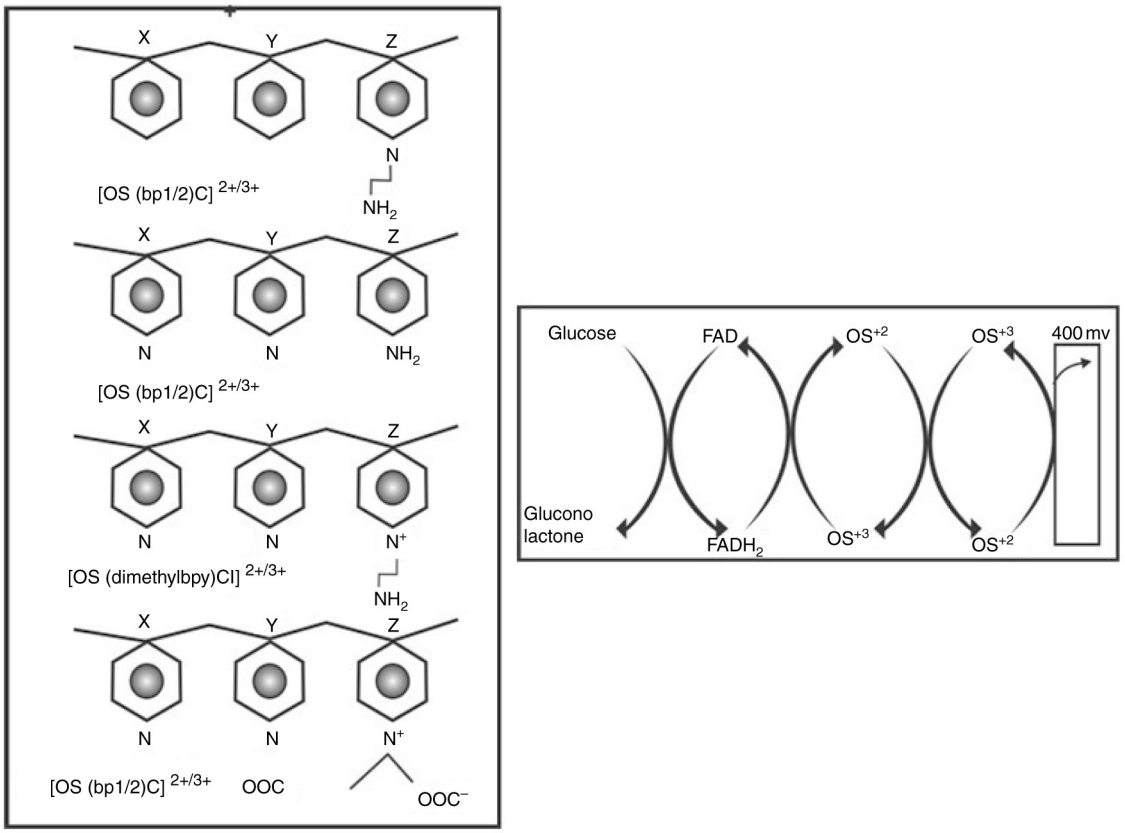

Figure 14.9. Structure of enzyme "wiring" polymers. Variation of X, Y, and Z allow the properties of the polymer to be customized and the redox cycles occurring at a three-dimensional redox epoxy-wired enzyme electrode. The wired enzyme is a flavin (FAD)-containing oxidase enzyme.

Ion selective electrodes utilized in biosensing devices can be classified as follows:

1. *Normal pH electrodes*: These are normal hydrated glass membrane electrodes, which sense cations in a concentration dependent manner by producing a transverse electric potential arising due to competition for binding sites.
2. *Glass pH electrode for gases*: These electrodes are composed of gas permeable membrane selective for specific gases like carbon dioxide, hydrogen sulfide, or ammonia. The measurement is based on pH differences between electrode and the membrane arising due to diffusion of these gases through the membrane.
3. *Solid-state electrode*: In these electrodes, the glass membrane is replaced by conductive membrane made of silver compounds. These electrodes have been used in the determination of cyanide and iodide ions.

Recently ion selective electrodes have been used in the production of ISFETs, which have been conjugated with enzymes to develop biosensors.

3 Nanosensors

Nanosensor can be defined as any sensing device which incorporates a nanoscale component, that is, a component having dimensions within 1–100 nm at least in one dimension (Jianrong et al., 2004). Nanomaterials can be used in sensors both to enhance sensing as well as transduction.

3.1 Nanomaterials: Ideal Properties for Use in Sensors

The physical, chemical, and biological properties of nanomaterials are remarkably different from their macroscopic counterparts (Ravichandran, 2010). These properties impart unique characteristics to the nanomaterials which can be exploited for biosensor design to improve the specificity, accurary, linearity, response time, simplicity, continuous monitoring ability, reproducibility, portability, and cost (Li et al., 2011)

3.1.1 Size

The size of nanomaterials is comparable to biological macromolecules. Therefore, these materials are well suited to detect analytes present in the microenvironments which are not accessible to the macro sensors (Wei et al., 2009). Due to their nanoscale size, nanoparticles are ideal candidates for direct localized detection. A number of biosensors are now available which do not require preprocessing of the sample, and therefore can be used for a direct estimation of analyte. Small size of the sensor element also enables miniaturization of the sensor increasing the portability for onsite testing. The detection is, therefore, possible not only at the source, but also at the distribution/consumer level. With the help of microfluidic systems, detections are possible utilizing small sample volumes in addition to also effectively reducing the cost and time required for analysis. The fabrication cost of the electrode is also reduced due to reduction in volume of expensive materials (Wei et al., 2009). Owing to the small size, the multiple nanosensors may be equipped in single instrument to provide multianalyte detection or to obtain spatial distribution of a single analyte (Wei et al., 2009). The favorable size and optical properties allow nanosensors to be incorporated into food packaging for noninvasive, visual analysis of food quality during storage, transportation, and consumption (Duncan, 2011).

3.1.2 Electrical Properties

There is enhanced electron transfer due to high electrical conductivity; therefore, the sensitivity of the sensor is greatly increased for example, gold nanoparticles (Majdalawieh et al., 2014). Electrochemical measurements are considerably quick in nanoelectrodes due to radial (nonplanar) diffusion compared to macroelectrodes operating via planar diffusion (Wei et al., 2009).

3.1.3 Optical Properties

The optical properties of nanoparticles are considerably different from the respective bulk materials owing to the quantum effect. There is higher energy difference between the conduction and the highest valence band due to the compact packing density of electrons in nanoparticles resulting release of higher energy upon return of electron from excited state to ground state for the spectral shift. The semiconductor properties are also affected leading to changes in electrochemical phenomena like surface Plasmon resonance (Warriner et al., 2014). Quantum dots, a type of nanoparticles have found a new application as a substitute for enzyme labeling. Quantum dots are able to absorb more photons compared to organic fluorescent dyes, provide more brightness, and release the energy in the form of photo luminescence omitting the requirement of an additional assay step (Pisanic Ii et al., 2014). In addition, they are photostable and provide high signal to noise ratio (Syed, 2014). As the emission peak is dependent upon the size of nanocrystal, different sizes of quantum dots can be used to obtain different color labels for multiplexing.

3.1.4 Chemical Properties

The chemical reactivity is also enhanced compared to macromaterials, due to small size enabling fast detection in both liquid and gaseous phases (Vaseashta, 2006).

3.1.5 Magnetic Properties

Magnetic nanoparticles labeled with a suitable capture reagent, for example, antibodies can be used to concentrate the analyte, a technique called IMS (immunomagnetic separation). Some nanoparticles behave as ferromagnetic particles when an external magnetic field is applied because of having a lower number of unpaired electrons than the bulk material. These properties are useful in generating magneto-resistive nanosensors. A large number of analytical approaches are being used in food industry requiring a sample preconcentration to achieve detectable signal.

3.1.6 Semiconductor Property

A large number of enzymes used in biosensors are oxidoreductases producing an electroactive product detected amperometrically. These enzymes require mediators to prevent interference from other electroactive constituents in the sample. Use of semiconductor nanoparticles allow direct wiring of enzyme redox centers to the electrode by passing the requirement of exogenous mediator and reducing response time of the sensor (Palanisamy et al., 2012; Hu et al., 2012).

3.1.7 Enhanced Sensing Surface Area

Nanoparticles have a high surface area volume ratio; large surface areas work more efficiently for immobilization of bioaffinity agent. The higher surface area increases the number of bioaffinity agents that can be incorporated per unit volume and therefore increase the sensitivity of the device. Due to large surface area of nanoelectrodes, nanosensors can be used to detect analytes in poorly conducting media even in the absence of suitable electrolyte (Wei et al., 2009) particularly useful for detecting analytes in complex and variable food matrices (Singh et al., 2009).

3.1.8 High Accuracy

Any minute changes in the sensor environment are transduced accurately for reliable detection of the analyte due to enhanced sensing surface.

3.1.9 Quick Response

Use of nanoparticles allows direct wiring of enzyme redox centers to the electrode by passing the requirement of exogenous mediator and reduce response time of the sensor (Palanisamy et al., 2012; Hu et al., 2012). These properties of nanomaterials have improved the design and efficiency of biosensors to meet the expectations of growing market demands (Fig. 14.10).

3.2 Types of Nanosensors

Nanomaterials can improve sensor design at various levels. They can improve sensing abilities as well as transduction properties. Likewise, nanosensors can be classified into following types:

3.2.1 Colorimetric Metal Nanoparticle Detectors

Colorimeteric sensors offer simplicity and ease of operation and detection. Certain nanoparticles can undergo easily visible color changes in response to presence of analyte and this property can

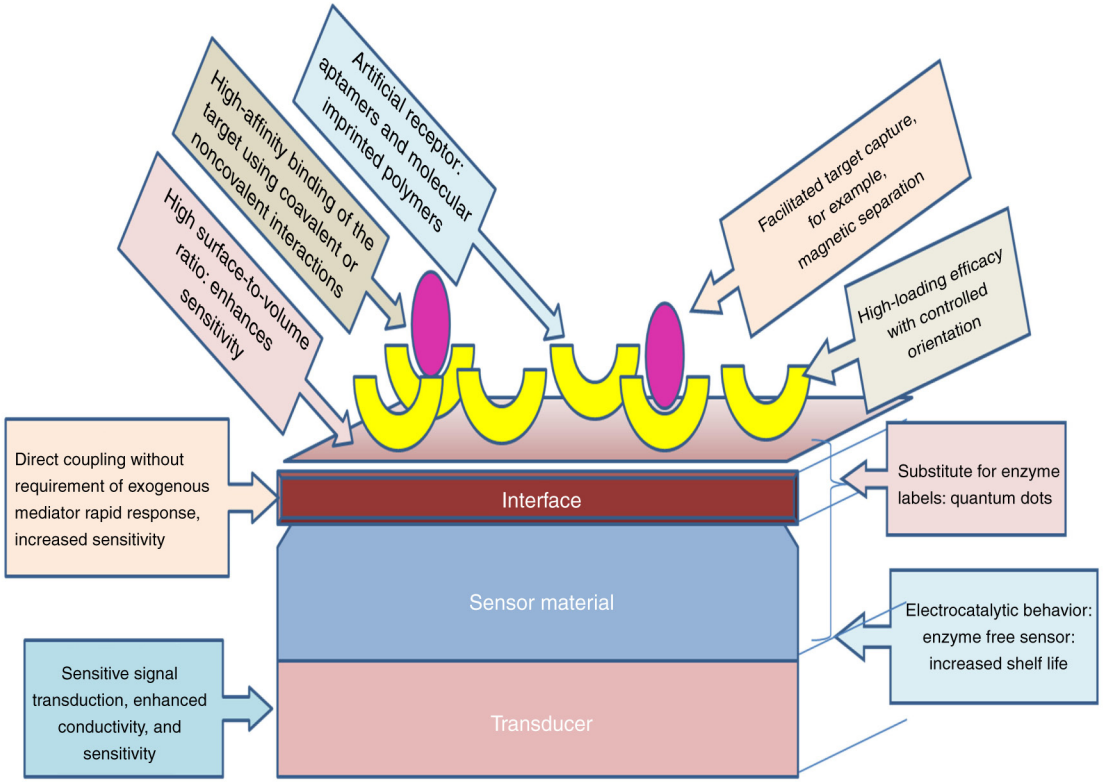

Figure 14.10. Design and efficiency of biosensors with nano sensors and nanomaterials.

be utilized to develop nanoparticle-based colorimetric assay (Ai et al., 2009; Kuang et al., 2011). The formation of nanoparticles can also be controlled by presence of analyte to produce a visible effect proportional to the concentration of analyte (Cao et al., 2010).

3.2.2 Carbon Nanotube Biosensor

CNT-based nanomaterials have the unique capability to alter their properties in presence of some chemical species but are otherwise chemically inert. They are morphologically flexible, biocompatible, and their size is comparable to biomolecules, making them an ideal candidate for biosensor design and applications having high surface area: weight ratio; conducting properties; quick, accurate, and reversible measurements; easy derivatization; and ability to generate electro-chemiluminiscence in aqueous solutions (Vaseashta, 2006). Carbon nanotubes can be used as a substitute owing to their superior mechanical properties for silicon-based chips in nanoelectromechanical systems (Sapmaz et al., 2003).

3.2.3 Electronic Nose

The quality of certain food items is ascertained primarily by their aroma. Traditionally the aroma quality is analyzed by a trained panel who decodes the complexity and heterogeinity of the aroma and rates them on the basis of experience. This type of analysis might not be reproducible and may vary from person to person (Peris and Escuder-Gilabert, 2009). The other alternative is analytical analysis of the headspace using gas chromatography. This method has higher reliability but has high operating cost and suffers from the disadvantage of long analysis time per analysis (García et al., 2006). Electronic noses, which mimic the human nose in sensing the aroma and rate it according to the pattern of different volatile components, bypass the problem of reliability in expert panel-based methods and cost of analytical methods. An electronic nose (Persaud and Dodd, 1982; Gan et al., 2005) comprises three components: a sensory component, which is usually an array sensor enabled to sense different types of aroma imparting volatile compounds; a unit to collect the signals generated from the sensory unit; and, finally, a software to recognize the pattern of VOC's to ascertain food quality (García et al., 2006) (Fig. 14.11).

The sensory component of electronic noses can be surface acoustic wave sensor, metal oxide semiconductor sensor, or quartz resonator. They can be used for the analysis of VOC arising from both liquid and solid food samples (Schaller et al., 1998;

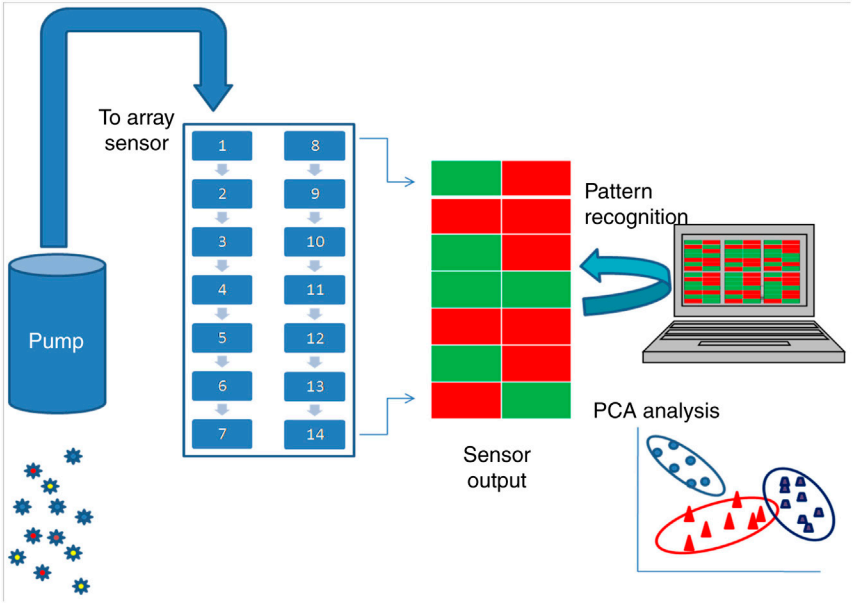

Figure 14.11. Diagrammatic view of e-nose (electronic nose).

Frías et al., 2002). Electronic noses offer several advantages including portability, low detection time, low cost per analysis, and high reliability, but the presence of high concentration of interfering substances, for example, ethanol and water in wines may reduce their sensitivity (Francis and Cynkar, 2000). Electronic noses have been incorporated directly in packaging material to quickly sense the presence of pathogens by their metabolic products (Ravichandran, 2010). Some notable examples of utility of e-nose in ascertaining food quality are listed in Table 14.2.

3.2.4 *Electronic Tongue*

An electronic tongue is similar to an electronic nose and works for nonvolatile analytes. E-tongues can be used to analyze the quality of foodstuffs like wine, beer, and tea. The quality of these foodstuffs is determined by detecting their bitterness, sourness, or astringency, which is mainly attributed to the presence of polyphenols. These can be incorporated directly in packaging material to quickly sense the "taste," mimicking the physico-chemical interaction of food molecules with taste buds present on tongue (Ravichandran, 2010).

Table 14.2 Selected Applications of e-nose in the Food Industry

Food Item	Type of Analysis	References
Wines	Quality	Penza and Cassano (2004); Buratti et al. (2004)
Cheese	Detection of mould	Ampuero and Bosset (2003)
Milk	Quantification of off flavors; identification of single strains of disinfectant–resistant bacteria in mixed cultures	Ampuero and Bosset (2003)
Meat products	Quality changes	Vestergaard et al. (2007)
Vegetable oils	Flavor analysis	Gan et al. (2005)
RBD palm olein	Storage stability	Gan et al. (2005)
	Lard adulteration	Man et al. (2005)
Wheat	Age and insect damage	Zhang and Wang (2007)
Sesame oil	Maize oil adulteration	Hai and Wang (2006)
Virgin olive oils	Adulteration	Oliveros et al. (2002)
Porcine meat loaf	Sensory quality	Hansen et al. (2002)

As in the case of electronic nose, multiple analytes are tested in a single run to get a blueprint pattern of sample composition, thereby reducing the detection time to a large extent, which is very crucial in food industry (Ravichandran, 2010). E-tongue sensors based on amperometric transducer are of four types metal, conducting polymer, phtalocyanine film, and biosensors. Similarly the detection mode can be fixed potential or pulse sweeping potential.

Metal microchip based e-tongue is composed of capillary electrophoresis coupled with screen-printed electrode. E-tongues based biosensor are composed of an enzyme, solid electrode, and biochemical transducer. The conductivity of conducting polymer sensors might vary with the analyte (Scampicchio et al., 2008), and they are sensitive to humidity but they offer advantages such as rapid adsorption desorption and partial selectivity. Electrodes are composed of coordination compounds containing a transition metal with phthalocyanine film (Baldwin et al., 2011) and used to sense bitterness in olive oils (Apetrei et al., 2004). The performance of metal sensor, biosensor, or conductive polymer based e-tongue can be enhanced by utilizing nanodimensions to increase surface-to-volume ratio to reduce the detection limits. Miniaturized sensor arrays are now being developed. Often, a lipid membrane is used for recognition, which translates the relevant substances into electric potential across membrane. The amount of lipids present in the membrane can be varied to optimize detection limit (Iiyama et al., 2009).

3.2.5 Nanocantilevers

Nanocantilever biosensors can respond to mechanical bending caused by a slight change in temperature, pH, DNA hybridization, interaction between antigen and antibody, adsorption of pathogen, or formation of self-assembled monolayers at the sensor surface (Fritz, 2008). The detection is based on physical and chemical signaling stimulated by biological interactions such as those between antigen–antibody, enzyme–substrate, and ligand–receptor. They are made up of silicon-based materials such as silicon nitride or silicon dioxide forming the bottom layer and a gold-made reflective top layer (Fritz, 2008). They are used for detection of chemical contaminants, toxins, and antibiotic residues in food (Ravichandran, 2010). Vibrational frequency can also be customized as per biomass of pathogenic microorganism to simultaneously detect multiple pathogens (Ravichandran, 2010).

Cantilever sensors consist of a cantilever beam sensitive to their environment having bending in order of nanometer and the sensors are called as nanomechanical sensor (Fritz, 2008). A position sensitive detector (PSD) is used to transform the mechanical bending into positional change of laser spot. Alternatively, a

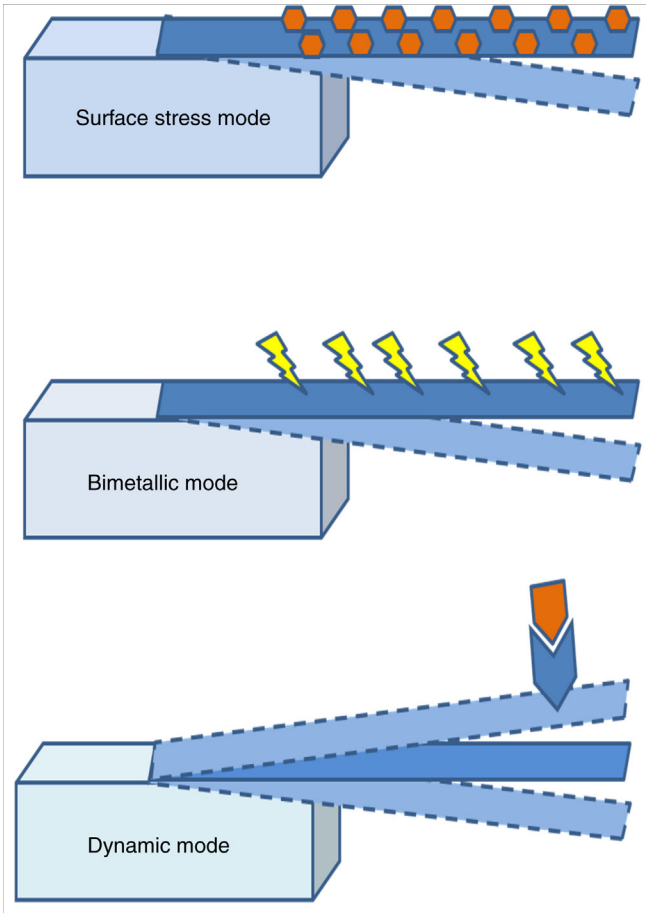

Figure 14.12. Different modes of nanocantelevers.

peizoresistive readout may be used for measuring change in resistance upon mechanical bending (Fritz, 2008). Nanocantilevers can be operated in different modes: surface stress mode, bimetallic mode or dynamic mode (Fig. 14.12).

Surface stress mode is the most commonly used in which molecules bind to the top and bottom surface of the cantilever asymmetrically, causing cantilever bending. The two surface layers may be functionalized for enhanced asymmetric binding using silane chemistry by layering amino or mercapto silane, which can be further cross-linked to desired receptor molecule via their end groups. Alternatively, electrostatic binding of positively charged molecules to the negatively charged silicon dioxide may be achieved (Fritz, 2008). The gold surface may be functionalized utilizing thiol chemistry by exploiting the affinity of gold to sulfur groups. Thus thiol-labeled DNA or cysteine-rich proteins may be bound

to this surface acting as receptor for the complementary DNA or specific antibody (Fritz, 2008). The layers can also be covered with inert coating like thiolated polyethylene glycol to prevent adsorption (Fritz, 2008). The bending of beam due to differential thermal expansion of the two surfaces is sensed in bimetallic mode. Temperature changes as little as 10^{-5} K can be sensed using this mode by careful optimization (Ziegler, 2004). Dynamic mode is based on the property of cantilevers to behave as harmonic oscillators, which can be excited at their resonant frequency, depending on the effective mass of the oscillator. When the analyte is bound to the surface of the cantilever, its effective mass increases, decreasing the resonant frequency and the mass change can be measured even up to single molecule (Yang et al., 2000).

There are several advantages of cantilever biosensors including their amenability to microfabrication. These sensors are label free, allowing them to detect unmodified molecules and, therefore, can be used for real-time monitoring. They can be applied to a wide variety of molecules by appropriate functionalization with small sample volumes. However, the requirement of sophisticated functionalization and limited theoretical description and low repeatability in liquid samples limit their use (Craig et al., 2013).

3.2.6 Optical

Fiber optic nanoprobe: Nanoprobes consist of an optical fiber of nanometer radius whose outer wall is coated with silver/aluminum or gold and the tip is functionalized by silanization to enable it to covalently bind with antibody. Fluorescent analyte is detected by the probe, which is mounted on an inverted microscope coupled to a photomultiplier tube (PMT) detector (Vo-Dinh et al., 2001). Cells are maintained in a viable state by heating microscope stage to 37°C. The optical nanoprobe sensor can be used to manipulate subcellular locations owing to their small size.

3.2.7 Nanoelectromechanical System (NEMS)

Nanoelectromechanical systems (NEMS) are the successors of microelectromechanical systems (MEMS). They work by converting electrical signal into nanoscale mechanical motion and mechanical motion into electrical signal. The devices offer advantages like rapid response, portability, and low cost because of nanoscale moving parts. They can be used as active cells by devices and can be used to control the environment during storage. They are able to detect chemical or biochemical signals using advanced transducers (Ravichandran, 2010). The MEMS technology for detection of trans food content has been commercialized by Polychromix, Wilmington, MA, USA (Ritter, 2005).

3.2.8 Aptamer Sensor

Aptamers are single-stranded oligonucleotide sequences or peptide molecules with the capability to recognize various specific target molecules ranging from small ions to large proteins with high affinity and specificity. They are the novel biomolecular recognition elements, which can be utilized as receptors in biosensors (among other applications) due to small size, cost efficiency, and design flexibility. Aptamer-based biosensors are able to detect analytes which were, up to now (using antibody technology) very difficult to measure or even detect, like toxic or nonimmunogenic substances (Figs. 14.13 and 14.14). Immunobiosensor with EC, optical (SPR fluorescence) or mass-sensitive (piezoelectric microbalance) transducers are gaining extensive research interest. Aptamer has a wide range of molecular and therapeutic targets,

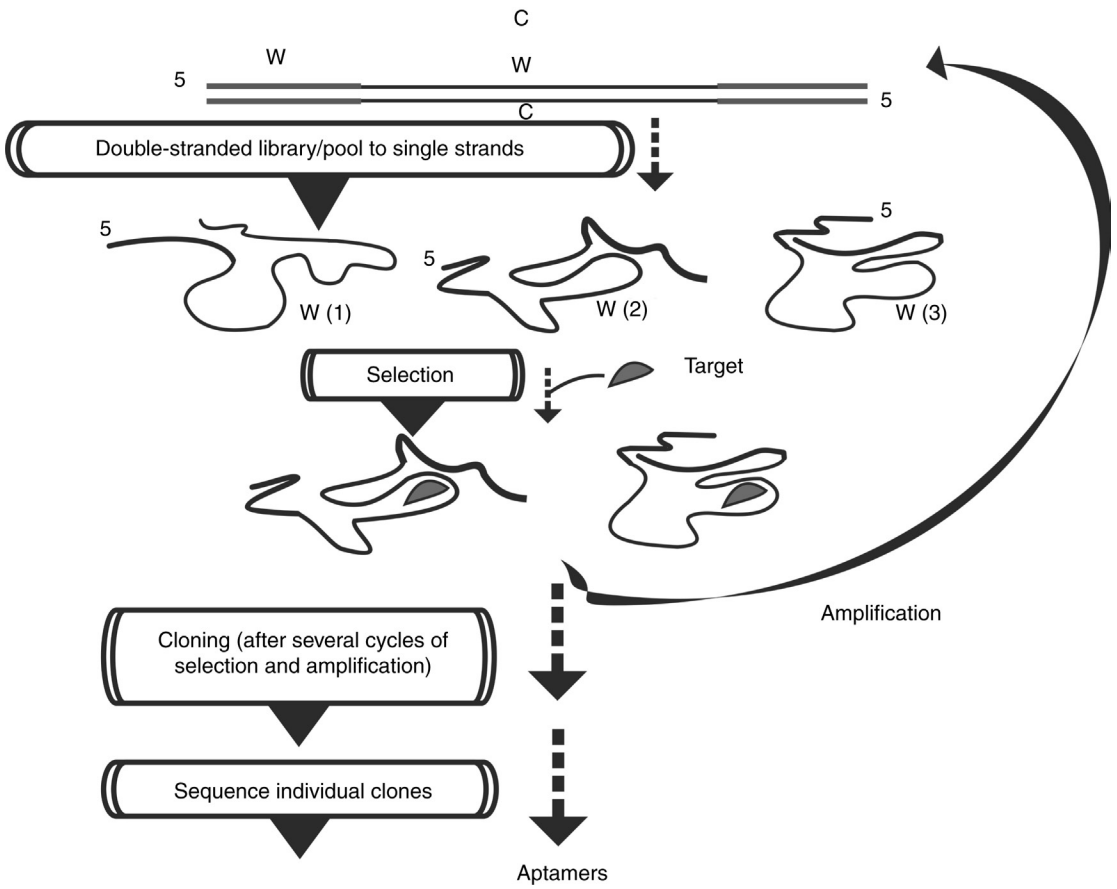

Figure 14.13. The approach can be used to isolate high-affinity aptamers for a wide variety of protein and small molecules.

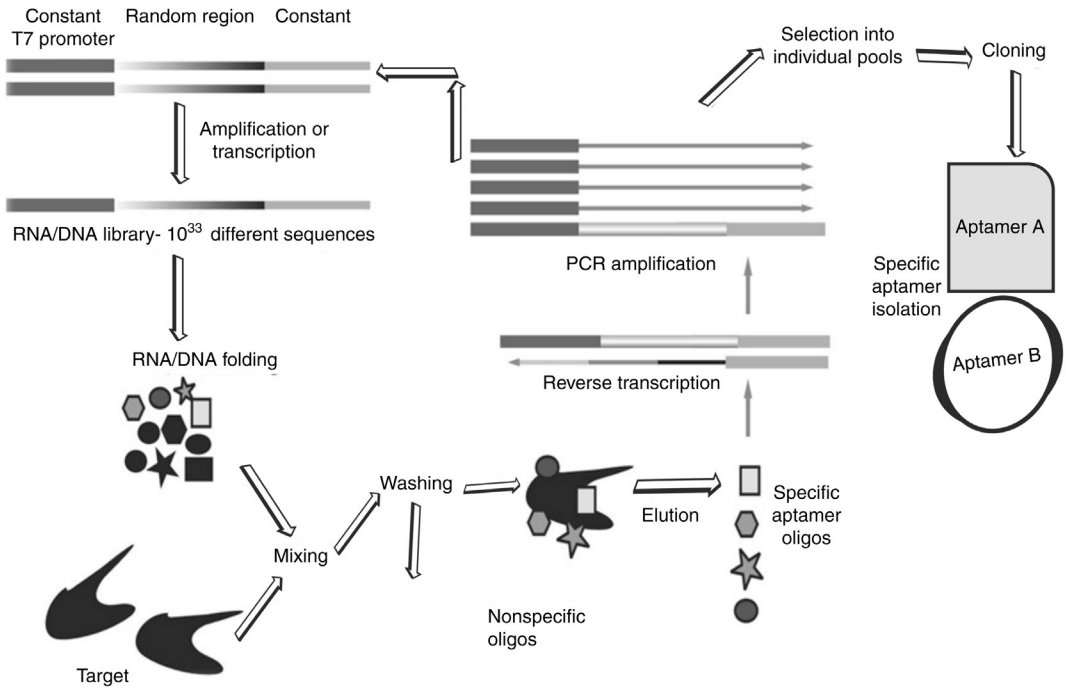

Figure 14.14. Aptamers can be used to select specific regions.

including amino acids, any class of proteins (enzymes, membrane proteins, viral proteins, cytokines, growth factors, and immunoglobulins), drugs, metal ions, other small bio/organic/inorganic small molecules, and even whole cells. Moreover, combinatorial chemical synthesis offers a wide variety of methods for aptamer sequence modifications such as the terminal tagging chemical groups (Selvakumar and Thakur, 2012). A major limitation of aptamer sensors, is the potential of biodegradation or bio-fouling when used in complex biological matrices containing high levels of nucleases which can be overcome by encapsulating the aptamer sensor in suitable polymer (Li et al., 2015).

Aptamers resemble antibodies but they undergo structural modification after binding with their target. Aptamers are developed by systematic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold, 1990).

3.2.9 Surface-Enhanced Raman Scattering–Based Sensor

SERS nanosensors are based on the principle of Raman spectroscopy where the Raman scattering signal of analytes adsorbed on metal surfaces is amplified 100–1000-folds. In Raman

spectroscopy, the molecules are identified on the basis of their characteristic spectral fingerprint arising due to the unique pattern of molecular vibrations (Duncan, 2011). In order to overcome the major limitation of Raman scattering, the roughened metal surfaces can be used in the proximity of analyte molecules to enhance the signal called the surface-enhanced Raman scattering. The effect arises out of interaction of localized electric fields generated due to photoexitation of surface plasmon of the metal and the molecular electronic states of the analyte. Rough surfaces having lots of curvatures works well for SERS. Thus metallic nanostructures are particularly useful for SERS-based sensing as the interaction is dependent on orientation of the analyte with respect to the surface. Nanomaterials like fractal-like or patterned gold nanostructures (Lin et al., 2008), AuNPs, AgNPs, and Au–Ag core–shell nanoparticles (Ravindranath et al., 2011) have been utilized for the detection. SERS-based nanosensors have been reported for detection of chemicals like melamine (Liu et al., 2010), malachite green, crystal violet (He et al., 2008a), perchlorate (Gu et al., 2009), bacteria (He et al., 2008b; Liu et al., 2008; Wang et al., 2010; Ravindranath et al., 2011, Wang et al., 2011, Craig et al., 2013) and viruses (Fan et al., 2010; Zheng and He, 2014). SERS can be used for rapid screening of samples in conjuction with HPLC analysis to eliminate false-positive samples (Duncan, 2011). The advantages include high sensitivity, minimal sample preparation requirements, high throughput screening, label-free detection, short analyses time, and real-time analysis, but sophisticated instrumentation is required for analysis (Craig et al., 2013).

3.2.10 Liposome Nanovesicles

Liposomes are spherical molecules with an aquesous interior surrounded by a phospholipid bilayer (Fig. 14.15). Nanoliposomes offer advantages of being biocompatible and biodegradable in addition to being small in size. They can be used to improve solubility and bioavailability of bioactive agent and prevent their undesirable interactions with other molecules (Mozafari, 2010). Nanovesicles have been used frequently for encapsulation and delivery in food industry. Regarding biosensing applications, these vesicles can be conveniently used in immunoassays to store a large number of fluorescent molecules, amplifying the signal greatly, thus increasing the sensitivity of the assay (Monroe, 1990). For immunoassay, these polar headgroups of constituting phospholipids can be conjugated to the antibody of interest. This conjugation can be covalent, biotin-streptavidin-based or Fc-binding protein (protein G/protein A) based (Chen et al., 2005). Initially

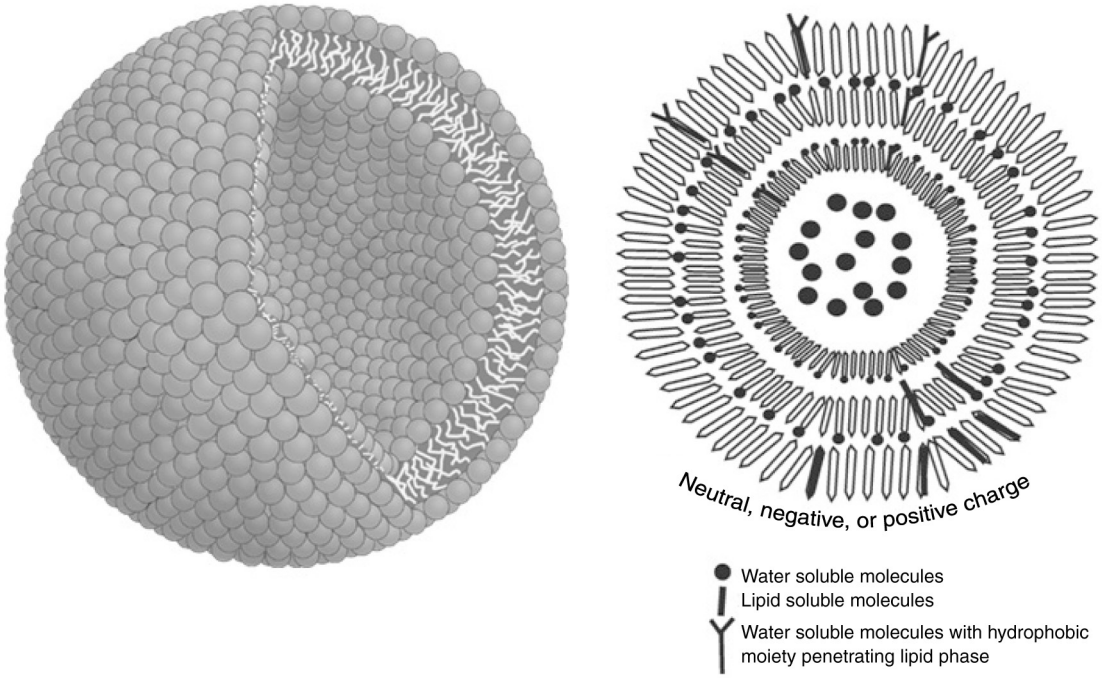

Figure 14.15. Diagrammatic representation of a liposome and inner view showing three bilayer phospholipid membranes alternating with aqueous spaces.

used in solid phase analysis, liposomes have now been used in continuous immunuassays called flow-injection immunoassays (Locascio-Brown et al., 1990).

3.2.11 Fluorescence Enzyme–Linked Immuno Sorbent Assay

A variant of the conventional ELISA method is the fluorescence enzyme–linked immuno sorbent assay (F-ELISA) technique, which has been utilized to detect very low concentrations of proteins in blood. It is called Fluorescence ELISA or F-ELISA as it involves use of an enzymatic reporter, which generates a fluorescent product. The method involves production of microscopic beads with protein-specific antibodies coated on their surface. The beads are placed isolated from each other in femtolitre volume reaction chambers. As the immunocomplex is captured, it is labeled with the fluorogenic enzymatic reporter to generate a fluorescent signal that can be detected using fluorescnence imaging. Quantitative data can be obtained by counting the number of fluorescent wells relative to the total number of wells (Rissin et al., 2010).

3.2.12 Nanowire Biosensor

Nanowires can be defined as unidimensional nanostructures with large aspect ratios useful for manipulating electrons, photons, plasmons, phonons, and atoms (Guo et al., 2013). Nanowires have high surface-to-volume ratio, minimum power consumption, and the potential to be packed in high-density nanoscale devices. They can be used for efficient transport of electrons for optical excitation. Owing to their high surface-to-volume ratio and quantum confinement effect, minor perturbations are sufficient to strongly affect their electrical properties enabling single molecule detection. 1-D structure can be used direct due to limited loss of signal intensities, label free readout, and are particularly suitable for rapid and real-time monitoring (Wanekaya et al., 2006). Optical detection is aided with nanowires because of their high mechanical flexibility, sidewall smoothness, and diameter uniformity (Guo et al., 2013).

3.2.13 Ion Channel Switch

Ion channel switch biosensors utilize electrical transduction due to flux of ions through the channel. The interactions can be measured at the membrane surface to which the ion channel is tethered. Anlaytes such as bacteria, protein, DNA, and drugs can be quantitatively analyzed using this sensor (Woodhouse et al., 1999). Ion channel switch biosensor has been used for rapid screening of influenza A virus in clinical samples (Oh et al., 2008).

3.2.14 Viral

Pathogen detection using biosensor involves specific surface functionalization using molecular probes specific for the target. Antibodies and nucleic acid probes have been routinely utilized for this purpose. However, these probes are susceptible to environmental conditions and are sometimes cross-reactive, costly, and technical expertise is required for the operation. Bacteriophages have been exploited as alternative probes for pathogen detection due to their specificity, selectivity to the host, and ease of amplification. Additionally, as phages can propagate only in live bacteria, they reduce the chances of false positives (Brovko et al., 2012). Once the phage is bound to the host, the host cell can be detected by labeling the phage using fluorescent dyes which can be visualized using fluorescent microscopy or detected using flow cytometery. Phages can be utilized for the replacement of antibodies in detection involving SPR. Genetically engineered phages with modified surface properties to display specific molecules (phage display) or to emit detectable signal (reporter

phages) have been utilized for probing. Phage amplification and phage-induced lysis-based methods have also been developed (Cho et al., 2014). However, drawbacks such as induction of lysis of host, drying effect resulting in loss of capturing ability, limit their use (Singh et al., 2013).

3.2.15 PEBBLE: Probes Encapsulated by Biologically Localized Embedding

Spherical optical nanosensors or PEBBLEs range in size from 20 to 600 nm in diameter and consist of fluorescent indicators entrapped in a matrix consisting of polyacrylamide/polydecylmethacrylate or sol–gel (silica, ormosil) nanoparticle (Clark et al., 1999). They encapsulate the sensing element within an inert matrix, thereby protecting it. Owing to their small size, specificity, and sensitivity comparable to macroscopic ion selective electrodes, they can be used for sensing ions in cellular environment with much better response time and detection limit (Buck et al., 2004). PEBBLEs have been developed for intracellular detection of small analytes like protons, calcium, magnesium, zinc, potassium, sodium, chloride, and hydroxyl ions as well as oxygen and glucose.

3.2.16 Nanoshell Biosensor

Metal nanoshells are composed of a core of dielectric nanoparticle like silica encapsulated by an ultrathin metal shell usually made up of gold. These nanoshells have unique optical properties that are amenable to fine-tuning. By manipulating the size and composition of each layer of nanoshell, they can be made to absorb or scatter light in different regions of electromagnetic spectrum. These particles can be easily conjugated with antibodies and other probes and are also effective substrates for SERS, making them an ideal candidate for biosensor design (Hirsch et al., 2006). The restriction of "free-electron-like" metal substrates such as Au, Ag, and Cu nanostructures in SERS can be overcome by using a shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) technique. This technique has a potential to make SERS applicable to surface with any composition and morphology by utilizing Au-core silica-shell nanoparticles (Au@SiO(2) NPs) (Li et al., 2013).

3.2.17 Array Biosensors

Array biosensors are composed of a solid substrate harboring a two-dimensional array of recognition sites (biochips). Biochips can have a separate detection system as in array plate biochips or an integrated detection system such as integrated

circuit microsensor chip as in the case of detector array biochips (Vo-Dinh et al., 2001). Biochips offer advantages of multiplexed detection and can be conveniently used for medical diagnosis.

3.2.18 Microfluidic Devices/Lab on a Chip

Microfluidic devices are nanosensors in miniature format. The main advantage includes high sensitivity, greatly reduced working volumes, and scope of miniaturization to increase portability for on-site monitoring (Ravichandran, 2010). Silicon-based microfluidic devices are called laboratory on chip technology and has been used to test food additives like benzoate, sorbate, beta hydroxyl benzoic acid esters, glutamate by carrying out electrophoresis in chip set up (Bodor et al., 2001).

4 Detection Using Nanosensors

The nanosensors have shown great promise for the fabrication of novel biosensors with faster response and higher sensitivity than that of planar sensor configurations, due to their small dimensions combined with a dramatically increased contact surface and strong binding with biological and chemical reagents. Such nanosensors have important applications in food industry.

4.1 Detection of Leakage or Spoilage

Production, processing, and shipment of food products could be made more secure through the use of nanosensors for pathogen and contaminant detection. Nanomaterials are being developed with enhanced mechanical and thermal properties to ensure better protection of foods from exterior mechanical, thermal, chemical, or microbiological effects.

4.1.1 Oxygen

To increase the shelf life, a variety of food items are packaged under vacuum or under modified gaseous atmosphere (Valdés et al., 2009). Leakage of oxygen inside food packaging causes its deterioration owing to lipid oxidation and microbial growth. It may also cause discoloration, change in texture, odor, and flavor (Bowles and Lu, 2014). The traditional method of measurement of oxygen in package is destructive in nature where package is punctured to extract atmosphere of the package and loaded into electrochemical fuel cell for analysis (Bültzingslöwen et al., 2002).

A photoactivated indicator ink developed by Mills (2005) provides users with a method to directly detect modified atmosphere

packages (MAPs) which might be damaged during transport or storage. The ink contains a redox active dye—methylene blue—and TiO_2 or SnO_2 nanoparticles, which change color in response to oxygen and allow users to visually analyze the package qualitatively before buying. An oxygen-sensing device has been developed with methylene blue TiO_2 nanocomposite deposited on glass slide, followed by spin-coating of triethanolamine embedded in hydroxy ethyl cellulose under UV irradiation, resulting in bleaching of blue color due to reduction of methylene blue to leuco form. In the presence of molecular oxygen, the dye is reoxidized and blue color reappears (Lee et al. 2005; Gutiérrez-Tauste et al., 2007; Mills and Hazafy, 2008, 2009).

Luminescent nanostructures such as polystyrene-block-vinyl pyrrolidone nanobeads have also been utilized to sense oxygen. The detection is based on quenching of luminescence of the luminescent nanobeads (stained with metal ligand complex). Although not directly applied in food industry but being used in monitoring bioreactors (Borisov and Klimant, 2009).

4.1.2 Moisture

A noninvasive method for detecting moisture level in package was devised by employing a matrix of copper nanoparticles covered by silicon tenside film and a wetting agent. The strip color is based on the change of color of internanoparticle separation due to swelling of polymer matrix in humid environment (Luechinger et al., 2007).

4.1.3 Carbon Dioxide

Carbon dioxide is usually added in packaging to reduce bacterial growth (Bültzingslöwen et al., 2002). To monitor the MAPs, CO_2 is traditionally analyzed using infrared absorption spectrometry. But the analysis leads to destruction of packaging and also does not guarantee complete quality control (Bültzingslöwen et al., 2002). A nondestructive method based on immobilized fluorescent pH indicator using modified silica matrix has been described (Bültzingslöwen et al., 2002). Analysis of the lifetime of luminescent dye was carried out using polymer nanobeads, which encapsulate a fluorophore. This CO_2 sensor has a wide detection range of 0.8–100% having low (0.6%) cross-sensitivity with oxygen.

4.1.4 Gaseous Amine

Gaseous amines such as trimethylamine and others are widely used as indicators of fish and meat spoilage. A nanosensor composed of nanofibrils of perylene-based fluorophores has been used

to detect gaseous amines even at very low level (Che et al., 2008; Che and Zhang, 2009). The same could also be determined with SnO_2 composites and TiO_2 microrods whose conductance was dependent on changes in gaseous amine levels (Zhang and Zhang, 2008). The detection limit is found to be low 1 μg mL^{-1} and the sensor is selective and stable with prompt response and recovery.

A quartz crystal microbalance (QCM)-based sensor was developed to detect Trimethylamine (TMA) by depositing a polyaniline TiO_2 nanocomposite on electrode (Zheng et al., 2008). Surfactant-modified ZnO/polyvinyl pyrrolidone composite films are spin-coated on Al electrodes and utilize for sensing TMA by Tang et al. (2006) with a detection limit as little as 0.4 μg mL^{-1}.

4.1.5 Hypoxanthine

Hypoxanthine, a major catabolic product of ATP, imparts a bitter taste in fish and is an indicator of fish freshness. In flatfish, it can be used as measure of duration of icing (Valdés et al., 2009). An amperometric biosensor using gold nanoparticles based on activity of xanthine oxidase was developed by Cubukcu et al. (2007) for determination of hypoxanthine in tuna fish samples. Similar approach was used by Agui et al. (2006) for the amperometric determination of hypoxanthine in stored samples of sardines and chicken meat.

4.1.6 Volatile Organics

A sensor series composed of SnO nanobelts or ZnO-TiO_2 nanocomposites has been utilized for simultaneous detection of volatile organic compounds like acetone, ethanol and carbon monoxide (Comini et al., 2005, 2006; Barreca et al., 2007).

4.1.7 Ethylene Gas

Ethylene is a well-known indicator of fruit ripening, softening, and senescence. Continuous monitoring of ethylene allows storage and distribution centers to manage their produce according to its status. WO_3–SnO_2 nanocomposites have been used for the detection of ethylene gas and consequent ripening of the fruit (Pimtong-Ngam et al., 2007). A WO_3–SnO_2 binary oxide sensor is inserted in the middle of a quartz tube. Ethylene gas is detected using conductivity measurements at 300°C. There is a linear response between 2 and 8 μg mL^{-1} with sensor.

4.2 Detection of Pathogens and Their Products

4.2.1 Pathogens

New technology allows government regulatory agencies to identify a bacterial pathogen and trace it back to its source more

rapidly. The majority of foodborne disease outbreaks result from unintentional contamination of a product as a result of inappropriate processing or handling (WHO, 2007). According to data from Centers for Disease Control and Prevention, one-sixth of the US population becomes ill due to foodborne diseases (CDC, 2011). The conventional method to detect microbial contamination includes culture dependent and biochemical identification, immunological detection, and PCR-based detection (Velusamy et al., 2010). Culture-based methods are accurate and reliable but are time consuming and labor intensive. They usually take 7–10 days for confirmatory results (Chu et al., 2008; Velusamy et al., 2010). Immunological methods on the other hand are highly sensitive and faster but have narrow detection range (Craig et al., 2013). PCR-based methods are also very sensitive but might give errors arising from nontarget amplification and require pathogen enrichment. Therefore, rapid, sensitive, and portable pathogen detection is the need of the hour (Craig et al., 2013). SERS-based approach captures pathogens of interest using selective biomolecules, imparting specificity to the sensor. They can be easily used to differentiate between species and strains (Velusamy et al. 2010, 2012). Although there has been significant progress in biosensor design, simultaneous detection of multiple pathogens is often accompanied by compromises in either precision or sensitivity (Ravindranath et al. 2011).

4.2.1.1 *E. coli*

E. coli O157:H7 is major foodborne pathogen which causes an estimated 70,000 infections annually in the USA due to consumption of contaminated raw clover sprouts, ground beef, salads, frozen food samples, organic spinach, and spring-mix blends (CDC, 2015). The infections due to production of microbial toxins damage the intestinal lining, causing stomach cramps and anaemia called haemolytic uremic syndrome and hemorrhagic colitis. Sensing techniques that enable culture enrichment or amplification are of great interest (Valdés et al., 2009) as the minimum dose of *E. coli* O157:H7 that can cause infection is fewer than 100 organisms (Tuttle et al., 1999).

The quartz crystal microbalance (QCM) DNA sensor method involves immobilization of a thiolated, *E. coli* O157:H7 specific ssDNA (eaeA gene) on the surface of QCM sensor through self-assembly (Mao et al., 2006). The hybridization of the ssDNA probe to the complementary target DNA (in presence of pathogen) resulted in mass change, which could be sensed in the form of variation in resonant frequency of the QCM. Streptavidin conjugated Fe_3O_4 nanoparticles are used to amplify the frequency change to enhance the mass. *E. coli* O157:H7 eaeA gene fragments

are amplified using asymmetric PCR with biotin labeled primers detected to sense upto 2.67×10^2 colony forming unit (cfu) mL^{-1}. Functionalized –COOH multiwall carbon nanotubes have been utilized in association with Nafion modified glassy carbon electrode (GCE) for detection of coliforms amperometrically (Cheng et al., 2008). A suspension of Nafion (perfluorosulfonated cation-exchanger polymer) solution/MWCNT is poured on the carbon electrode and the solvent is allowed to evaporate to generate the modified GCE. Galactosidase enzyme, characteristic of coliforms when reacts with *p*-aminophenol β-galactopyranoside substrate added in the bacterial solution, hydrolyzes it to *p*-aminophenol detected by the MWCNT/Nafion modified GCE. The method having a detection limit of 10 cfu mL^{-1} for the detection of *E. coli* in river water is normally recommended in the food industry due to rapidity and sensitivity.

Zhang et al. (2007) recommended bismuth nanofilm to modify a glassy carbon electrode for *E. coli* detection for medical, environmental and food applications. The assay is based on the presence of the enzyme β-D-glucuronidase in *E. coli*, which catalyzes the hydrolysis of externally added substrate 4-nitrophenyl β-D-glucuronidase to 4-nitrophenol (4-NP). The reaction is detected in the form of a reduction peak at –0.53 V. The assay is completed in just 3 h and could detect upto 100 cfu mL^{-1} *E. coli* cells.

Immobilized self-assembled peptide nanotubes (PNTs) on carbon paste electrode have been developed to detect *E. coli* O157:H7 electrochemically (Cho et al., 2008). FITC labeled anti-*E. coli* antibodies are then attached to the electrode. The antigen–antibody interaction is sensed by cyclic voltammetry using redox probe; the device is highly portable allowing easy field sampling (Cho et al., 2008).

Impedance-based biosensor for rapid detection of *E. coli* O157:H7 in ground beef has been recommended in which a gold microelectrode is coupled with magenetic (Fe/Fe_2O_3), antibody coated nanoparticles using biotin streptavidin chemistry (Varshney and Li, 2007). Once the target is captured onto the magnetic nanoparticles (via antigen–antibody interaction), the nanoparticles are concentrated on an interdigitated array microelectrode for detection. The method did not require a redox probe, however the LOD (limit of detection) is quite high (3×10^5 cfu mL^{-1}). A further improvement on this type of sensor is to embed labeled magnetic nanoparticles in custom made microfluidic flow cell to achieve lower LOD (Varshney et al., 2005; Varshney et al., 2007).

Temur et al. (2010) employed a SERS-based sandwich immunoassay along with gold nanoparticles to detect *E. coli* with a limit of detection of 5 cfu mL^{-1}. In addition, the method has proved to

be highly specific for detection of *E. coli* against *Enterobacter aerogenes* and *Enterobacter dissolvens*. Combined with a SERS-based sandwich immunoassay along with antibody labeled magnetic nanoparticles was employed to enumerate *E. coli* with a linear correlation from 10^1–10^4 cfu mL^{-1} and a LOD of 8 cfu mL^{-1} and analysis time less than 70 min (Guven et al., 2011).

Detection of *E. coli* using optical method has also been reported by Ravindranath et al. (2009). Abdalhai et al. (2015) have developed an electrochemical genosensor for *E. coli* O157:H7 in beef sample. Cho et al. (2015) proposed a simple and rapid technique to detect low levels of *E. coli* O157:H7 in pure culture and ground beef-based on SERS. They utilized two antibody-conjugated nanoparticle complexes (gold and magnetic) to capture and separate target bacteria. The bacteria complexed with the nanoparticles were then localized using silver intensification and analyzed by SERS having high sensitivity (up to 10 cfu mL^{-1}) and short detection time (1–3 h).

4.2.1.2 Salmonella

Salmonella is the second most frequently occurring pathogen causing foodborne illnesses in the USA (CDC, 2015). Infection of *Salmonella enteritidis* and *S. typhimurium* causes Salmonellosis—an enteric disease. *Salmonella infantis* has also been reported to cause foodborne outbreaks associated with the egg and especially the chicken meat. Villamizar et al. (2008) developed a network of single-walled carbon nanotubes SWCNTs to construct a field effect transistor sensor for detecting *S. infantis*. SWCNTs were conjugated with anti-*Salmonella* antibodies. The interaction between antigen–antibody resulted in a decrease in electric current. The detection limit of this biosensor was 100 cfu mL^{-1} of the pathogen in 1 h.

4.2.1.3 *Mycobacterium avium* spp. paratuberculosis

Mycobacterium avium spp. paratuberculosis has been quantitatively detected from contaminated milk by observing effects of magnetic particle agglomeration (Kaittanis et al., 2007).

4.2.1.4 Brucella

Brucella has been detected in blood serum of infected cattle with synthesized magnetic nanoparticles (Fornara et al., 2008).

4.2.1.5 *Listeria monocytogenes*

An IMS-based sensor utilizing magnetic nanoparticles has been developed by immobilizing the specific antibodies on the surface of the functionalized iron oxide nanoparticles. The sensor

is utilized for detection of *L. monocytogenes* in artificially contaminated milk samples (Yang et al., 2007). Electrochemical detection of *L. monocytogenes* has also been reported (Wang et al., 2009).

4.2.1.6 *Vibrio parahaemolyticus*

Outbreaks involving *V. parahaemolyticus* are prevalent in areas where raw and undercooked shellfish is consumed in daily diet. An amperometric immunosensor has been described for the detection of *V. parahaemolyticus* (Zhao et al., 2007). The sensor was constructed by employing a composite of agarose doped gold nanoparticles to coat electrode. This membrane is then used to immobilize HRP-labeled anti-*V. parahaemolyticus* antibody. The reduction of cathodic peak current in relation to the presence of *V. parahaemolyticus* was used to sense the pathogen.

4.2.1.7 *Bacillus cereus*

A direct-charge transfer (DCT) biosensor based on antigen–antibody interaction and direct electron flow to generate a resistance signal has been employed to detect the foodborne pathogen, *B. cereus* (Pal et al., 2007). It utilizes *B. cereus* specific antibodies as sensing element and polyaniline nanowire as electrical transducer with a sensitivity of 10–100 cfu mL^{-1} for pure cultures of *B. cereus*.

4.2.1.8 Simultaneous Detection of Multiple Pathogens

Simultaneous detection of multiple pathogens is of particular interest as it saves time and effort to check for presence of different pathogens in addition to saving the sample. Several multipathogen detection nanosensors have been developed.

A SERS-based nanosensor has been described to detect multiple pathogens like *E. coli*, *Salmonella*, or *Listeria* in several matrices in real time (Liu et al., 2008; Weidemaier et al., 2015). The assay bypasses the requirement of wash steps required for complex samples. In addition, the method allows continuous monitoring of pathogen through enrichment process. The SERS-labeled immunoassay reagents are added in the enrichment vessel, and the real-time signal is monitored through the wall of the vessel, which also enables a bio-contained food safety testing (Liu et al., 2008; Weidemaier et al., 2015).

A sensitive multiplex fluorescence immunoassay using multicolor quantum dot probes labeled with the specific antibodies to simultaneously detect three pathogens *S. enteritidis*, *Staphylococcus aureus,* and *E. coli* have been developed to test multiple food samples (Wang et al., 2015). Simultaneous detection of different strains of *Shiga*-toxin producing *E. coli* (STEC) have been

demonstrated using optical biosensing with gold nanoparticles functionalized with oligonucleotide (Quintela et al., 2015). SERS-based strategy has been utilized to detect *E. coli*, *E. coli* O157:H7, *Staphylococcus epidermidis*, *E. coli* DH 5α, *S. aureus,* and *Salmonella typhimurium* individually and in mixture (Chu et al., 2008). They generated AgNP substrate by oblique angle deposition method. SERS bands common to all bacterial species and specific to different species could be identified. PCA was applied to discriminate between Gram stain types, species, and strains. The reliability was improved by applying linear discrimination analysis. *E. coli, S. typhimurium,* and *S. aureus* at a concentration of 10 cfu mL^{-1} were detected using SERS probes composed of silver nanospheres formed by assembly of silver nanocrystals. Fan et al. (2011) utilized SERS-based strategy involving silver nanosubstrates to differentiate *E. coli* O157:H7, *Listeria monocytogenes*, *S. epidermidis*, and *Enterococcus faecelis* and compared two approaches, one involving gold-coated microscope slide and second involving internal deposition of AgNPs inside cells. It was concluded that the latter strategy was able to enhance the Raman signals to a large extent compared to the former by detecting and discriminating even single cell (Fan et al., 2011).

Wang et al. (2011) developed a SERS-based immunomagnetic sensor for multipathogen detection in food matrix where *Salmonella enterica* serovar Typhimurium and *S. aureus* were detected in spinach wash water and peanut butter sample using silica-coated magnetic probes labeled with pathogen specific antibodies. Gold nanoparticles integrated with Raman reporters and labeled with antibodies against same pathogen were utilized for the sandwich assay and obtained LOD of 10^3 cfu mL^{-1}. Ravindranath et al. (2011) developed a SERS-based method utilizing different nanoprobes labeled with Raman reporter molecules in addition to pathogen specific antiligands. When the specific pathogen is present, these probes quickly bind to the pathogen surface and filter out on Millipore nanoporous membrane of 450 nm pore size, while the unbound probe is washed out. The probes attached to the captured bacteria can then be used to directly carry out Raman spectra analysis without the requirement of additional processing steps. This strategy was successfully used to detect *S. typhimurium*, *S. aureus,* and *E. coli* O157:H7 by incorporating gold nanoparticles functionalizd with *S. typhimurium* aptamers, silver NP functionalized with anti-*S. aureus* antibodies and core-shell NPs functionalized with anti-*E. coli* O157:H7 antibodies. The nanoparticles were coupled with Raman reporters and the pathogen was subsequently detected using confocal Raman approach and an LOD ranging from 10^2–10^3 cfu mL^{-1} was achieved (Ravindranath et al., 2011).

4.3 Toxins

4.3.1 Microcystin-LR

Traditionally, Microcystin-LR toxin produced by cyanobacterium *Microcystis aeruginosa* in water has been detected using ELISA but now this method can be replaced with a 10 times more sensitive method using carbon nanotubes coated with anti-MCLR antibodies (Wang et al., 2009). The binding of the toxin on the surface initiates a change in electrochemical conduction, which can be detected up to 0.6 nM sensitivity (Wang et al., 2009).

4.3.2 Aflatoxin in Milk

A highly sensitive immunosensor that detects aflatoxin B17 in milk has been developed. This sensor is based on piezoelectric AuNP (Jin et al., 2009).

4.3.3 Ochratoxin A

Ochratoxin-A foodborne fungal contaminant can be detected electrochemically by utilizing chitosan nanocomposite along with cerium oxide nanoparticle immuosensor (Kaushik et al., 2009). A plasmonic-based optical biosensor for Ochratoxin-A has been developed by Todescato et al. (2014); tested in different matrices like dried milk, juices, and wheat mix. A sensitive Ochratoxin-A sensor based on aptamer has been developed by employing Ochratoxin-specific aptamer in conjunction with complementary DNA and double-strand specific fluorescent dye pico green to detect the toxin (Lv et al., 2014).

4.3.4 Staphylococcal Enterotoxin B

Nanowire transistors (Mishra et al., 2008) and carbon nanotubes-based optical immunodetection (Yang et al., 2008) is being used for enterotoxin B detection.

4.3.5 Cholera Toxin

Carbon nanotubes-based technology has been developed for detection of cholera toxin with very high sensitivity (Viswanathan et al., 2006).

4.3.6 Botulinum Toxin Serotype A

Botulism has been of major concern due to its possible bioterrorism applications. Quantum dots method labeled with the respective antibody has been developed for higher LOD (Warner et al., 2009).

4.4 Banned Dyes and Adulterants

4.4.1 Urea

Urea is added in milk to increase solid not fat value (SNF) and nitrogen content. This misleads the conventional testing of protein content. More than 70 mg dL^{-1} urea in milk can lead to indigestion, urinary tract obstruction, renal failure, gastrointestinal bleeding, and cancer (Nikoleli et al., 2010). Urease functionalized AuNPs-based bio-sensors (Nair et al., 2013) and unmodified gold nanoparticles-based aptamer sensors (Kumar et al., 2015) have been developed for detection of urea.

4.4.2 Melamine

Melamine in dairy products can lead to urinary calculi, renal failure and may result in death in infants (Cheng et al., 2010; Lin et al., 2008). Melamine adulteration has lead to major outbreaks related with contaminated infant formula milk in China in 2008 and contaminated pet food in United States in 2007, leading to market recalls and huge economic losses. Melamine levels need to be monitored (Smoker and Krynitsky, 2008; Turnipseed et al., 2008) in order to maintain the safe level in infant formula (up to 1 mg L^{-1}) and food products (up to 2.5 mg L^{-1}). Nanosensor-based approaches have been utilized to monitor melamine levels in food products replacing time consuming traditional chromatographic detection. An optical sensor has been developed which is based on the property of cyanuric acid to selectively bind melamine. Presence of melamine causes concentration dependent aggregation AuNPs fuctionalized with cyanuric acid resulting in a visible color change from red to blue. This sensor is highly sensitive and can detect melamine up to a concentration as low as 2.5 ppb (Ai et al., 2009). Another approach was based on formation of AuNP formation in presence of reductant. In presence of melamine, the reductant is unable to cause formation of AuNP with no change in color to red (Cao et al., 2010). Melamine has also been detected colorimetrically by using AuNPs and ether modified thiols (Kuang et al., 2011). A visual method based on 3-mercaptopriopionic acid MA molecules conjugated on AuNP surfaces to form MA-modified AuNPs (MA-AuNPs), acting as nanoprobes has been used for the detection of melamine in infant formula (Cai et al., 2014).

Li et al. (2014) have overcome the problems of random spreading and dilution of liquids by fabricating a super hydrophobic-oleophobic mesh-like surface from Ag nanowires for SERS-based detection of melamine and Sudan I in water and toluene. Spiked milk samples were also successfully analyzed using this sensor.

4.4.3 Food Colorants

Ponceau 4R and Allura Red in soft drinks (Zhang et al., 2010) and Sudan 1 in ketchup or chilli powder (Mo et al., 2010) has been detected using carbon nanotubes. The colorant specific oxidation peak undergoes intensity changes on the basis of intensity of analyte. Sunset yellow and chrysoidine have been detected using a SERS-based method utilizing a substrate composed of SiO2@Au nanoshells with a detection limit of 1 ppm and 0.5 ppm respectively (Xie et al., 2014).

4.5 Pharmacological Residues

A SERS-based technique utilizing dendritic AgNPs has been reported for detection of ciprofloxacin, enrofloxacin, and chloramphenicol (He et al., 2009) replacing time-consuming traditional chromatographic antibiotic detection. The nanosubstrate is composed of AgNPs deposited on a gold-coated glass slide. The three antibiotics could be resolved by PCA and LOQ by around 20 ppb. Chloremphenicol and crystal violet, used as an illegal antifungal agent in aquaculture, has also been detected using Klarite and Q-SERS substrates (Lai et al., 2011b) with an LOD of 20–50 ppb. An immuno-chromatographic test strip based on Ru(phen)3(2+)-doped silica fluorescent nanoparticle (FN) has been developed for detection of enrofloxacin (ENR) residues in chicken meat (Huang et al., 2013) and for furazolidone, enrofloxacin, and malachite green (an industrial dye) in tilapia fillets using SERS (Zhang et al., 2012). Clenbuterol, a drug used illegally to promote growth in food animals can impair lung and heart functions in humans. A competitive SERS immnunoassay has been developed with 4,4'-dipyridyl, and clenbuterol antibody labeled AuNPs attached to a substrate for competitive combination to detect this drug in pig urine (Zhu et al., 2011). Sulpha drugs such as sulfamethazine, sulfamerazine, and sulfamethoxazole are used commonly to promote growth of farm animals and to control disease outbreaks. These have been detected using Klarite SERS-active substrate with an LOD of 10 ng mL^{-1} (Lai et al., 2011a). Leanness of swine carcass is improved using drug raptopamine, which is banned in China and European Union due to the potential health risk though legal in USA (Zhai et al., 2011). Overdose of Theophyllin, a drug widely used for airway obstruction treatment can be lethal (Liu et al., 2011b; Mitenko et al., 1973). Theophyllin is found in certain food products such as tea, coffee, and chocolate and has been detected using AgNPs coated with biomimetic receptors with an LOD of 10 μM in spiked green tea samples (Liu et al., 2011b). Phenolic estrogens have been detected indirectly in infant formula using SERRS (surface-enhanced resonance Raman scattering) with an LOD of 0.1 ppb (Han et al., 2011).

4.6 Pesticides

There is a growing concern over the presence of trace amounts of pesticide residues in products derived from the crops. These residues might lead to long-term heath effects (Craig et al., 2013). Therefore, to assure safety of consumers, monitoring of pesticides in agricultural produce on a routine basis is very important. Nanosensor-based rapid, sensitive, and portable methods are being developed to replace routinely used time-consuming chromatographic method for pesticide analysis (Vongsvivut et al., 2010).

4.6.1 Parathion and Paraoxon

Parathion is a moderately toxic organophosphorus insecticide. Wang and Li (2008) developed a ZrO_2/Au nanocomposite film electrode for determination of Parathion with the LOD of 3 ng mL^{-1}. The sensor requires a preabsorption of parathion on electrode and detection was based on square wave voltametry.

Paraxon is a metabolite of organophosphorous insecticide parathion and has neurotoxic property. Constantine et al. (2003) developed an optical nanosensor for detecting paraoxon. The in-solution detection was carried out in a film assembly composed of layers of alternately arranged film of chitosan, organophosphorous hydrolase, and thioglycolic acid capped CdSe quantum dots. Exposure to organophosphorous compound led to its hydrolysis aided by OP hydrolase resulted in altered conformation and subsequent decrease luminescence of quantum dots. A similar approach based on CdSe–ZnS quantum dots was developed by Ji et al. (2005). However, real samples need to be tested to validate the effectiveness (Valdés et al., 2009). Vamvakaki and Chaniotakis (2007) used inhibition of activity of enzyme acetyl cholinesterase to detect Paraxon. The enzyme was encapsulated into liposome along with a pH sensitive fluorophore pyramine. Upon addition of substrate thioacetlycholine, hydrolysis resulted in decrease in pH, which could be sensed by pyramine. The change in pH reduction in presence of pesticide could be monitored fluorometrically.

4.6.2 Amitrole

Amitrole is a herbicide with a maximum detectable residue limit of 0.01 mg kg^{-1} by employing a carbon paste electrode modified with Fe(II)-phthalocyanine (FePh) nanoparticles to detect amitrole using square wave voltametry (Siswana et al., 2006). Crystal violet and malachite green are banned antimicrobials, which are used in aquaculture to improve production. If not used within prescribed limits, they can be detected in fish grown in contaminated water (Zheng and He, 2014).

4.6.3 Fonofos (Organophosphorous Pesticide)

A highly sensitive SERS-based technique for detecting fonofos pesticide utilizing metal colloids and AgNPs has been developed by Vongsvivut et al. (2010).

4.6.4 Sulphur-Containing Pesticides

A SERS-based approach have been used to identify sulphur containing pesticides in grapes, apple, mango, pear, and peach peels (Liu et al., 2011a). Silver-coated AuNPs have been used with strong absorption of plasmonic resonance to achieve much higher enhancement of Raman scattering than the individual AuNPs or AgNPs in this technique.

4.6.5 Tricyclazole

Quantification of tricyclazole was based on SERS and a portable Raman instrument is used to carry out the analysis in paddy rice (Tang et al., 2012). Pesticides that enter through water are detected using nanoscale liposome-based detector (Vamvakaki and Chaniotakis, 2007).

4.7 Contamination Caused by Packaging Material

Melamine used in packaging material may also enter food under inappropriate storage/ handling conditions. As already described earlier, melamine contamination can be detected by using AuNPs (Ai et al., 2009; Cao et al., 2010; Kuang et al., 2011).

4.8 Allergens

Gliadin (inflammation causing protein in patients suffering from celiac disease) and gluten can be detected using nanostructured silver island films in close proximity to rhodamine labeled antigliadin antibodies. The metal-enhanced fluorescence is detected using enhanced fluorescence linked immuno-sorbent assay (Staiano et al., 2009) while peanut allergens by Speroni et al. (2010).

5 Nanobiosensors in Food Technology Market

The nanotechnology market in future will be driven by enhanced sensitivity and high throughput detection. The food and medical industries will offer maximum opportunities in this regard in areas of quality management and control during production, processing, packaging, storage, and transportation. The growth in

Table 14.3 Commercialized Nanobiosensors for the Food Industry

Contaminant	Broad Category	Method of Detection	Commercial Name	References
Trans fat content	NEMS	Digital transform spectrometer	Polychromix	Ritter (2005)
Pathogens in food and water	Cantilever	Sensing ligand receptor interactions	European funded project: BioFinger	Jain (2008)
Flavor analysis of vegetable oils	Surface acoustic wave electronic nose	SAW vapor print	Z-Nose	Gan et al. (2005)

revenue generation is expected to be concomitant with advancements in nanotechnology. Nanosensors incorporating improved nanomaterials showing better characteristics will be the major thrust in the market. Factors such as improved design, reduced complexity, and wireless architechture will also play a critical role. As these nanosensors begin to prove their worth, their demand will be enhanced as evident from the example of nanobots—the nanosensors containing tiny transducer chips for storage. The total market share for nano sensor in 2012 was $1.2 billion and some important companies involved with nanosensor research and development are (1) Dow Corning, (2) Samsung, (3) Boeing, (4) Lockheed Martin, (5) IBM, (6) Motorola, (7) Agilent, (8) start-ups (Nanomix and Ambri). Few nanobiosensors that have been commercialized are listed in Table 14.3.

6 Safety and Challenges

Toxicity of nanoparticles is dependent upon a large number of factors including the size, structure, shape, elemental constituents, mass concentration, solubility, surface area, surface charge, and surface chemistry (Tiede et al., 2008). Enhanced bioavailability of certain nutrients or food additives might have unfavorable effects (Ravichandran, 2010).

Nanomaterials used in food packaging are usually not inhaled or ingested by the end users, although there is a risk that they may enter food. If this happens, their use should be based on acceptable daily intake (ADI) (Ravichandran, 2010). In addition, the effect of these nanomaterials on the normal microbial flora of gut and

mouth needs to be assessed beforehand (Sozer and Kokini, 2009). A second possible exposure is via the inadvertent release of nanomaterials in the environment such as disposal of smart surfaces. This type of exposure may have unforeseen, untested effects (Ravichandran, 2010). Therefore, the long-term fate and disposal of smart surfaces should be considered prior to use. Presently there are no worldwide-accepted rules or regulations regarding nanotechnology-based food products. Manufacturers have to follow general existing regulations for food products; however, the guidelines will vary from country to country. The USFDA does not specifically cover nanoparticles. The Institute of Food Science and Technology (UK), European Commission reiterates the requirement of modification of existing laws for example, ingredient labeling to allow consumer to take informed decisions. TA Swiss (Swiss center for technology assessment) allows use if additives appear on a positive list of tested items identifiable by an E-number, which is again not nanomaterial-specific (Ravichandran, 2010). The list of generally accepted as safe (GRAS) additives should be reexamined when used at a nanoscale level (Ravichandran, 2010). Ultimate success of any nanomaterial-based product depends on consumer acceptance. Public needs to be groomed to increase acceptability of these 'Atomically modified foods." They should be allowed to take informed decisions by incorporating the nanomaterial component in the labeling (Ravichandran, 2010).

7 Future Prospects

The most promising breakthrough of the development of online or on-site, sensitive, low-cost, rapid methods for routine use are expected to be made in the area of nanosensor technology. Many prototypes for food diagnostic application in the food and drink industry are currently being developed. They have high potential for automation and allow the construction of simple and portable equipment for fast analysis. These properties will open up many applications within quality and process control, quality and safety control of raw materials, and for HACCP monitoring. New technology must have high sensitivity, high specificity, high precision (repeatability), at the same time rapid, robust, and cheap. There is currently no method that will fulfill all requirements. A variety of nanosensor platforms are available for sensing applications in food industry that offer specific, sensitive, and cost-effective detection of contaminants. However, these technologies have to prove their worth for real food samples. The major challenge among the detection systems used is the refinement of sample preparation steps so that even an unskilled person can

perform the analysis. An ideal sensor would be one in which the sample preparation module is integrated with the on-site sensing system. Thus, versatile, multifunctional sensing systems, which can work in a variety of complex food matrices are the need of the hour. Although multiplexing has been initiated with the e-nose technology for detection and analysis of complex aroma compounds, further work is required to delineate sample processing steps for different types of food matrices so that the same sensor could work in different types of food samples. Efforts are also likely to be made to reduce the material cost in nanosensor-based systems and to integrate sensors in packaging material for direct consumer analysis. Future applications might include protein quality and the detection of allergens, genetically modified proteins, BSE prions, pathogens, and biocide residues.

8 Conclusions

Nanomaterials have no doubt been the major drivers in the field of biosensing in food industry catering to the specific needs of the industry including safety testing, real-time monitoring of food products during storage and transportation and on-site testing to ensure consumer satisfaction. With their widely acclaimed qualities such as appropriate and easily alterable size, unique electrical, optical, chemical, magnetic, and semiconducting properties and enhanced sensing surface area that can be easily functionalized, they surpass the routinely used biosensors in terms of sensitivity and accuracy, quick response, and direct localized detection. A range of nanosensor platforms is now available with widely differing detection principles. The simplest nanosensors are colorimetric metal nanoparticle detectors, which produce a visible reaction upon reaction with analyte. Carbon nanotubes have superior mechanical properties and have found application in nanoelectromechanical system (NEMS). Electronic noses and electronic tongues mimic the human nose and tongue by decoding patterns of volatile and nonvolatile compounds respectively. Nanocantilevers work by generating mechanical bending of nanodevices in response to the presence of analyte. Optical nanosensors can be used to probe subcellular locations using fluorescence phenomenon and a fiber-optic nanoprobe. Aptamer sensors can be used to design biological recognition molecules similar to antibodies based on custom requirements. SERS utilize Raman scattering signal of analytes adsorbed on nanomaterial surfaces. Liposome nanovesicles have been used to amplify fluorescent signals in immunosensing applications. PEBBLE can be used for intramolecular detection of small analytes. Microfluidic

devices/lab-on-a-chip devices offer advantages of working with small sample volumes and rapid detection. Nanosensors have been applied for various applications including detection of pathogens, toxins, spoilage, banned chemicals, pharmacological residues, pesticides, packaging material residues, and allergens. In future, further advancements are likely to be directed at decreasing the cost of the sensor, increasing the rapidity for on site testing, automated analysis, as well as streamlining the sample preparation methods for diverse food matrices. Prospective applications include development of nanosensors for detecting genetically modified proteins, BSE prions, and biocide residues.

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