CHAPTER TWO

Novel Biosensors for the Rapid Detection of Toxicants in Foods

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Abstract

The modern environmental and food analysis requires sensitive, accurate, and rapid methods. The growing field of biosensors represents an answer to this demand. Unfortunately, most biosensor systems have been tested only on distilled water or buffered solutions, although applications to real samples are increasingly appearing in recent years. In this context, biosensors for potential food applications continue to show advances in areas such as genetic modification of enzymes and microorganisms, improvement of recognition element immobilization, and sensor interfaces. This chapter investigates the progress in the development of biosensors for the rapid detection of

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food toxicants for online applications. Recent progress in nanotechnology has produced affordable, mass-produced devices, and to integrate these into components and systems (including portable ones) for mass market applications for food toxicants monitoring. Sensing includes chemical and microbiological food toxicants, such as toxins, insecticides, pesticides, herbicides, microorganisms, bacteria, viruses and other microorganisms, phenolic compounds, allergens, genetically modified foods, hormones, dioxins, etc. Therefore, the state of the art of recent advances and future targets in the development of biosensors for food monitoring is summarized as follows: biosensors for food analysis will be highly sensitive, selective, rapidly responding, real time, massively parallel, with no or minimum sample preparation, and platform suited to portable and handheld nanosensors for the rapid detection of food toxicants for online uses even by nonskilled personnel.

1. INTRODUCTION

A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. Chemical sensors usually contain two basic components connected in series: a chemical recognition element (receptor) and a physicochemical transducer. The recognition system translates the chemical information (i.e., concentration of the analyte) into a chemical or physical output signal. The transducer (i.e., a physical detection system) serves to transfer the signal from the output domain of the recognition element to the electrical, optical, or piezoelectric domain. A biosensor is a self-contained integrated device which is capable of providing specific quantitative analytical information using a biological recognition element (e.g., enzymes, antibodies, natural receptors, and cells) which is retained in direct spatial contact with a transduction element. Recent advances in the technology of artificial receptors have prompted a clear distinction between chemical sensors and biosensors. The latter utilize a transduction element of biological origin; however, since there has not been much development in engineered molecules, both terms are and can be used in the literature for this class of devices. The chemical sensors should be clearly distinguished from an analytical system which incorporates additional separation steps, such as liquid chromatography (LC) or additional hardware and/or sample processing such as specific reagent introduction, e.g., flow injection analysis (FIA). Biosensors have not yet made a large impact in the area of environmental, food, or biomedical applications, but clearly offer advantages in comparison to standard analytical methods, such as minimal sample preparation and

handling, real-time detection, rapid detection of the analytes of concern, use of nonskilled personnel, etc. Because of the importance of the ability of biosensors to be repeatedly calibrated, the term multiple-use biosensor is limited to devices suitable for monitoring both the increase and decrease of the analyte concentrations. Thus, single-use devices which cannot rapidly and reproducibly be regenerated should be named single, etc.

Environmental and food biosensors come in literally thousands of forms and types based on a wide range of physical and chemical principles with varying types of usable outputs. Typical environmental contaminants monitored are metals, volatile organic compounds, biological contaminants, and radioisotopes. The field applications of sensors are also extremely varied. Among the key trends in the environmental and food sensors business is miniaturization down to the nanoscale, continuous and/or real-time sensing capabilities, wireless networked operation, rapid processing, and increased sensitivity or flexibility. Areas of environmental focus include vehicular emissions, combustion of fossil fuels, agricultural runoff, industrial and mine waste disposal, ocean spills and dumping, and climate change and weather monitoring. However, very few examples of portable biosensors have been reported in the literature being used online and can rapidly detect environmental pollutants directly in the gas phase.

The specific objective of this monograph is mainly to exploit progress in nanosciences to deploy nanotechnology in affordable, mass-produced sensors, and to integrate these into components and systems (including portable ones) for mass market applications in environmental and food monitoring. The sensing targets include chemical and microbiological environmental toxicants, such as chlorinated hydrocarbons, heavy metals, polychlorinated dibenzodioxins (commonly called dioxins), nitrogen oxides (NO and NO_2), components of the photochemical smog which is a noxious mixture of air pollutants (SO_2, aldehydes, nitrogen oxides, peroxyacyl nitrates, etc.), chlorofluorocarbons (CFCs), hydrogen sulfide, water pollution (by the discharge of wastewater from commercial and industrial waste), discharges of untreated domestic sewage, insecticides, pesticides, and herbicides.

2. ELECTROCHEMICAL BIOSENSORS

Electrochemical biosensors can be categorized according to the measured electrical parameter:

- potentiometric biosensors that measure the difference between two potentials at equilibrium (in volts);

- amperometric (or voltammetric) biosensors, which measure the current (or charge) due to electron transfer (in amperes or coulombs);
- conductometric biosensors (chemiresistors) that measure the resistance (in ohms) or conductance (the reciprocal of resistance); and
- impedimetric biosensors that measure the impedance (in ohms).

The biosensing elements can be tissue parts, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids (NAs), aptamers, recombinant antibodies, synthetic receptors, biomimetic catalysts, combinatorial ligands, and also imprinted polymers as biomimetic elements. The specific molecular recognition that leads to high selectivity and specificity is based on the affinity between complementary structures such as enzyme with substrate or antibody with antigen. The result of this interaction is the conversion of a biochemical signal into a quantifiable electrical response which is a function of concentration.

A short history regarding the discovery and developments of electrochemical biosensors is given in Taylor and Schultz (1996).

Potentiometric biosensors, which measure a potential difference at zero current, i.e., at equilibrium, are of two types, in which either ion-selective electrodes (ISEs) or electrolyte–insulator–semiconductors are used in order to transduce the biological reaction into an electrical signal. For both of these transducer types, the key part is an ion-selective membrane in contact with the analyte-containing solution, the membrane being responsible for the selectivity to target ions in the presence of interfering ions in the sample being analyzed. The main phenomenon responsible for generation of the response, i.e., the potential difference across the membrane, is ion exchange between the two membrane/solution phases, which depends on the activity of the target ion (analyte) in these phases (Eisenman, 1967). For an ISE and in the simplest case, the potential difference across the membrane is measured by an internal reference electrode immersed in the inner solution whose composition is constant and an external reference electrode which is immersed in the analyte-containing solution. An ISE biosensor can consist, for example, of a membrane with immobilized enzyme surrounding a pH electrode (Newman & Setford, 2006), where the enzymatic reaction occurs next to the thin sensing glass membrane causing a change in pH.

This potential is described by the relevant Nernst equation:

$$E = E_0 + \frac{RT}{zF} \ln a$$

where E is the measured potential (V), E_0 is a characteristic constant for the ion-selective/external electrode system, R is the gas constant, T is the absolute temperature (K), z is the signed ionic charge, F is the Faraday constant, and a is the activity of the ionic species of interest. The logarithmic dependence of the potential on the ionic activity, often replaced by concentration, is responsible for the wide analytical range and for the high accuracy and precision of these sensors.

The majority of potentiometric biosensors for detection of environmental pollutants have used enzymes that catalyze the consumption or production of protons. Biosensors based on ISEs were reported for detection of insecticides such as aldicarb, carbaryl, carbofuran, and dichlorvos, the principle consisting in cholinesterase enzyme inhibition (Marty, Garcia, & Rouillon, 1995). Heavy metal ions can also be measured using the enzyme urease coupled to an ammonium ion sensor. Because the activity of urease is sensitive to heavy metal ions, inhibition of enzyme activity can be used to estimate the total concentration of these ions (Shi, Stein, & Schwedt, 1997).

A few examples of microorganism biosensors for toxicant detection based on potentiometric transducers are summarized in Table 1 (Lei et al., 2006).

In voltammetric and amperometric sensors, the measurement is based on the transfer of electrons across the sensor–electrolyte interface, the current measured being directly proportional to the number of electroactive species which are being oxidized or reduced.

For *voltammetric sensors*, a current–voltage profile is recorded, the current being measured as a function of applied potential using a potentiostat. Occasionally, the potential response to an applied current is recorded using a galvanostat. Unlike potentiometric sensors, where a potential difference is

Table 1 Examples of Microorganism Biosensors for Toxicant Detection Based on Potentiometric Transducers (Lei, Chen, & Mulchandani, 2006)

Target	Microorganism	Transducer Type
Organophosphates	*Flavobacteium* sp.	pH electrode
	Recombinant *E. coli*	
Penicillin	Recombinant *E. coli*	pH electrode
Urea	*Bacillus* sp.	NH_4 electrode
Trichloroethylene	*Pseudomonas aeruginosa*	Chloride electrode
Ethanol	*Saccharomyces ellipsoideus*	Oxygen sensor

measured at equilibrium, usually three electrodes are necessary: working (sensor), auxiliary, and reference electrodes; the reference electrode can also serve as auxiliary electrode if the currents are very small (Brett & Oliveira-Brett, 2011). The applied potential is the driving force for the electron transfer reaction and the current results from the electrochemical oxidation or reduction of the electroactive species. The current should be directly proportional to the bulk concentration of the electroactive species. Voltammetric techniques that are commonly employed including linear sweep and cyclic voltammetry, differential pulse voltammetry (DPV), and square wave voltammetry (SWV); a description of the fundamentals of these voltammetric techniques can be found in Brett and Oliveira-Brett (1998). With voltammetric sensors the information obtained is diverse, detection limits can be low (down to nanomolar with preconcentration), and more than one electroactive species, if each reacts at different applied potentials, can be determined in the same experiment. Thus, in the most easily applicable situations for these types of sensor, there is no need for prior separation of components of complex mixtures.

Enzyme biosensors have been used in food toxicity and environmental monitoring. The main analytes monitored by amperometric enzymatic biosensors for environmental and food applications are summarized in Table 2. Some applications in these areas are:

Heavy metals: Inhibition-based biosensors for heavy metal determination have been the subject of reviews (Amine, Mohammadi, Bourais, & Palleschi, 2006; Turdean, 2011). An amperometric biosensor based on urease has been developed for the detection of mercury, cadmium, and arsenic at a screen-printed electrode with rhodinised carbon (Pal et al., 2009). Cadmium, copper, lead, and zinc have been determined in the micromolar range by inhibition of glucose oxidase at a poly(neutral red) carbon film modified electrode (Ghica & Brett, 2008) for detection in milk.

Pesticides and herbicides: Enzymatic biosensors based on inhibition are also used to measure pesticides, mostly acetylcholinesterase in combination with choline oxidase (Andreou & Clonis, 2002; Arvinte et al., 2006; Choi, Kim, Lee, Min, & Lee, 2001). A remote biosensor online monitoring of organophosphate (OP) pesticides has been developed using organophosphate hydrolase (OPH) (Wang & Chen, 1995) and the capability of various pesticides to inhibit tyrosinase has been reported (Smit & Rechnitz, 1993; Wang & Chen, 1995).

Toxins: Aflatoxins (AFs) are a group of highly toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*

Table 2 Application of Amperometric Enzyme Biosensors in Environmental and Food Toxicity

Target	Enzyme	References
Cd^{2+}, As^{2+}, Hg^{2+}	Urease	Pal, Bhattacharyay, Mukhopadhyay, and Sarkar (2009)
Cd^{2+}, Co^{2+}, Pb^{2+}, Zn^{2+}	Glucose oxidase	Ghica and Brett (2008)
Methyl paraoxon	Acetylcholinesterase	Arvinte, Rotariu, and Bala (2006)
Organophosphate	Organophosphate hydrolase	Wang, Chen, Mulchandani, and Chen (1999)
Cyanide	Tyrosinase	Smit and Rechnitz (1993)
Hydrazine	Tyrosinase	Wang and Chen (1995)
Aflatoxin B1	Acethylcholinesterase	Rejeb et al. (2009)
Phenol, 4-chlorophenol, catechol	Tyrosinase	Malzahn, Windmiller, Valdes-Ramirez, Schöning, and Wang (2011)
E. coli	Tyrosinase	Hasebe, Yokobori, Fukasawa, Kogure, and Uchiyama (1997)
Biogenic amines	Polyamine oxidase	Esti et al. (1998)
Ethanol	Alcohol oxidase	Barsan and Brett (2008)
Acetaldehyde	Aldehyde dehydrogenase	Ghica, Pauliukaite, Marchand, Devic, and Brett (2007)
Hypoxanthine	Xanthine oxidase	Torres, Ghica, and Brett (2013)

fungus; an aflatoxin B_1 sensor has been developed for measurement in olive oil based on inhibition of acetylcholinesterase (Rejeb et al., 2009).

Phenolic compounds: A considerable number of organic pollutants, that are found widely distributed in the environment, have phenolic structures. Phenol, 4-chlorophenol, and catechol were determined by a tyrosinase-containing carbon ink printed on a wearable neoprene (Malzahn et al., 2011). Phenolic compounds in olive oil have been measured by DPV using a screen-printed electrode with immobilized tyrosinase (Capannesi, Palchetti, Mascini, & Parenti, 2000).

Microorganisms: Bacteria, viruses, and other microorganisms are found widely in polluted untreated and treated waters and constitute hence a public

health problem. An amperometric biosensor for the detection of *Escherichia coli* in wastewater was developed based on tyrosinase-catalyzed oxidation of polyphenolic compounds, which are produced microbiologically from salicylic acid (Hasebe et al., 1997).

Biogenic amines: Biogenic amines, mainly putrescine, cadaverine, spermidine, histamine, and tyramine, are not only biosynthesized in animal and plant cells but also produced by microbial decarboxylation of amino acids. Amperometric biosensors for amines were reported for determination in fish using screen-printed electrodes with monoamine oxidase and putrescine oxidase (Chemnitius & Bilitewscki, 1996) and in fruits (Esti et al., 1998) using diamine oxidase and polyamine oxidase immobilized on polymeric membranes.

3. PIEZOELECTRIC BIOSENSORS

Several anisotropic crystals exhibit the piezoelectric effect—mechanical deformation results in oriented dipoles and electric voltage. In the opposite, applying alternating voltage excites vibrations and at resonance frequency equal to the natural vibrations, transfer of energy from the electric field to the crystal is most efficient and the energy remains preserved in the oscillating system. A thin plate of quartz (AT-cut) is covered with metal (gold) electrodes on both sides and inserted in a holder for simplified manipulation and contacting.

A typical piezoelectric quartz crystal is shown in Fig. 1. A thin plate of quartz (AT-cut) is covered with metal (gold) electrodes on both sides and inserted in a holder for simplified manipulation and contacting.

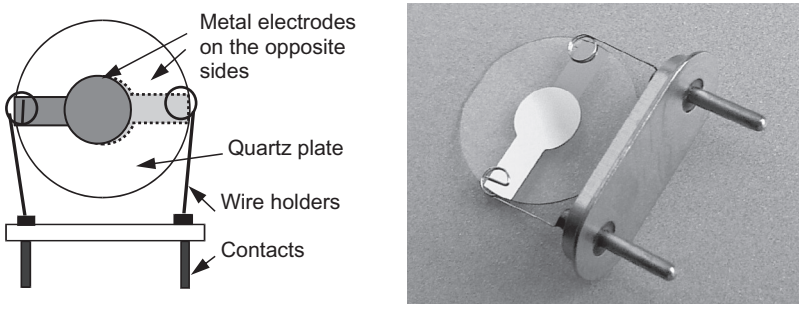

Fig. 1 Piezoelectric quartz crystal resonator: schematic description (*left*), photo of the polished smooth plate with metal electrode on top (*right*).

The piezoelectric transducers are used as chemical sensors since the discovery of the relationship between mass of adsorbed films and the resonant frequency by Sauerbrey:

$$\Delta f = -\frac{2f_0^2 \Delta m}{A\sqrt{\rho_q \mu_q}} = -2.26 \times 10^6 f_0^2 \frac{\Delta m}{A} \quad (1)$$

The change Δf of the resonant frequency f_0 is directly proportional to the mass change Δm, the numeric constant applies to calculations using Δf in Hz, f_0 in MHz, and Δm in $\mathrm{g\,cm^{-2}}$. The typical working frequencies are from 5 to 20 MHz. The amount of molecules bound on the sensitive area of electrodes can be easily quantitatively measured as a decrease of frequency; according to recent studies, the microbalance response is more complex.

Table 3 summarizes the recent most interesting applications of piezoelectric quartz sensors in the areas of interest. In addition to immunosensors, the examples of DNA sensors are presented. The presented examples demonstrate that piezoelectric biosensor can be combined with different recognition strategies not limited to classic immunosensors and hybridization sensors. The list of measured compounds and biological objects is quite illustrative.

4. ENZYMATIC BIOSENSORS

The specificity of every biosensor is due to the specificity of the bioreceptor molecule used. *Enzymes* have been the most widely used bioreceptor molecules in biosensor applications. Enzymes are globular proteins containing from 62 to over 2500 amino acids and act as catalysts for biochemical reactions. Their activity is determined by their chemical composition and three-dimensional structure. An enzyme is capable of recognizing only a specific target substrate (a substrate is a molecule upon which an enzyme acts) because the enzyme and the substrate have complementary geometric shapes that fit exactly into one another. During interaction, the substrate binds with the enzyme active site, giving rise to an enzyme–substrate complex. The substrate is converted into one or more products that are released from the active site of the enzyme is ready to accept new substrate species.

Different enzymatic reactions commonly exploited for fabrication of biosensors are listed in Table 4.

Over the last years, nanomaterials (metal and metal oxide nanoparticles, nanowires, carbon nanotubes, and quantum dots), by virtue of their unique

Table 3 Selected Piezoelectric Biosensors for the Assay of Diverse Analytes in Food Samples

Analyte	LOD	Time	Sensing Principle/Sample Matrix	References
Alcohol volatiles (*Salmonella* produced)	<5 ppm	10 min	Biomimetic peptide (odorant binding protein), gas analysis/beef	Sankarana, Panigrahi, and Mallik (2011)
Chloramphenicol	0.2 ng mL^{-1}	30 min	PPy-chloramphenicol, competitive immunosensor/milk, meat, and honey	Karaseva and Ermolaeva (2012)
Cryptosporidium parvum	3×10^5 cells mL^{-1}	1.5 h	Direct immunosensor	Poitras, Fatisson, and Tufenkji (2009)
Cymbidium mosaic potexvirus	25 ng mL^{-1}	5 min	SWCN-Tween-Ab, direct immunosensor/orchid leaves	Chen et al. (2010)
Desulfotomaculum sp.	1.8×10^4 CFU mL^{-1}	70 min	Magn. NP-vancomycin labels cells, magn. attached to PZ/sea mud	Wan, Zhang, and Hou (2010)
Enterotoxin A	7 ng mL^{-1}	25 min	Sandw immunosensor/milk	Salmain, Ghasemi, Boujday, and Pradier (2012)
Escherichia coli	10^7 CFU mL^{-1}	5 h	Growth curve/milk, chicken stock	Plata, Contento, and Ríos (2011)
E. coli O157:H7	53 CFU mL^{-1}	2 h	Magn. NP–Ab capture, sandw immunosensor, AuNP-Ab2, particle enlargement/milk	Shen et al. (2011)
Formaldehyde	1 ppm	<10 min	Polyethylene/nanofiber cellulose/air	Hu, Chen, Liu, Ding, and Wang (2011)

GMO (*pflp* gene)	$0.25\,ng\,\mu L^{-1}$	PCR +1 h	Thiol-oligonucleotide probe, PCR, hybridization/tobacco	Karamollaoglua, Öktema, and Mutlu (2009)
Hemorrhagic septicaemia virus	1.6 nM	PCR +20 min	SA/biotin-probe, RT-PCR, hybridization/fish	Hong, Jeong, and Hong (2010)
Lactobacillus sp.	$100\,CFU\,mL^{-1}$	4 h	Microbe-induced coagulation/milk	Jang, Chang, Lee, Lee, and Hsu (2009)
Microcystin-LR	1 ng mL	3 h	Vesicles-dendrimer-Ab, sandw immunosensor, Ab2-AuNP/surface water	Xia, Zhang, and Jiang (2011)
Shrimp allergen	$330\,ng\,mL^{-1}$	10 min	AuNP-thiol-Ab, direct immunosensor/food	Xiulan et al. (2010)
Toxicity of nanomaterials	$3\,\mu g\,mL^{-1}$	1–6 h	DH82 macrophage cells, phagocytosis, apoptosis/cytotoxicity	Wang et al. (2011)
Vibrio harveyi	$10^3\,CFU\,mL^{-1}$	(?)	Direct immunosensor/shrimp culture	Buchatip et al. (2010)

Abbreviations: *Ab*, antibody; *Ab2*, secondary Ab; *GMO*, genetically modified organisms; magn., magnetic; *NP*, nanoparticle; sandw, sandwich immunocomplex; *SA*, streptavidin; *SWCNT*, single-walled carbon nanotubes.

Table 4 Common Enzymes and Reactions Used in Enzymatic Biosensors

Enzyme	Enzymatic Reaction
Alcohol oxidase	ethanol + O_2 → acetaldehyde + H_2O_2
Glucose oxidase	glucose + O_2 → gluconic acid + H_2O_2
Lactose oxidase	lactose + O_2 → pyruvate + H_2O_2
Alcohol dehydrogenase	ethanol + NAD^+ → acetaldehyde + NADH + H^+ acetaldehyde + NAD^+ → acetic acid + NADH + H^+
Lactate dehydrogenase	lactate + NAD^+ → pyruvate + NADH
Acetylcholinesterase	acetylcholine + H_2O → choline + acetic acid
Choline oxidase	choline + O_2 → betaine aldehyde + H_2O_2
Horseradish peroxidase	H_2O_2 + electron donor → $2H_2O$ + oxidized donor (electron donor = phenols, aromatic amines, luminol, etc.)
Urease	urea + H_2O → CO_2 + $2NH_3$
Tyrosinase	phenol + $1/2O_2$ → quinone + H_2O
Lipase	triglycerides + $3H_2O$ → *g*lycerol + 3 fatty acids
Nitrate reductase	NO_3^- + NADH + H^+ → NO_2^- + NAD^+ + H_2O
Alkaline phosphatase	*p*-nitrophenyl phosphate + H_2O → *p*-nitrophenol + $H_2PO_4^-$
Creatinine diaminase	creatinine + H_2O → NH_4^+ + *N*-methylaydantonin

physical and chemical properties, have attracted considerable attention for the design of electrochemical and optical enzymatic biosensors that exhibit high sensitivity and stability. Biomolecules have been immobilized on nanostructure materials by traditional enzyme immobilization methods (adsorption, entrapment, or covalence). The rationale behind using nanoscale structures for immobilization is to reduce diffusion limitations, to enhance the electron transfer between the enzyme redox center and the electrode surface and to maximize the functional surface area in order to increase enzyme loading (and hence the catalytic activity of attached enzymes).

Carbon nanotubes possess remarkable electrical, mechanical, and structural properties. They have an outstanding ability to mediate fast electron transfer kinetics for a wide range of electroactive species involved in enzymatic reactions, such as hydrogen peroxide or NADH. In addition, carbon nanotubes chemical functionalization can be used to attach almost any

desired chemical species to them. This has permitted the realization of composite electrodes comprising carbon nanotubes based electrodes with immobilized enzymes. In such electrodes, the carbon nanotubes mainly serve as transducers, communicating the signal effectively from the active enzyme centers to the substrate.

The vast majority of enzymatic biosensors existing today exploit either electrochemical (amperometric and potentiometric) or optical (absorbance, fluorescence, and chemiluminescence) transduction to convert the action of the bioreceptor molecule into a measurable signal. Other transducers (such as enzymatic thermistors) and detection modes (electrochemical impedance) do exist but are rather specialized and outside the scope of this work. Different enzymes commonly used in biosensors, their substrates, the species detected and the detection modes used are listed in Table 5.

5. ANTIBODY-BASED BIOSENSORS

Molecules of all antibodies have the general structural principle based on paired heavy and light polypeptide chains that allows to integrate them into the common chemical class of immunoglobulins. In most cases, the biosensors are used by immunoglobulins G (IgG) prevailing in serum. Y-shaped IgG molecule consists of three structural blocks: one branching constant block and two identical variables. Disulfide bonds provide linkage of polypeptide chains of these blocks together.

Molecular diversity of immunoglobulins is provided by variable structural blocks through three mechanisms:

- The multiplicity of genes encoding the variable domains, the one gene only is selected and expressed in each antibody-producing cell.
- Additional somatic combination of segments by which these genes are divided.
- Mutations in the course of B-cells differentiation and their transformation into plasma cells.

The combined effect of these three processes provides high variability: several 1000 kinds of heavy and light chains of immunoglobulins (their number varies slightly depending on animal specimen) can be formed in the body. Aggregation of light and heavy chains leads to the formation of the active antigen-binding antibody site which may have millions of variants estimate the range of 2×10^7–10^8 (Conroi, Hearty, Leonard, & O'Kennedy, 2016, De Genst, Messer, & Dobson, 2014). Contact with some antigen

Table 5 Different Enzymes Commonly Used in Biosensors, Their Substrates, the Species Detected, and the Detection Mode Used

Substrate	Enzyme	Species Detected	Detection
Choline	Choline oxidase	H_2O_2	Amperometry
Ethanol	Alcohol oxidase	H_2O_2	Amperometry
Formaldehyde	Formaldehyde dehydrogenase	NADH	Amperometry
Glucose	Glucose oxidase	H_2O_2, O_2	Amperometry
Glutamine	Glutamine oxidase	H_2O_2	Amperometry
Glycerol	Glycerol dehydrogenase	NADH, O_2	Amperometry
Hypoxanthine	Xanthine oxidase	H_2O_2	Amperometry
Lactate	Lactate oxidase	H_2O_2	Amperometry
Oligosaccharides	Glucoamylase, glucose oxidase	H_2O_2	Amperometry
Phenol	Polyphenol oxidase	Quinone	Amperometry
Hormones	Alkaline phosphatase (as label)	*p*-Nitrophenol	Amperometry
Aspartame	L-Aspartase	NH_3	Potentiometry
Fats	Lipase	Fatty acids	Potentiometry
Glucose	Glucose oxidase	Gluconic acid	Potentiometry
Urea	Urease	NH_4^+, CO_2	Potentiometry
Nitrite	Nitrite reductase	NH_4^+	Potentiometry
Penicillin	Penicillinase	H^+	Potentiometry
Sulfate	Sulfate oxidase	HS^-	Potentiometry
Glucose	Glucose oxidase	Gluconic acid	ISFET
Penicillin	Penicillinase	Penicillic acid	ISFET
Triolein	Lipase	Fatty acids	ISFET
Ethanol	Alcohol dehydrogenase	NADH	Absorbance
Glucose	Glucose oxidase	O_2	Absorbance

Table 5 Different Enzymes Commonly Used in Biosensors, Their Substrates, the Species Detected, and the Detection Mode Used—cont'd

Substrate	Enzyme	Species Detected	Detection
Urea	Urease	Ammonia	Absorbance
Lactate	Lactate monooxygenase	NADH	Absorbance
Penicillin	Penicillinase	Penicillinic acid	Absorbance
Blutamate	Glutamate dehydrogenase	NADH	Absorbance
Pesticides	Acetylcholinestarase	Acetic acid	Absorbance
Chlorophenols	Alkaline phosphatase	*p*-Nitrophenol	Absorbance
Choline	Horseradish peroxidase	H_2O_2	Chemiluminescence
Ethanol	Alcohol dehydrogenase	NADH	Electrochemiluminescence

activates the line of antibody-producing cells, which secretes immunoglobulins that can be bound with this antigen and neutralize it. Available diversity of antibodies provides the potential for the immune response to the compounds of all kinds.

The immune response varies from organism to organism and depends on the physiological characteristics of the animal as well as the kinds of antigens it contacts during its lives. Absolute replication of polyclonal antibody preparation properties during immunization of different animals is impossible. Ideally, to reproduce immunization results it would be better to use linear and specific pathogen free (SPF) animals. However, both the first and especially the second solution increase significantly the cost of antibody production process and are not prominent in the commercial (along with research) practice.

Even after successful immunization, a small fraction of serum immunoglobulins is the antibodies to immunogen; usually they amount to a few percent. Therefore, the preparation of antibodies without "ballast" nonspecific immunoglobulins is preferable to be used in biosensors. To clean the preparation of antibodies, it is usually circulated through the antigen-containing

sorbent and then the bound molecules are eluted. These obtained affine antibodies are specific for a particular antigen, but anyway are the mixture of molecules with different structure of the antigen-binding sites that interact variously with the target antigen (Conroi et al., 2016; De Genst et al., 2014).

The history of antibodies to detect different compounds is much longer than the history of biosensors. By the time of biosensor development the antisera were the only available source of antibodies, and all of the first publications on immunosensors describe the use of polyclonal antibodies specifically.

The majority of immunosensors use antibodies or antigen immobilized on the sensitive surface of the sensor. In the case of immobilized antigen, the recommendations for immobilization are not common and depend on its nature. If the chosen format of analysis is based on immobilization of antibodies (or their derivatives) at the surface of the biosensor and registration of its binding to the antigen in the test sample, the requirements for immobilization method are rather universal. The sensor should provide the maximum ratio of the response to the concentration of the analyte in the sample, thus achieving the lowest detection limits. The maximum compact immobilization of antibodies at the surface of the sensor but not leading, however, to structure change in the active site of the antibody or restriction of its availability to interact with antigen is preferable. Note that it is undesirable to improve the antigen-binding capacity using the procedures that reduce the detected signal—for example, to form additional layers that reduce conductivity of the electrochemical sensor. On the contrary, the spatial convergence of the label and the sensitive surface allow in some cases to detect immune complexes without separation of bound and unbound label (Dzantiev et al., 2004; Ivnitski & Rishpon, 1996).

The simplest way to immobilize the antibody is by physical adsorption. Even though in many systems, this process is accompanied by denaturation or significant conformational changes of antibodies. In addition, according to application of the sensor produced by noncovalent immobilization, especially in nonphysiological media, the number of antibodies dissociated from its surface increases. A clear alternative is covalent coupling of antibodies to chemically activated surface of the sensor. Generally, the methods that are used randomly direct the coupled antibodies against the surface attachable—depending on which reactive group of antibodies is in contact with it.

6. ION CHANNEL SWITCH- AND LIPID FILM-BASED BIOSENSORS

Most of biosensors based on lipid membranes are cost efficient, easy-to-use, fast responding, and are good alternative to mostly expensive, time consuming standard analytical and screening methods (i.e., chromatographic procedures or mass spectroscopy). These devices will be able to be either regenerated for multiple uses or be used as disposable sensors in a single format.

Studies are currently focused on the development and evaluation of methods for the incorporation of ion channels, enzymes, antibodies, or receptors in electrochemical, optical, or mass-sensitive detectors with lipid film as a recognition element or as an ion channel switch-based devices. An example of development of lipid films based sensors are glass fiber filter supported BLMs with incorporated artificial receptor that has been developed by our group for the rapid detection of carbofuran in fruits and vegetables (Nikolelis, Ntanos, Nikoleli, & Tampouris, 2008; Nikolelis, Raftopoulou, Psaroudakis, & Nikoleli, 2008; Nikolelis, Raftopoulou, Simantiraki, et al., 2008). The same principle will be fully extended for the detection of heavy metals, insecticides, and other toxicants in foods. Novel trends include nanofabricated surfaces composed of carbon nanotubes or nanoporous alumina. Substantial attention was made also to formation of hybrid sBLM supported on micro- and nanoporous substrates. The signal generation, especially those connected with redox reactions have been enhanced by modification of lipid layer by nanoparticles.

Stabilized lipid films were prepared by polymerization that was previously described in the literature (Nikolelis & Mitrokotsa, 2002; Nikolelis, Raftopoulou, Nikoleli, & Simantiraki, 2006). Stabilized lipid films were firstly prepared by polymerization by heating at 60°C (Nikolelis & Mitrokotsa, 2002). A schematic version of polymerization stage and preparation of polymerized lipid membranes is shown in Fig. 2. In recent works, however, the polymerization took place by using UV irradiation instead of the thermal polymerization (Nikolelis et al., 2006). The latter method had the advantage that a recognition element, e.g., enzyme could be incorporated prior to polymerization without loss of its activity. Briefly, 0.8 mL of a mixture containing 4% (w/v) egg phosphatidylcholine 4% (w/v) and partially oxidized cholesterol in *n*-hexane were mixed with 0.07 mL of

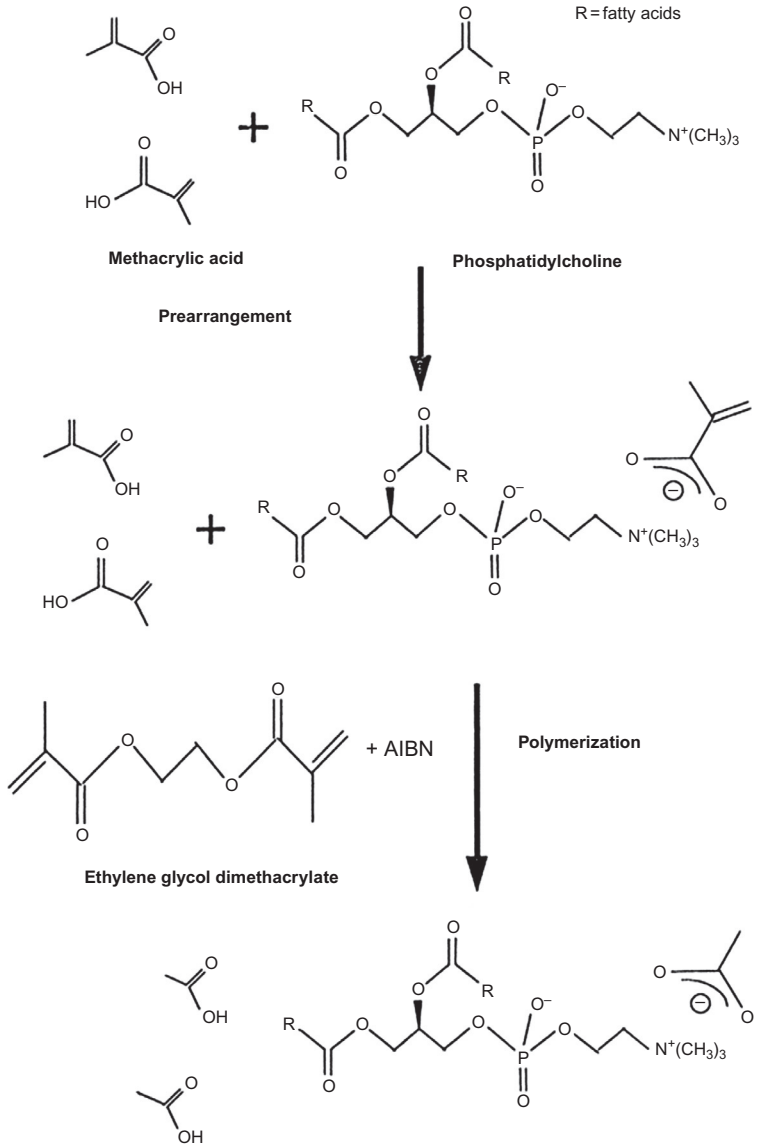

Fig. 2 A schematic version of polymerization stage and preparation of polymerized lipid membranes. *From Nikolelis, D. P., & Mitrokotsa, M. (2002). Stabilized lipid film based biosensor for atenolol.* Biosensors & Bioelectronics, 17, *565–572 with permission.*

methacrylic acid, 0.8 mL of ethylene glycol dimethacrylate, 8 mg of 2,2′-azobis-(2-methylpropionitrile), and 1.0 mL of acetonitrile. The mixture was sparged with nitrogen for about 1 min and sonicated for 30 min. For the preparation of the stabilized lipid films, 0.15 mL of this mixture was

spread on the microfilter. The filter with the mixture was then irradiated using the UV deuterium lamp. Raman spectrometry was used to monitor the kinetics of the process of polymerization. These membranes were stable in storage in air for repetitive uses.

The development of a device based on lipid membranes for analytical applications may include the following steps:

1. Development of stabilized lipid membranes such as those supported on a metal, on ultrafiltration membranes, or on a polymer such as polymethylacrylate, etc.
2. Determination of the optimal method to convert the lipid membranes into systems sensitive to desired analytes. This can be achieved by incorporation of a receptor.
3. Performance of experimental studies to find out whether the permeability of the lipid membranes is altered by an analyte/lipid membrane interaction and to what extent. Investigations were performed to define the optimum experimental conditions for maximized response.
4. Development of the techniques for the direct selective and automated monitoring or rapid screening (in a single format) of compounds of biomedical, pharmaceutical, environmental, and industrial interest, such as insecticides, herbicides, toxins, gas pollutants, etc., based on the results of the above studies. Therefore, a considerable improvement in terms of cost efficiency, availability, and speed of biomedical, pharmaceutical, environmental, and industrial monitoring procedures can be made.

A schematic configuration of the stabilized supported lipid membranes that can be used to detect various analytes in a nonstopped flow technique is presented in Fig. 3.

The stabilized supported lipid membranes were used for the FIA of pesticides (Nikolelis, Simantiraki, Siontorou, & Toth, 2005). The principle of the method is based on the degree of inhibition of the enzyme by the pesticide. Carbofuran was chosen as a typical pesticide. The novelty of the method is based on the fact that an "air segmented" flow injection technique can be used as compared to previous systems that are based on lipid film technology but are not stable in air. This allows the determination of the pesticide using the degree of inhibition and reactivation of enzyme by injections of substrate. Carbofuran was determined at concentration levels of 10^{-7}–10^{-9} mol L^{-1}. The investigation of the effect of potent interferences included a wide range of compounds usually found in foods and also proteins and lipids. The technique was applied in various real samples of fruits, vegetables, and dairy products. The recovery ranged between c.96% and 106% which shows no interferences from the matrix.

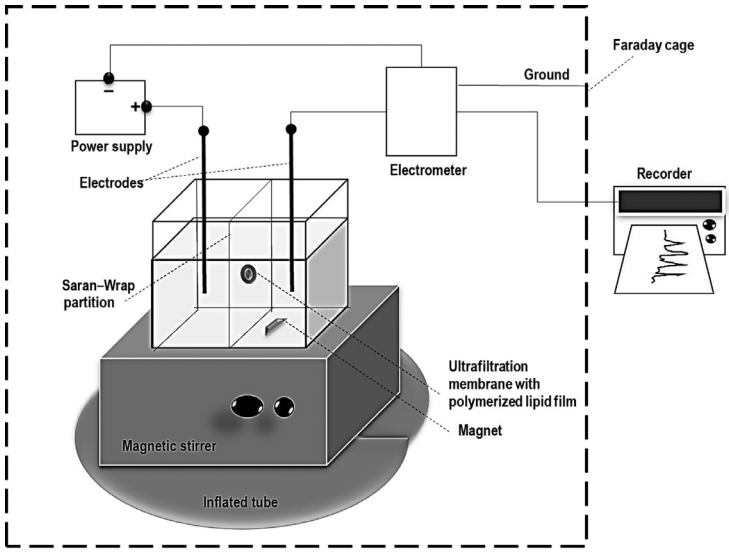

Fig. 3 Simplified version of the experimental set-up used. The microporous ultrafiltration membrane (diameter of approximately 9 mm) with the polymerized lipid film was placed between the two plastic layers of the Saran–Wrap, with the filter centered on a 0.32 mm orifice. The Saran–Wrap is clamped between two Perspex blocks and extends their limits, so no leakage of the electrolyte solution will occur around the edges of the blocks. Stirring can be made with a magnetic flea in a small well. *From Nikolelis, D. P., Simantiraki, M. G., Siontorou, C.G., & Toth, K. (2005). Flow injection analysis of carbofuran in foods using air stable lipid film based acetylcholinesterase biosensor,* Analytica Chimica Acta, 357 *(1–2), 169–177 with permission.*

However, most efficient biosensors for selective detection of various compounds are based on artificial or natural receptors incorporated into lipid films. The supported lipid films modified by calixarenes allowed sensitive and rapid detection of insecticides in fruits and vegetables (Nikolelis, Ntanos, et al., 2008; Nikolelis, Raftopoulou, Psaroudakis, et al., 2008; Nikolelis, Raftopoulou, Simantiraki, et al., 2008). Recent applications in this field include the construction of a disposable chemosensor for the selective rapid detection of carbofuran (Nikolelis, Ntanos, et al., 2008; Nikolelis, Raftopoulou, Psaroudakis, et al., 2008; Nikolelis, Raftopoulou, Simantiraki, et al., 2008) and food hormones (i.e., naphthalene acetic acid—NAA) in fruits and vegetables (Nikolelis, Ntanos, et al., 2008; Nikolelis, Raftopoulou, Psaroudakis, et al., 2008; Nikolelis, Raftopoulou, Simantiraki, et al., 2008) and of zinc in waters (Nikolelis, Raftopoulou, Psaroudakis, & Nikoleli, 2009).

A miniaturized potentiometric urea lipid film-based biosensor on graphene nanosheets has been recently reported in the literature (Nikoleli et al., 2012). Structural characterization of graphene nanosheets for miniaturization of potentiometric urea lipid film-based biosensors has been studied through atomic force microscopy and transmission electron microscopy measurements. UV–vis and Fourier-transform IR spectroscopy have been utilized to study the pre- and postconjugated surfaces of graphene nanosheets. The presented potentiometric urea biosensor (Fig. 4) exhibits good reproducibility, reusability, selectivity, rapid response times ($\sim$4 s), long shelf life, and high sensitivity of c.70 mV/decade over the urea logarithmic concentration range from 1×10^{-6} M to 1×10^{-3} M.

The investigations of electrochemical interactions of NAA with stabilized lipid films supported on a methacrylate polymer on a glass fiber filter with incorporated auxin-binding protein 1 receptor for the development of a biosensor for the rapid detection of this compound in fruits was described (Bratakou et al., 2016). NAA was injected into the flowing streams of a carrier electrolyte solution, the flow of the electrolyte solution stops and an ion current transient was obtained; the magnitude of the signal was correlated to NAA concentration, which could be determined at the micromolar level. NAA preconcentrates at the lipid membrane surface, which causes dynamic alterations of the electrostatic fields and phase structure of membranes. The response times were c.5 min and NAA was determined at concentration levels of micromolar. The effect of potent interferences included a wide

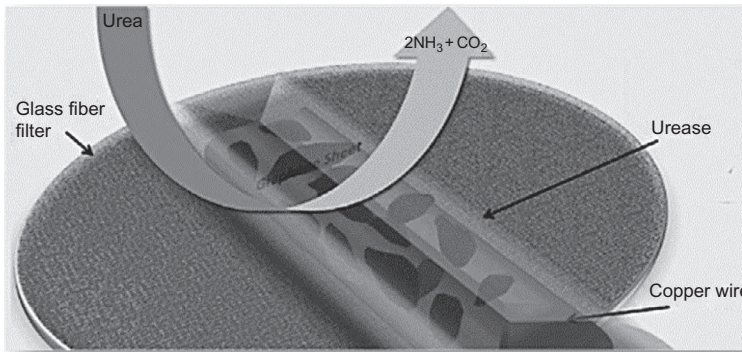

Fig. 4 Schematic image of the urea biosensor. *From Nikoleli, G.-P., Israr, M. Q., Tzamtzis, N., Nikolelis, D. P., Willander, M., & Psaroudakis, N. (2012). Structural characterization of graphene nanosheets for miniaturization of potentiometric urea lipid film based biosensors,* Electroanalysis, 24*(6), 1285–1295 with permission.*

range of compounds. The results showed no interferences from these compounds in concentration levels usually found in real samples. The method was applied for the determination of NAA in fruits, and the reproducibility of the method was checked in samples of various fruits. A quantitative method for the detection of NAA in fruits that can be complimentary to HPLC methods was also provided. These lipid films can be used as portable sensors for the rapid detection of NAA in fruits by nonskilled personnel.

The air stable lipid films supported on a methacrylate polymer on a glass fiber filter with incorporated artificial receptor has been used for detection of zinc (Nikolelis et al., 2009). This minisensor was constructed for the electrochemical FIA of zinc. The device can detect the analyte in a drop (75 μL) of sample. Zinc was injected into flowing streams of a carrier electrolyte solution. A complex formation between the calix[4]arene phosphoryl receptor and zinc takes place. This enhances the preconcentration of zinc at the lipid membrane surface which in turn causes structural changes of membranes. As a result, ion current transients were obtained and the magnitude of these signals was correlated to the substrate concentration. The response time was approximately 5 s and zinc was determined at concentration levels of nmol L^{-1}. The analytical curve was linear in the concentration range 0.1–1.2 μmol L^{-1} with detection limit of 0.05 μmol L^{-1} and a relative standard deviation lower than 4%. The effect of potent interferences included a wide range of other metals. As an analytical demonstration, trace concentrations of Zn(II) were successfully detected in real samples of waters without any laborious and time consuming treatment.

7. OLIGONUCLEOTIDE AND NUCLEIC ACID-BASED BIOSENSORS

Natural nucleic acids (deoxyribonucleic acid—DNA; and ribonucleic acid—RNA) are macromolecules composed of nucleotide chains. Nucleotides consist of a sugar ring (deoxyribose in DNA and ribose in RNA), a phosphate group, and a NA ring (which contains a basic nitrogen group and hence is the base in the nucleotide). These nucleotides form phosphodiester bonds between the 3′ group of one sugar molecule and 5′ group of another. The sugar phosphate backbone does not change, but the base group can be different throughout the chain. There are four NA bases in DNA nucleotides, to give four different nucleotides: adenine, cytosine, guanine, and thymine. Similarly, there are four NA bases in RNA, to give four different nucleotides: adenine, cytosine, guanine, and uracil. The bases can

be either a purine (adenine or guanine) or a pyrimidine (thymine, cytosine, or uracil). DNA is a double helix and has two complementary antiparallel strands, whereas RNA is, in many of its biological roles, single-stranded. The role of NAs in nature is of tremendous importance since they are responsible for carrying genetic information from one generation to another. Passage of the genetic information is done by Watson–Crick base pair recognition of complementary strands. Each adenine in one strand makes two hydrogen bonds with thymine (or uracil) in the complementary strand, and each guanine makes three hydrogen bonds with cytosine. Additionally, the DNA sequence is matched with its complementary sequence in RNA which is further used to make a protein that satisfies the genetic code. The process of building the RNA from the DNA is called transcription, whereas the process of building the protein based on the RNA is called translation.

In recent years, NAs have been incorporated into a wide range of biosensors and bioanalytical assays, due to their wide range of physical, chemical, and biological activities. A biosensor is defined as a compact analytical device incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer. A NA-based biosensor employs as the sensing element an oligonucleotide, with a known sequence of bases, or a complex structure of DNA or RNA.

Short DNA oligonucleotide chains as well as genomic DNA have been employed to develop biosensor for toxicity evaluation. The elaboration of new highly sensitive, specific, and rapid methods of determination of environmentally toxic compounds has always been a challenge to analytical chemists. This is due to the complex ecological situation and the demand for diagnosing the consequences of the impact of biologically active contaminants upon the human organism. For these reasons, the development and characterization of rapid, sensitive, and inexpensive assays for detection of chemically induced damage to DNA caused by pollutants is an important issue in environmental monitoring. A significant number of short-term tests for genotoxicity/mutagenicity have been developed to determine the extent of environmental hazards in polluted water and sediments. These bioassays have been used in well-constructed batteries of cytotoxicity and genotoxicity tests to complement traditional approaches. These tests typically fall into one of several classes depending on their mechanism and end point. These classes include: bacterial mutagenesis, cultured mammalian cell mutagenesis, chromosomal damage, and DNA damage. Despite the description of short term, many of these assays are expensive to run, require sophisticated

technical expertise, and are not well suited to be adapted to screening applications. In recent years, there has been an enormous increase in the use of NAs as a tool in the recognition or monitoring of chemical compounds of environmental interest that may affect the genome. In this context, DNA-based biosensors can be a valid approach to detect of DNA damage and can be designed to be rapid, inexpensive, and user friendly. Similarly, the effect of drugs or natural organic substances on NAs can be exploited by using DNA damage biosensors. Different kind of transducers have been employed, each of them has their own advantages and disadvantages. In particular, electrochemical DNA biosensors can be used: (a) for the detection of DNA strand breaks and base damage and (b) for detection of electroactive substances that specifically interact with DNA (covalently and/or no covalently). The measurement of DNA damage using electrochemical biosensors has been demonstrated using the direct measurement of oxidation–reduction properties of the bases or indirectly using electrochemical probes.

Guanine is the most easily oxidized of the four DNA bases and has an oxidation potential of 1.06 V vs SCE at pH 7. The three remaining nucleobases have more positive oxidation potentials. Guanine and adenine bases in DNA can be oxidized on solid electrodes such as mercury, glassy carbon, and pyrolytic graphite.

Biosensors were also used to measure toxic aromatic amines, oxidative damage, bioactivated benzo(a)pyrene, hydrazine, triazine-based herbicides, and fluorine derivatives (Fojta, Kubicarova, & Palecek, 2000; Lucareli, Palchetti, Marrazza, & Mascini, 2002; Oliveira-Brett & da Silva, 2002; Vlyskoiil, Labuda, & Barek, 2010; Wang et al., 1996; Wang, Jansson, Schenkman, & Rusling, 2005; Wang et al., 1997; Wang & Rusling, 2003). Recently, DNA damage biosensor has been applied to the detection of the antioxidant effect of apple and orange juices (Ziyatdinova & Labuda, 2012).

8. TISSUE, MICROORGANISMS, ORGANELLES, AND CELL-BASED BIOSENSORS

Living bacteria can be immobilized by physicochemical methods onto the measuring surface of the sensors. The method used for immobilization is very important since it may influence bioactivity and cell viability. Horváth, Pedersen, Skivesen, Selmeczi, and Larsen (2003) tested a grating-coupled planar optical waveguide sensor by monitoring the adhesion of *E. coli* K12 cells to the sensor surface. Yeh and Ramsden (2010) investigated a simple method for determining the number of bacteria adsorbed on a planar optical waveguide.

Wei et al. (2007) developed a biosensor based on surface plasmon resonance (SPR) for the rapid identification of *Campylobacter jejuni* in broiler samples. They examined the specificity and sensitivity of commercial antibodies against *C. jejuni* with six *Campylobacter* strains and six non-*Campylobacter* bacterial strains. Antigen–antibody interactions were studied using enzyme-linked immunosorbent assay (ELISA) and a commercially available SPR biosensor platform (Spreeta™). *Campylobacter* cells killed with 0.5% formalin had significant lower antibody reactivity when compared to live cells, or cells inactivated with 0.5% thimerosal or heat (70°C for 3 min) using ELISA. The SPR biosensor showed a good sensitivity with commercial antibodies against *C. jejuni* at 10^3 CFU mL^{-1} and a low cross reactivity with *Salmonella* serotype *typhimurium*. The sensitivity of the SPR was similar when testing spiked broiler meat samples. However, research is still needed to reduce the high background observed when sampling meat products.

A novel SPR biosensor using lectin as bioreceptor was developed by Wang, Ye, Si, and Ying (2013) for the rapid detection of *E. coli* O157:H7. The selective interaction of lectins with carbohydrate components from bacterial cells surface was used as the recognition principle for the detection. Five types of lectins from *Triticum vulgaris*, *Canavalia ensiformis*, *Ulex europaeus*, *Arachis hypogaea*, and *Maackia amurensis* were employed to evaluate the selectivity of the approach for binding *E. coli* O157:H7 effectively. A detection limit of 3×10^3 cfu mL^{-1} was obtained for determination of *E. coli* O157:H7 when used the lectin from *T. vulgaris* as the binding molecule. Furthermore, the proposed biosensor was used to detect *E. coli* O157:H7 in real food samples. Results showed that the lectin-based SPR biosensor was sensitive, reliable, and effective for detection of *E. coli* O157:H7, which hold a great promise in food safety analysis.

A whole cell array biosensor for the efficient detection of neurotoxic OP compounds was developed through the immobilization of recombinant *E. coli* cells containing periplasmic expressing organophosphorus hydrolase (OPH) onto the surface of a 96-well microplate using mussel adhesive protein (MAP) as a microbial cell-immobilizing linker (Kim, Choi, Seo, Lim, & Cha, 2013). Both the paraoxon-hydrolyzing activity and fluorescence microscopy analyses demonstrated that the use of MAP in a whole cell biosensor increased the cell-immobilizing efficiency and enhanced the stability of immobilized cells compared to a simple physical adsorption-based whole cell system. Scanning electron microscopic analyses also showed that the *E. coli* cells were effectively immobilized on the MAP-coated surface without any pretreatment steps. The whole cell array biosensor system, prepared using optimal MAP coating (50 μg cm^{-2}), and cell loading (4 OD_{600}),

detected paraoxon levels as low as 5 μM with high reproducibility, and its quantitative detection range was 5–320 μM. The MAP-based whole cell array biosensor showed a good long-term stability for 28 day with 80% retained activity and a reusability of up to 20 times. In addition, paraoxon in tap water was also successfully detected without a reduction in sensitivity. Their results indicate that the proposed MAP-based whole cell array system could be used as a potential platform for a stable and reusable whole cell biosensor.

Cell-based assays are currently considered central to toxicity and environmental exposure testing. One of the main advantages of cell-based approaches is their possibility to detect not only specific substances but also to supply functional information, i.e., information about general cytotoxicity. Up to now, sensors incorporating living cells face the drawback of a steady and high risk of contamination and cell death, caused by changes in the cellular environment.

A water-based carbon screen-printing ink formulation, containing the redox mediator cobalt phthalo-cyanine (CoPC) and the enzyme glucose oxidase (GOx), was investigated for its suitability to fabricate glucose microbiosensors in a 96-well microplate format (Pemberton et al., 2012): (1) the biosensor ink was dip coated onto a platinum (Pt) wire electrode, leading to satisfactory amperometric performance; (2) the ink was deposited onto the surface of a series of Pt microelectrodes (10–500 mm diameter) fabricated on a silicon substrate using MEMS (microelectromechanical systems—MEMS) microfabrication techniques: capillary deposition proved to be successful; a Pt microdisc electrode of Z100 mm was required for optimum biosensor performance; and (3) MEMS processing was used to fabricate suitably sized metal (Pt) tracks and pads onto a silicon 96-well format base chip, and the glucose biosensor ink was screen printed onto these pads to create glucose microbiosensors. When formed into microwells, using a 340 μL volume of buffer, the microbiosensors produced steady-state amperometric responses which showed linearity up to 5 mM glucose (CV = 6% for $n = 5$ biosensors). When coated, using an optimized protocol, with collagen in order to aid cell adhesion, the biosensors continued to show satisfactory performance in culture medium (linear range to 2 mM, dynamic range to 7 mM, CV = 5.7% for $n = 4$ biosensors). Finally, the operation of these collagen-coated microbiosensors, in 5-well 96-well format microwells, was tested using a five-channel multipotentiostat. A relationship between amperometric response due to glucose, and cell number in the microwells, was observed. These results indicate that microphotolithography and screen-printing techniques can be combined successfully to produce microbiosensors capable of

monitoring glucose metabolism in 96-well format cell cultures. The potential application areas for these microbiosensors are discussed.

Sulfur-oxidizing bacteria can be used for the continuous monitoring of toxicity and their use circumvents many of the obstacles associated with conventional assays (Hassan & Oh, 2010; Oh, Hassan, & Van Ginkel, 2011; Van Ginkel, Hassan, & Oh, 2010).

9. NANOTECHNOLOGY AND NANOFABRICATION APPLICATIONS IN CHEMICAL SENSING

Recently, with the revolutionary advent of nanotechnology a number of new nanomaterials have been synthesized and their properties have been investigated. Moreover, these novel nanomaterials are being used strongly in the biosensor technology. The use of nanomaterials in biosensing describes the consolidation of different sciences such as material science, molecular engineering, chemistry, and biotechnology. The nanomaterials have great impact on the working performance of biosensor by improving the sensitivity and recognition of biomolecules at small volumes. The most advantageous properties of nanomaterials for the biosensing applications include larger surface to volume ratio, different physical and chemical properties from microsurface and easy to detect target analyte in small volumes.

The material science is showing a very close relation with biostuff due to the favorable properties exhibited by synthesized materials. In order to have a highly sensitive and specific biosensor, the choice of substrate to be used is very important for evaluating the performance of biosensor. Several materials at nanoscale have been utilized for the development of biosensors due to their distinctive and typical physical, mechanical, chemical, optical, and magnetic characteristics which significantly improve the sensitivity and the recognition of biomolecules sensing. Such nanomaterials include gold and magnetic nanoparticles, carbon nanotubes, and quantum dots.

A work that describes a miniaturized potentiometric NAA sensor on graphene nanosheets with incorporated lipid films was reported in the literature (Bratakou et al., 2016). Auxin-binding protein 1 receptor immobilized on the stabilized lipid films provided adequate selectivity for detection over a wide range of hormone concentrations, fast response time of c.5 min, and detection limit of 10 nM. The proposed sensor is easy to construct and exhibits good reproducibility, reusability, selectivity, long shelf life, and high sensitivity of c.56 mV/decade of hormone concentration. The reliability of the biosensor was successfully evaluated using a wide range of NAA-spiked fruits and vegetables.

An article that describes a miniaturized potentiometric carbofuran chemical sensor on graphene nanosheets with incorporated lipid films appeared in the literature (Bratakou, Nikoleli, Nikolelis, & Psaroudakis, 2015). The graphene electrode was used for the development of a very selective and sensitive chemical sensor for the detection of carbofuran by immobilizing an artificial selective receptor on stabilized lipid films. The artificial receptor was synthesized by transformation of the hydroxyl groups of resorcin[4] arene receptor into phosphoryl groups. This chemical sensor responded for the wide range of carbofuran concentrations with fast response time of c.20 s. The presented potentiometric carbofuran chemical sensor is easy to construct and exhibits good reproducibility, reusability, selectivity, rapid response times, long shelf life, and high sensitivity of c.59 mV/decade over the carbofuran logarithmic concentration range from 10^{-6} to 10^{-3} M.

A miniaturized potentiometric saxitoxin sensor on graphene nanosheets with incorporated lipid films. Anti-STX, the natural saxitoxin receptor, immobilized on the stabilized lipid films was recently described in the literature (Bratakou et al., 2017). An adequate selectivity for detection over a wide range of toxin concentrations, fast response time of c.5–20 min, and detection limit of 1 nM is achieved. The proposed sensor is easy to construct and exhibits good reproducibility, reusability, selectivity, long shelf life, and high sensitivity of c.60 mV/decade of toxin concentration. A schematic configuration of saxitoxin is presented in Fig. 5. The method was implemented

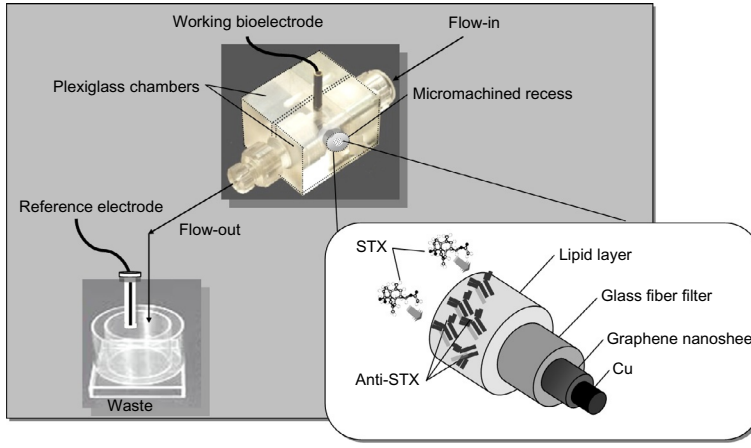

Fig. 5 Schematic configuration of the saxitoxin electrochemical biosensor and the bioelectrode edge surface. *From Bratakou, S., Nikoleli, G. -P., Siontorou, C. G.,Karapetis, S., Nikolelis, D. P., Tzamtzis, N. (2017). Development of an electrochemical biosensor for the rapid detection of saxitoxin based on air stable lipid films with incorporated anti-STX using graphene electrodes.* Electroanalysis, 29, *990–997 with permission.*

and evaluated in lake water and shellfish samples. This novel ultrathin film technology is currently adapted to the rapid detection of other toxins that could be used in bioterrorism.

10. MOLECULARLY IMPRINTED POLYMER (MIPS)-BASED BIOSENSORS

Molecular imprinting is a versatile technique for the preparation of synthetic receptors, on the basis of complexes between a template molecule and polymerizable monomers. After polymerization process, the products are called as MIPs. These polymers are prepared by using cross-linked polymers containing the cavities specific to an analyte. These cavities are created by copolymerization of cross-linking monomers and functional monomers along with an imprinting molecule or template. In the molecularly imprinting technique, the first important step is the interaction of template molecule with functional monomer before polymerization. The monomer–template interactions may occur with two different way covalent or noncovalent interactions. The second important step is to remove the template from the resulting polymer for the generation of vacant recognition sites to complementary in shape and functional groups to the template. The MIP then selectively rebinds to the analyte compound. A schematic illustration of molecularly imprinting is given in Fig. 6. In a way, MIP technologies imitate

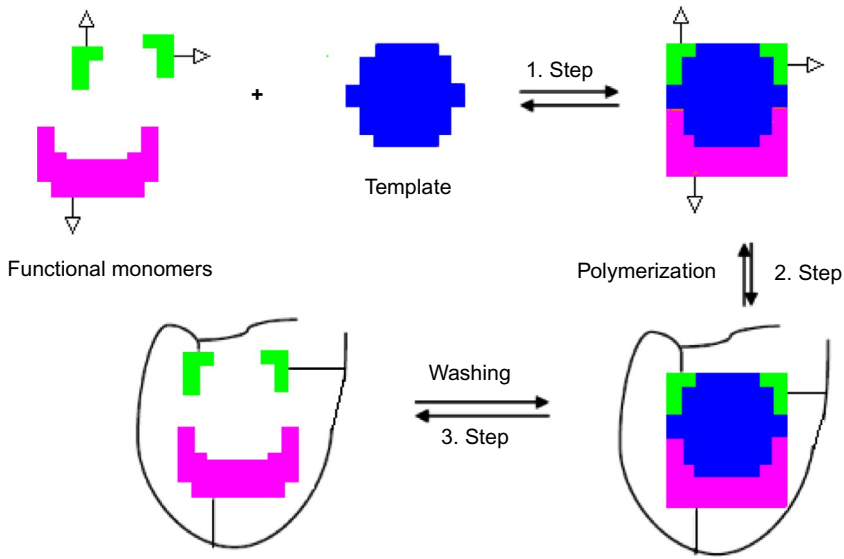

Fig. 6 Schematic illustration of molecular imprinting.

biological antibody systems which the function on the basis of specific binding sites where an antigen binds strongly to an antibody.

Table 6 summarizes the various biosensing devices that detect food toxicants detected by using different techniques in combination with MIP.

11. LAB-ON-A-CHIP (LOC) AND MICROFLUIDIC TECHNOLOGY

The ability to perform laboratory operations on a small scale using miniaturized (LOC) devices is a promising biosensing technique. The advantages of LOC are the small time of analysis, the low reagent costs, and the reduced amount of chemical wastes. The application of portable, easy-to-use, and highly sensitive LOC biosensors for real-time detection could offer significant advantages over current analytical methods. Integrated optics based biosensors have become the most suitable technology for LOC integration due to their ability for miniaturization, their extreme sensitivity, robustness, reliability, and their potential for multiplexing and mass production at low cost.

A LOC is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size. LOCs deal with the handling of extremely small fluid volumes down to less than picoliters. LOC devices are a subset of MEMS devices and often indicated by "Micro Total Analysis Systems" (μTAS) as well. Microfluidics is a broader term that describes also mechanical flow control devices like pumps and valves or sensors like flowmeters and viscometers. However, strictly regarded "Lab-on-a-Chip" indicates generally the scaling of single or multiple lab processes down to chip format, whereas "μTAS" is dedicated to the integration of the total sequence of lab processes to perform chemical analysis. The term "Lab-on-a-Chip" was introduced later on, when it turned out that μTAS technologies were more widely applicable than only for analysis purposes.

After the invention of microtechnology (~1954) for realizing integrated semiconductor structures for microelectronic chips, these lithography-based technologies were soon applied in pressure sensor manufacturing (1966) as well. Owing to further development of these usually complementary metal–oxide–semiconductor compatibility limited processes, a tool box became available to create micrometer- or submicrometer-sized mechanical structures in silicon wafers as well: the MEMS era (also indicated with microsystem technology—MST) had started.

Table 6 Food Toxicants Detected by Using Different Techniques in Combination With MIP

Analyte (Template)	Functional Monomer	Detection Limit	Method	References
4-Nonylphenol	2 amino thiophenol	$3.20 \times 10^{-7}\,\mathrm{mol\,L^{-1}}$	Amperometry	Huang et al. (2011)
Ametryn	Methacrilic acid	—	HPLC	Koohpaei, Shahtaheri, Ganjali, RahimiForushani, and Golbabaei (2008)
Parathion	Methacrilic acid	5.0×10^{-10} M	SWV	Alizadeh (2009)
Pirimicarb	Methacrilic acid	—	Spectrometry	Gao, Wang, An, and Liu (2008)
Paraoxon	PTMOS, APTES, TEOS, and TMOS	—	Colorimetry	Marx and Zaltsman (2003)
Methidathion	*N*,*N*′-methylene bisacrylamide	$0.02\,\mathrm{mg\,L^{-1}}$	HPLC	Bakas et al. (2012)
DEP	Methacrilic acid	—	Chromatography	Kang, Xu, Zhou, and Pan (2012)
Chloramphenicol	Methacrilic acid	2.0×10^{-9} M	DPV	Alizadeh, Ganjali, Zare, and Norouzi (2012)
Vomitoxin	Polypyrrole	$>1\,\mathrm{ng\,mL^{-1}}$	SPR	Choi, Chang, Lee, and Chun (2011)
Patulin	4,4′-Azobis(4-cyanopentanoic acid) ACPA	$10\,\mu\mathrm{g\,kg^{-1}}$	HPLC	Zhao, Jia, Yu, and Sun (2011)
Mycophenolic acid	4-Vinylpyridine	$0.17\,\mu\mathrm{g\,kg^{-1}}$	LC/MS	Smet et al. (2011)
Zearalenone	1-Allypiperazine	—	LC/MS	Mausia et al. (2011)
Fumonisin B	DEAEM	$22\,\mu\mathrm{g\,kg^{-1}}$	HPLC	Smet, Dubruel, Peteghem, Schacht, and Saeger (2009)
Moniliformin	Methacrilic acid	—	HPLC	Appell, Kendra, Kim, and Maragos (2007)

Abbreviations: Analyte: *DEP*, Diethyl(3-methylureido) (phenyl)methylphosphonate. Functional monomers: *PTMOS*, phenyl trimethoxysilane; *APTES*, minopropyltriethoxysilane; *TMOS*, tetramethylorthosilicate; *TEOS*, tetraethyl orthosilicate; *ACPA*, 4-4′Azobis(4-cyanopentanoic acid); *DEAEM*, 2-(diethylamino) ethyl methacrylate. Measurement techniques: *HPLC*, High performance liquid chromatography; *SWV*, square wave voltammetry; *DPV*, differential pulse voltammetry; *SPR*, surface plasmon resonance; *LC/MS*, liquid chromatography/mass spectrometry.

A big boost in research and commercial interest came in the mid-1990s, when μTAS technologies turned out to provide interesting tooling for genomics applications, like capillary electrophoresis and DNA microarrays. A big boost in research support also came from the military, especially from DARPA (Defense Advanced Research Projects Agency), for their interest in portable bio/chemical warfare agent (CWA) detection systems. The added value was not only limited to integration of lab processes for analysis but also the characteristic possibilities of individual components and the application to other, nonanalysis, lab processes. Hence the term "Lab-on-a-Chip" was introduced.

The basis for most LOC fabrication processes is photolithography. Initially most processes were in silicon, as these well-developed technologies were directly derived from semiconductor fabrication. Because of demands, e.g., specific optical characteristics, bio- or chemical compatibility, lower production costs, and faster prototyping, new processes have been developed such as glass, ceramics and metal etching, deposition and bonding, polydimethylsiloxane processing (e.g., soft lithography), thick-film and stereolithography as well as fast replication methods via electroplating, injection molding, and embossing. Furthermore, the LOC field more and more exceeds the borders between lithography-based MST, nanotechnology, and precision engineering.

Microfluidics deals with the behavior, precise control, and manipulation of fluids that are geometrically constrained to a small, typically submillimeter, scale. Typically, *micro* means one of the following features:

- small volumes (μL, nL, pL, fL)
- small size
- low energy consumption
- effects of the microdomain

Typically fluids are moved, mixed, separated, or otherwise processed. Numerous applications employ passive fluid control techniques like capillary forces. In some applications external actuation means are additionally used for a directed transport of the media. Examples are rotary drives applying centrifugal forces for the fluid transport on the passive chips. Active microfluidics refers to the defined manipulation of the working fluid by active (micro)components as micropumps or microvalves. Micropumps supply fluids in a continuous manner or are used for dosing. Microvalves determine the flow direction or the mode of movement of pumped liquids. Often processes which are normally carried out in a lab are miniaturized on a single chip in order to enhance efficiency and mobility as well as reducing sample and reagent volumes.

The ability to create precise and carefully controlled chemoattractant gradients makes microfluidics the ideal tool to study motility, chemotaxis, and the ability to evolve/develop resistance to antibiotics in small populations of microorganisms and in a short period of time. These microorganisms including bacteria (Ahmed, Shimizu, & Stocker, 2010) and the broad range of organisms that form the marine microbial loop (Seymour, Simó, Ahmed, & Stocker, 2010), responsible for regulating much of the oceans' biogeochemistry.

12. WATER-SOLUBLE VITAMIN AND DRUG RESIDUE DETERMINATION

Vitamin C (ascorbic acid) content in foodstuffs is an indicator of its freshness and nutritive value. Its natural availability is as L-ascorbic acid (since the D-ascorbic acid does not occur in nature), possessing a significant activity in the processes of oxidation and reduction in living organisms. Owing to its extensive use in the food and beverages industries, more papers report its determination using biosensors, comparing with other vitamins.

Recently, in Wen, Xu, Liu, Li, and He (2012), an amperometric biosensor for vitamin C was reported based on the immobilization of ascorbate oxidase into a biocompatible sandwich-type composite film. The biocompatible conducting poly(3,4-ethylenedioxythiophene) composite film and highly stable and selective multiwalled carbon nanotubes–Nafion membrane were prepared as inner and outer films of biosensor and the enzyme molecules were immobilized between these two composite films. Content of ascorbic acid in commercial juices was determined, the developed biosensor being characterized by a very good bioelectrocatalytic performance toward the oxidation of vitamin C in solution, fast current response, low working potential, and high sensitivity ($187\,\mathrm{mA} \times \mathrm{M}^{-1}\,\mathrm{cm}^{-2}$), and also a wide linear range (4.0×10^{-7} to $1 \times 10^{-3}\,\mathrm{M}$) and low detection limit ($0.087\,\mu\mathrm{M}$), showing a better performance than other similar biosensors. Chauhan, Dahiya, Priyanka, and Pundir (2010) reported the fabrication of an ascorbate amperometric biosensor based on the immobilization of ascorbate oxidase from *Lagenaria siceraria* onto an egg shell membrane, through glutaraldehyde coupling, and then mounting over a gold electrode for obtain the working electrode for ascorbate. A linear relationship between L-ascorbic acid concentration in the range $1 \times 10^{-5}\,\mathrm{M}$ and $4 \times 10^{-4}\,\mathrm{M}$ and current was obtained and further applied for quantification of L-ascorbic acid in various samples, including fruit juices. Other benefits offered by this developed biosensor

are the fast response time (10 s), good repeatability (200 assays), and long-term stability (a decrease of 50% of its initial sensitivity was observed after 4 months of storage).

Amperometric L-ascorbic acid biosensors equipped with enzyme micelle membrane were developed (Wang, Watanabe, & Uchiyama, 2008) and successfully tested for determination of vitamin C content in fruit juices. Inexpensive polystyrene (PS) membrane served as the support of enzyme (ascorbate oxidase) micelles, formed after previously bounding with poly-maleimidostyrene (PMS). Oxygen penetrated the permeable enzyme micelle membrane and was reduced at either at aminated glassy carbon electrode (AGCE) and gold electrode (AuE). The detection of ascorbic acid at both modified electrodes exhibited good response of catalytic reduction current of oxygen with high sensitivity and short response time (within 1 min). A good linear relationship was observed in the concentration range of 5 μM to 0.4 mM when AGCE was used and the applied potential was −0.5 V vs Ag/AgCl. Interferences from the reducing agents were avoided by using the determinations at cathodic potential.

12.1 Biotin, Folic Acid, Pantothenic Acid, and Vitamin B_{12}

SPR biosensor technology, using a ligand-binding protein interaction with the BIAcore® system (GE Healthcare Lifesciences), is already a golden standard for the analysis of several water-soluble vitamins (Blake, 2007). Representatives of the B vitamin group (biotin, folic acid, pantothenic acid, and vitamin B12), these organic compounds are widely detected with optical sensors based on SPR method, due to its multiple benefits such as robustness, sensitivity and versatility, delivering reliable, rapid, and relatively low-cost testing (Petz, 2009).

From the currently available instruments for SPR applications (Schasfoort & McWhirter, 2008), the Biacore Q equipment is dedicated to the qualitative or quantitative determination of analytes in food-related products and can be used in combination with specially developed Q flex kits (Petz, 2009), delivering rapid, reliable, and automated quantitation of vitamin content. Applications have been developed for biotin, folic acid, pantothenic acid, and vitamin B_{12}, and have been certified as performance tested methods by the AOAC Research Institute.

SPR analysis with Q flex kits is based on an inhibition immunoassay principle, since it was demonstrated as an accurate, rapid, and sensitive technique for the detection of small analytes (<1000 Da) as vitamins (Indyk &

Filonzi, 2000; Indyk et al., 2002). Sample preparation and analysis times are significantly reduced compared to traditional techniques and the vitamin content could be quantified in almost any food matrix, including colored or opaque samples. Several papers report application of Q flex kits for quantification of these four vitamins as well the riboflavin (vitamin B2) in supplemented infant formulas and within a collaborative study for determination of vitamin B12 content in fortified bovine infant milk, fortified soya-based infant formula powder, vitamin premix, and dietary supplements (Vyas & O'Kane, 2011; Vyas, O'Kane, & Dowell, 2012).

13. OPTICAL BIOSENSORS IN FOOD SAFETY AND CONTROL

These types of sensors are based on measuring responses to light emission or to illumination. Optical biosensors are based on well-founded methods including fluorescence, phosphoresence, light absorbance, photothermal techniques, chemiluminescence, SPR, total internal reflectance, light rotation, and polarization and could employ a number of techniques to detect the presence of a target analyte. As an example, these technical usages have been demonstrated to detect the presence of allergens, particularly peanuts, during food production (Otles & Yalcin, 2012; Rana, Jindal, Beniwal, & Chhokar, 2010).

The development of optical fiber sensors during recent years is related to two of the most important scientific advances: the laser and modern low-cost optical fibers. Recently, optical fibers have become an important part of sensor technology. Their use as a probe or as a sensing element is increasing in clinical, pharmaceutical, industrial, and military applications. Excellent light delivery, long interaction length, low cost, and ability not only to excite the target molecules but also to capture the emitted light from the targets are the main points in favor of the use of optical fibers in biosensors. Optical fibers transmit light on the basis of the principle of total internal reflection. Fiber optic biosensors are analytical devices in which a fiber optic device serves as a transduction element. The usual aim is to produce a signal that is proportional to the concentration of a chemical or biochemical to which the biological element reacts. Fiber optic biosensors are based on the transmission of light along silica glass fiber, or plastic optical fiber to the site of analysis. Optical fiber biosensors can be used in combination with different types of spectroscopic technique, e.g., absorption, fluorescence, phosphorescence, and SPR. Optical biosensors based on the use of fiber optics can be classified into two different categories: intrinsic sensors, where

interaction with the analyte occurs within an element of the optical fiber; and extrinsic sensors, in which the optical fiber is used to couple light, usually to and from the region where the light beam is influenced by the analyte.

OP neurotoxins comprise a unique class of contaminants and CWAs which have a high acute toxicity. These neurotoxins are powerful inhibitors of esterase enzymes, such as acetyl- and butyryl-cholinesterases or neurotoxic esterase. Simple methods for OP neurotoxins detection using fluorescence assays based on specific recognition of OP by OPH enzyme have been developed. A biosensor for direct detection of OP neurotoxins such as paraoxon has been reported by Simoniana, Good, Wang, and Wild (2005).

The biosensing method has been based on the change in fluorescence of a competitive inhibitor of the OPH enzyme when the inhibitor is displaced by the OP substrate. The change in fluorescence intensity was correlated with concentration of paraoxon presented in the solution. The sensitivity to paraoxon was obtained when enzyme inhibitor and OPH–gold nanoparticle conjugates were present at near equimolar levels.

An optical glutamate biosensor test strip based on stacked membranes of nafion/sol–gel (bottom layer) and chitosan (uppermost layer) was fabricated on a piece of paper as support to form a test strip by Muslim, Ahmad, Heng, and Saad (2012). The use of a stacked membrane system allows multiple immobilizations of sensing components directly without any covalent attachment via straight forward procedures. The uppermost membrane consisted of immobilized enzymes L-glutamate oxidase (GLOD) and horseradish peroxidase (HRP), which sensed the presence of L-glutamate and the bottom membrane contained the indicator dye, 3,3′,5,5′-tetramethylbenzidine. The test strip can be used to measure L-glutamate quantitatively by observing a color change from light green to dark green with increasing L-glutamate concentrations. Quantitative analysis could be performed by measuring the reflectance intensity of the color change at 550 nm. The glutamate biosensor test strip gave a linear response range of 0.01–0.30 mM to L-glutamate with a limit of detection of 5 μM. The strips were successfully applied for the estimation of L-glutamate in common food items such as sauces, soups, and processed food and flavor enhancers. Results of analysis of L-glutamate in various food samples using the glutamate test strip were comparable to a standard procedure employing HPLC method.

Cantilevers function by detecting differences in the stress, forces, or vibration frequency, occurring when molecules bind to the surface. For example, bacteria can be detected due to the additional mass resulting from the binding of specific antibodies immobilized on the cantilever surface and

the bacterial cell. There are several reports of sensitive detection of bacteria using cantilever technology such as a single *L. innocua* cell (Li, Ellington, & Chen, 2011), *E. coli* O157:H7 in spinach, spring lettuce mix and ground beef (LOD 100 CFU mL^{-1}) (Gupta, Akin, & Bashir, 2004) and an antibody functionalized piezoelectric-excited millimeter-sized cantilever sensor capable of identification of 1 E. coli O157:H7 cell mL^{-1} (Maraldo & Mutharasan, 2007). However differences in pathogen adherence to food matrices, which affects target binding to the sensor surface, can affect sensitivity (Campbell & Mutharasan, 2007). The scaling down of this technology to the nanoscale has increased the capacity of nanocantilevers for ultrasensitive, faster detection due to higher frequencies and better mass resolution.

SPR technology has been used for the detection of food borne pathogens using gold surfaces. Gold nanorods are elongated nanoparticles with distinct optical properties that depend on their shape. Compared to the single plasmon adsorption band of nanoparticles, excitation of surface plasmons by light in nanorods can be seen as two plasmon absorption bands, one corresponding to light absorption and scattering along the width of the particle, and the other along the length of the particle. Changes in the aspect ratio (ratio of the width of the object to its length) of a nanorod gives rises to plasmon adsorption bands at different positions hence different sized nanorods can be used as labels in a multiplex assay. The position and intensity of these bands can be affected by changes, for example binding events, in the dielectric constant around the vicinity of these nanorods, known as localized SPR (LSPR) or nanoSPR (Perez-Juste, Pastoriza-Santos, Liz-Marzan, & Mulvaney, 2005).

One of the most successful optical-based biosensor systems introduced has been the range of instruments supplied by BIAcore (Uppsala, Sweden). This instrument can be employed to study a wide range of biological interactions, both automatically and in real time. The instrument is based on SPR, whereby biomolecular binding events cause changes at a metal/liquid interface, usually involving a complex that includes a specific antibody against a target analyte. On binding, these changes (in the refractive index) are recognized by a shift in the SPR signal, indicating a presence of the target analyte in a sample solution. One particular advantage for sensors based on SPR is that the system does not require the presence of a labeled ligand (e.g., enzyme conjugated antibody) to function (Terry, White, & Tigwell, 2005). SPR sensor systems have been used extensively to investigate the presence of harmful contaminating microorganisms in food and to determine food quality. For example, an optically based biosensor was recently used to screen poultry liver and eggs for the presence of the drug nicarbazin, a feed additive

used to prevent outbreaks of coccidiosis in boiler chickens (McCarney, Traynor, Fodey, Crooks, & Elliot, 2003). The limits of detection for the sensor system were 17 and 19 ng g^{-1} for liver and eggs, respectively.

14. CONCLUSIONS AND FUTURE TRENDS

Although biosensors are exhibiting double-digit growth rates, they still have to overcome a number of challenges, including the following:

- New research focuses less into fundamental research due to impeding introduction of challenging applications
- Development of a single biosensor platform with multipurpose diagnostics capability has restricted biosensor applications
- Numerous problems encountered in successful commercialization of biosensors has encouraged conservative development strategies
- Competition from nonbiosensor technologies has hindered revenue growths
- Low rate of technology transfer and lower level of development has deterred the development of recent biosensors
- There has been very little development in the fabrication of portable chemical sensors for online uses.

Recently, the market thrust has shifted to nanosensors' capabilities to rapidly detect environmental toxicants and food toxicants. A recent report from market research firm In-Stat revealed that the media spotlight on this application may be premature: Despite the public's anticipation that biosensors with real-time detection will be able to monitor biochemical environmental toxicants, the technology has not caught up with expectations. Presently, biosensors in environmental monitoring stations nationwide can detect compounds like anthrax—but detection can take 12–24 h. The best ones on the market take 20 min.

Gas phase chemical detection is of critical importance for the sensing of highly toxic molecules, such as CWAs and toxic industrial chemicals. Beyond the ability to detect CWAs in a laboratory, i.e., in the context of large, technical, and relatively expensive apparatus, of significant interest is the detection of CWAs with SERS online. The advent of small, portable Raman spectrometers with dimensions close to that of a smartphone such as the ReporteR spectrometer (Intavec, Inc.) or the First Defender (Thermo Scientific, Inc.), and the development of standoff SERS detection has begun the transition from the lab to the field. Overall, SERS is a

promising method for the ultrasensitive detection of chemical environmental pollutants (Álvarez-Puebla and Liz-Marzán, 2010). Examples of experimental set-up for performing SERS online have been given in the literature by using a palm-size handheld spectrometer (Frontiera, Henry, Gruenke, & Van Duyne, 2011).

Currently in this chapter, we are looking into portable and handheld nanosensors, for example, such as dynamic DNA and protein arrays for rapid and accurate detection of environmental pollutants, pathogens, etc. Our challenges focus in:

1. High sensitivity—*detect* very small amounts of environmental pollutants such as heavy metals, pathogens, toxins, and chemical toxicants in the environment.
2. Highly selective—*discriminate* targets from other materials.
3. Massively parallel to detect multiple pathogens, minimize false positive, have rapid response, without sample preparation.
4. Nanosize and transportable or handheld, robust, simple to operate.
5. Inexpensive materials.
6. Adaptable to new biotoxicants, integrated chembiosensor.
7. Allowing for detection of single molecules.

Therefore, the state of the art of our devices is summarized as follows: Our nanosensors will be highly sensitive, selective, rapidly responding, real time, massively parallel, with no or minimum sample preparation, platform suited to portable, and handheld nanosensors for the rapid detection of environmental pollutants for online uses even by nonskilled personnel.

REFERENCES

Ahmed, T., Shimizu, T. S., & Stocker, R. (2010). Microfluidics for bacterial chemotaxis. *Integrative Biology*, *2*(11–12), 604–629.

Alizadeh, T. (2009). High selective parathion voltammetric sensor development by using an acrylic based molecularly imprinted polymer-carbon paste electrode. *Electroanalysis*, *21*, 1490–1498.

Alizadeh, T., Ganjali, M. R., Zare, M., & Norouzi, P. (2012). Selective determination of chloramphenicol at trace level in milk samples by the electrode modified with molecularly imprinted polymer. *Food Chemistry*, *130*, 1108–1114.

Álvarez-Puebla, R. A., & Liz-Marzán, I. M. (2010). Environmental applications of plasmon assisted Raman scattering. *Energy & Environmental Science*, *3*, 1011–1017.

Amine, A., Mohammadi, H., Bourais, I., & Palleschi, G. (2006). Enzyme inhibition-based biosensors for food safety and environmental monitoring. *Biosensors & Bioelectronics*, *21*, 1405–1423.

Andreou, V. G., & Clonis, Y. D. (2002). A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol–gel entrapped bromocresol purple for in-field use. *Biosensors & Bioelectronics*, *17*, 61–69.

Appell, M., Kendra, D. F., Kim, E. K., & Maragos, C. M. (2007). Synthesis and evaluation of molecularly imprinted polymers as sorbents of moniliformin. *Food Additives & Contaminants*, *24*, 43–52.

Arvinte, A., Rotariu, L., & Bala, C. (2006). Development of a pesticides biosensor using carbon-based electrode systems. *Chemicals as Intentional and Accidental Global Environmental Threats* (pp. 337–343). Springer, Dordrecht.

Bakas, I., Oujji, N. B., Moczko, E., Istamboulie, G., Piletsky, S., Piletska, E., et al. (2012). Molecular imprinting solid phase extraction for selective detection of methidathion in olive oil. *Analytica Chimica Acta*, *734*, 99–105.

Barsan, M. M., & Brett, C. M. A. (2008). An alcohol oxidase biosensor using PNR redox mediator at carbon film electrodes. *Talanta*, *74*, 1505–1510.

Blake, C. J. (2007). Analytical procedures for water-soluble vitamins in foods and dietary supplements: A review. *Analytical and Bioanalytical Chemistry*, *389*, 63–76.

Bratakou, S., Nikoleli, G.-P., Nikolelis, D. P., & Psaroudakis, N. (2015). Development of a potentiometric chemical sensor for the rapid detection of carbofuran based on air stable lipid films with incorporated calix[4]arene phosphoryl receptor using graphene electrodes. *Electroanalysis*, *27*, 2608–2613.

Bratakou, S., Nikoleli, G.-P., Siontorou, C. G., Karapetis, S., Nikolelis, D. P., & Tzamtzis, N. (2016). Electrochemical biosensor for naphthalene acetic acid in fruits and vegetables based on lipid films with incorporated auxin-binding protein receptor using graphene electrodes. *Electroanalysis*, *28*, 2171–2177.

Bratakou, S., Nikoleli, G.-P., Siontorou, C. G., Karapetis, S., Nikolelis, D. P., & Tzamtzis, N. (2017). Development of an electrochemical biosensor for the rapid detection of Saxitoxin based on air stable lipid films with incorporated anti-STX using graphene electrodes. *Electroanalysis*, *29*, 990–997.

Brett, C. M. A., & Oliveira-Brett, A. M. (1998). *Electroanalysis*. Oxford: Oxford University Press.

Brett, C. M. A., & Oliveira-Brett, A. M. (2011). Electrochemical sensing in solution—Origins, applications and future perspectives. *Journal of Solid State Electrochemistry*, *15*, 1487–1494.

Buchatip, S., Ananthanawat, C., Sithigorngul, P., Sangvaniche, P., Rengpipat, S., & Hoven, V. P. (2010). Detection of the shrimp pathogenic bacteria, Vibrio Harveyi, by a quartz crystal microbalance-specific antibody based sensor. *Sensors and Actuators B*, *145*, 259–264.

Campbell, G. A., & Mutharasan, R. (2007). A method of measuring E. coli O157:H7 at 1 cell/mL in 1 liter sample using antibody functionalized piezoelectric-excited millimeter-sized cantilever sensor. *Environmental Science & Technology*, *5*, 1668–1674.

Capannesi, C., Palchetti, I., Mascini, M., & Parenti, A. (2000). Electrochemical sensor and biosensor for polyphenols detection in olive oils. *Food Chemistry*, *71*, 553–562.

Chauhan, N., Dahiya, T., Priyanka, & Pundir, C. S. (2010). Fabrication of an amperometric ascorbate biosensor using egg shell membrane bound *Lagenaria siceraria* fruit ascorbate oxidase. *Journal of Molecular Catalysis B: Enzymatic*, *67*, 66–71.

Chemnitius, G. C., & Bilitewscki, U. (1996). Development of screen-printed enzyme electrodes for the estimation of fish quality. *Sensors and Actuators B*, *32*, 107–113.

Chen, Y. S., Hung, Y. C., Chiou, J. C., Wang, H. L., Huang, H. S., Huang, L. C., et al. (2010). Ultrasensitive detection of cymbidium mosaic potexvirus using a single-wall carbon nanotube-functionalized quartz crystal microbalance. *Japanese Journal of Applied Physics*, *49*, 105103.

Choi, S. W., Chang, H. J., Lee, N., & Chun, H. S. (2011). A surface plasmon resonance sensor for the detection of deoxynivalenol using a molecularly imprinted polymer. *Sensors*, *11*, 8654–8664.

Choi, J.-W., Kim, Y. K., Lee, I.-H., Min, J., & Lee, W. H. (2001). Optical organophosphorus biosensor consisting of acetylcholinesterase/viologen hetero Langmuir–Blodgett film. *Biosensors & Bioelectronics*, *16*, 937–943.

Conroi, P. J., Hearty, S., Leonard, P., & O'Kennedy, R. (2016). Antibody-based magnetoelastic biosensors: Potential devices for detection of pathogens and associated toxins. *Applied Microbiology and Biotechnology*, *100*(14), 6149–6163.

De Genst, E., Messer, A., & Dobson, C. M. (2014). Antibodies and protein misfolding: From structural research tools to therapeutic strategies. *Biochimica et Biophysica Acta (BBA)—Proteins and Proteomics*, *1844*(11), 1907–1919.

Dzantiev, B. B., Yazyninaa, E. V., Zherdeva, A. V., Plekhanovab, Y. V., Reshetilovb, A. N., Changc, S.-C., et al. (2004). Determination of the herbicide chlorsulfuron by amperometric sensor based on separation-free bienzyme immunoassay. *Sensors and Actuators B*, *98*, 254–261.

Eisenman, G. (1967). *Glass electrodes for hydrogen and other cations*. New York: Marcel Dekker.

Esti, M., Volpe, G., Massignan, L., Compagnone, D., La Notte, E., & Palleschi, G. (1998). Determination of amines in fresh and modified atmosphere packaged fruits using electrochemical biosensors. *Journal of Agricultural and Food Chemistry*, *46*(10), 4233–4237.

Fojta, M., Kubicarova, T., & Palecek, E. (2000). Electrode potential-modulated cleavage of surface-confined DNA by hydroxyl radicals detected by an electrochemical biosensor. *Biosensors & Bioelectronics*, *15*, 107–115.

Frontiera, R. R., Henry, A.-I., Gruenke, N. L., & Van Duyne, R. P. (2011). Surface-enhanced femtosecond stimulated Raman spectroscopy. *Journal of Physical Chemistry Letters*, *2*(10), 1199–1203.

Gao, B., Wang, J., An, F., & Liu, Q. (2008). Molecular imprinted material prepared by novel surface imprinting technique for selective adsorption of pirimicarb. *Polymer*, *49*, 1230–1238.

Ghica, M. E., & Brett, C. M. A. (2008). Glucose oxidase inhibition in poly(neutral red) mediated enzyme biosensors for heavy metal determination. *Microchimica Acta*, *163*, 185–193.

Ghica, M. E., Pauliukaite, R., Marchand, N., Devic, E., & Brett, C. M. A. (2007). An improved biosensor for acetaldehyde determination using a bienzymatic strategy at poly(neutral red) modified carbon film electrodes. *Analytica Chimica Acta*, *591*, 80–86.

Gupta, A., Akin, D., & Bashir, R. (2004). Detection of bacterial cells and antibodies using surface micromachined thin silicon cantilever resonators. *Journal of Vacuum Science & Technology B*, *22*(6), 2785–2791.

Hasebe, Y., Yokobori, K., Fukasawa, K., Kogure, T., & Uchiyama, S. (1997). Highly sensitive electrochemical determination of Escherichia coli density using tyrosinase-based chemically amplified biosensor. *Analytica Chimica Acta*, *357*, 51–54.

Hassan, S. H. A., & Oh, S. E. (2010). Improved detection of toxic chemicals by photobacterium phosphoreum using modified boss medium. *Journal of Photochemistry and Photobiology B: Biology*, *101*, 16–21.

Hong, S. R., Jeong, H. D., & Hong, S. (2010). QCM DNA biosensor for the diagnosis of a fish pathogenic virus VHSV. *Talanta*, *82*, 899–903.

Horváth, R., Pedersen, H. C., Skivesen, N., Selmeczi, D., & Larsen, N. B. (2003). Optical waveguide sensor for on-line monitoring of bacteria. *Optics Letters*, *28*, 1233–1235.

Hu, W., Chen, S., Liu, L., Ding, B., & Wang, H. (2011). Formaldehyde sensors based on nanofibrous polyethyleneimine/bacterial cellulose membranes coated quartz crystal microbalance. *Sensors and Actuators B*, *157*, 554–558.

Huang, J., Zhang, X., Liu, S., Lin, Q., He, X., Xing, X., et al. (2011). Development of molecularly imprinted electrochemical sensor with titanium oxide and gold nanomaterials enhanced technique for determination of 4-nonylphenol. *Sensors and Actuators B*, *152*, 292–298.

Indyk, H. E., & Filonzi, E. L. (2000). Use of an optical biosensor to determine water-soluble vitamins in infant formula. *Australian Journal of Dairy Technology*, *55*, 99.

Indyk, H. E., Persson, B. S., Caselunghe, M. C., Moberg, A., Filonzi, E. L., & Woollard, D. C. (2002). Determination of vitamin B12 in milk products and selected foods by optical biosensor protein-binding assay: Method comparison. *Journal of AOAC International*, *85*, 72–81.

Ivnitski, D., & Rishpon, J. (1996). A one-step, separation-free amperometric enzyme immunosensor. *Biosensors & Bioelectronics*, *11*, 409–417.

Jang, H. D., Chang, K. S., Lee, Y. G., Lee, S. J., & Hsu, C. L. (2009). Quantitative determination of lactobacillus spp. in milk using a series piezoelectric quartz crystal sensor. *European Food Research and Technology*, *229*, 349–355.

Kang, S., Xu, Y., Zhou, L., & Pan, C. J. (2012). Preparation of molecularly imprinted polymers: Diethyl (3-methylureido)(phenyl)methylphosphonate as a dummy template for the recognition of its organophosphate pesticide analogs. *Journal of Applied Polymer Science*, *124*, 3737–3743.

Karamollaoglua, I., Öktema, H. A., & Mutlu, M. (2009). QCM-based DNA biosensor for detection of genetically modified organisms (GMOs). *Biochemical Engineering Journal*, *44*, 142–150.

Karaseva, N. A., & Ermolaeva, T. N. (2012). A piezoelectric immunosensor for chloramphenicol detection in food. *Talanta*, *93*, 44–48.

Kim, C. S., Choi, B.-H., Seo, J. H., Lim, G., & Cha, H. J. (2013). Mussel adhesive protein-based whole cell array biosensor for detection of organophosphorus compounds. *Biosensors and Bioelectronics*, *41*, 199–204.

Koohpaei, A. R., Shahtaheri, S. J., Ganjali, M. R., RahimiForushani, A., & Golbabaei, F. (2008). Application of multivariate analysis to the screening of molecularly imprinted polymers (MIPs) for ametryn. *Talanta*, *75*, 978–986.

Lei, Y., Chen, W., & Mulchandani, A. (2006). Microbial biosensors. *Analytica Chimica Acta*, *568*, 200–210.

Li, B., Ellington, A. D., & Chen, X. (2011). Rational, modular adaptation of enzyme-free DNA circuits to multiple detection methods. *Nucleic Acids Research*, *39*(16), e110–114.

Lucareli, F., Palchetti, I., Marrazza, G., & Mascini, M. (2002). Electrochemical DNA biosensor as a screening tool for the detection of toxicants in water and wastewater samples. *Talanta*, *56*, 949–957.

Malzahn, K., Windmiller, J. R., Valdes-Ramirez, G., Schöning, M. J., & Wang, J. (2011). Wearable electrochemical sensors for in situ analysis in marine environments. *Analyst*, *136*, 2912–2917.

Maraldo, D., & Mutharasan, R. (2007). Preparation-free method for detecting E. coli O157: H7 in the presence of spinach, spring lettuce mix, and ground beef particulates. *Journal of Food Protection*, *70*(11), 2651–2655.

Marty, J.-L., Garcia, D., & Rouillon, R. (1995). Biosensors: Potential in pesticide detection. *Trends in Analytical Chemistry*, *14*, 329–333.

Marx, S., & Zaltsman, A. (2003). Molecular imprinting of sol gel polymers for the detection of paraoxon in water. *International Journal of Environmental Analytical Chemistry*, *83*, 671–680.

Mausia, T., Smet, D. D., Guorun, Q., Peteghem, C. V., Zhang, D., Wu, A., et al. (2011). Molecularly imprinted polymers as specific adsorbents for zearalenone produced by precipitation polymerization and applied to mycotoxin production. *Analytical Letters*, *44*, 2633–2643.

McCarney, B., Traynor, I. M., Fodey, T. L., Crooks, S. R. H., & Elliot, C. T. (2003). Surface plasmon resonance biosensor screening of poultry liver and eggs for nicarbazin residues. *Analytica Chimica Acta*, *483*, 165–169.

Muslim, N. Z. M., Ahmad, M., Heng, L. Y., & Saad, B. (2012). Optical biosensor test strip for the screening and direct determination of l-glutamate in food samples. *Sensors and Actuators B*, *161*, 493–497.

Newman, J. D., & Setford, S. J. (2006). Enzymatic biosensors. *Molecular Biotechnology*, *32*, 249–268.

Nikoleli, G.-P., Israr, M. Q., Tzamtzis, N., Nikolelis, D. P., Willander, M., & Psaroudakis, N. (2012). Structural characterization of graphene nanosheets for miniaturization of potentiometric urea lipid film based biosensors. *Electroanalysis*, *24*(6), 1285–1295.

Nikolelis, D. P., & Mitrokotsa, M. (2002). Stabilized lipid film based biosensor for atenolol. *Biosensors & Bioelectronics*, *17*, 565–572.

Nikolelis, D. P., Ntanos, N., Nikoleli, G.-P., & Tampouris, K. (2008). Development of an electrochemical biosensor for the rapid detection of naphthalene acetic acid in fruits by using air stable lipid films with incorporated auxin-binding protein 1 receptor. *Protein and Peptide Letters*, *15*, 789–794.

Nikolelis, D. P., Raftopoulou, G., Nikoleli, G.-P., & Simantiraki, M. (2006). Stabilized lipid membrane based biosensors with incorporated enzyme for repetitive uses. *Electroanalysis*, *18*, 2467–2474.

Nikolelis, D. P., Raftopoulou, G., Psaroudakis, N., & Nikoleli, G.-P. (2008). Development of an electrochemical biosensor for the rapid detection of carbofuran based on air stable lipid films with incorporated calix[4]arene phosphoryl receptor. *Electroanalysis*, *20*, 1574–1580.

Nikolelis, D. P., Raftopoulou, G., Psaroudakis, N., & Nikoleli, G.-P. (2009). Development of an electrochemical chemosensor for the rapid detection of zinc based on air stable lipid films with incorporated calix4arene phosphoryl receptor. *International Journal of Environmental Analytical Chemistry*, *89*, 211–222.

Nikolelis, D. P., Raftopoulou, G., Simantiraki, M., Psaroudakis, N., Nikoleli, G.-P., & Hianik, T. (2008). Preparation of a selective receptor for carbofuran for the development of a simple optical spot test for its rapid detection using stabilized in air lipid films with incorporated receptor. *Analytica Chimica Acta*, *620*, 134–141.

Nikolelis, D. P., Simantiraki, M., Siontorou, G. C., & Toth, K. (2005). Flow injection analysis of carbofuran in foods using air stable lipid film based acetylcholinesterase biosensor. *Analytica Chimica Acta*, *537*, 169–177.

Oh, S. E., Hassan, S. H. A., & Van Ginkel, S. W. (2011). A novel biosensor for detecting toxicity in water using sulfur-oxidizing bacteria. *Sensors and Actuators B*, *154*, 17–21.

Oliveira-Brett, A. M., & da Silva, L. A. (2002). A DNA-electrochemical biosensor for screening environmental damage caused by s-triazine derivatives. *Analytical and Bioanalytical Chemistry*, *373*, 717–723.

Otles, S., & Yalcin, B. (2012). Review on the application of nanobiosensors in food analysis. *Acta Scientiarum Polonorum. Technologia Alimentaria*, *11*, 7–18.

Pal, P., Bhattacharyay, D., Mukhopadhyay, A., & Sarkar, P. (2009). The detection of mercury, cadium, and arsenic by the deactivation of urease on rhodinized carbon. *Environmental Engineering Science*, *26*, 25–32.

Pemberton, R. M., Cox, T., Tuffin, R., Sage, I., Drago, G. A., Biddle, N., et al. (2012). Microfabricated glucose biosensor for culture well operation. *Biosensors and Bioelectronics*, *42*, 668–677.

Perez-Juste, J., Pastoriza-Santos, I., Liz-Marzan, L., & Mulvaney, P. (2005). Gold nanorods: Synthesis, characterization and applications. *Coordination Chemistry Reviews*, *249*, 1870–1901.

Petz, M. (2009). Recent applications of surface plasmon resonance biosensors for analyzing residues and contaminants in food. *Monatshefte Fur Chemie*, *140*, 953–964.

Plata, M. R., Contento, A. M., & Ríos, A. (2011). Simplified determination of bacterial contamination by Escherichia coli using a flow injection system with piezoelectric detection. *Microchimica Acta*, *172*, 447–454.

Poitras, C., Fatisson, J., & Tufenkji, N. (2009). Real-time microgravimetric quantification of Cryptosporidium parvum in the presence of potential interferents. *Water Research*, *43*, 2631–2638.

Rana, J. S., Jindal, J., Beniwal, V., & Chhokar, V. (2010). Utility biosensors for applications in agriculture—A review. *Journal of American Science*, *6*, 353–375.

Rejeb, I. B., Arduini, F., Arvinte, A., Amine, A., Gargouri, M., Micheli, L., et al. (2009). Development of a bio-electrochemical assay for AFB1 detection in olive oil. *Biosensors & Bioelectronics*, *24*, 1962–1968.

Salmain, M., Ghasemi, M., Boujday, S., & Pradier, C. M. (2012). Elaboration of a reusable immunosensor for the detection of staphylococcal enterotoxin a (SEA) in milk with a quartz crystal microbalance. *Sensors and Actuators B*, *173*, 148–156.

Sankarana, S., Panigrahi, S., & Mallik, S. (2011). Odorant binding protein based biomimetic sensors for detection of alcohols associated with Salmonella contamination in packaged beef. *Biosensors & Bioelectronics*, *26*, 3103–3109.

Schasfoort, B. M. R., & McWhirter, A. (2008). Chapter 3: SPR instrumentation. In B. M. R. Schasfoort & J. A. Tudos (Eds.), *Handbook of surface plasmon resonance* (pp. 35–80). Cambridge, UK: RSC Publishing.

Seymour, J. R., Simó, R., Ahmed, T., & Stocker, R. (2010). Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. *Science*, *329*, 342–345.

Shen, Z. Q., Wang, J. F., Qiu, Z. G., Min, J., Wang, X. W., Chen, Z. L., et al. (2011). QCM immunosensor detection of Escherichia coli O157:H7 based on beacon immunomagnetic nanoparticles and catalytic growth of colloidal gold. *Biosensors & Bioelectronics*, *26*, 3376–3381.

Shi, R. B., Stein, K., & Schwedt, G. (1997). Determination of mercury(II) traces in drinking water by inhibition of an urease reactor in a flow injection analysis (FIA) system. *Fresenius' Journal of Analytical Chemistry*, *357*, 752–755.

Simoniana, A. L., Good, T. A., Wang, S.-S., & Wild, J. R. (2005). Nanoparticle-based optical biosensors for the direct detection of organophosphate chemical warfare agents and pesticides. *Analytica Chimica Acta*, *534*, 69–77.

Smet, D. D., Dubruel, P., Peteghem, C. V., Schacht, E., & Saeger, S. D. (2009). Molecularly imprinted solid-phase extraction of fumonisin B analogues in bell pepper, rice and corn flakes. *Food Additives & Contaminants: Part A*, *26*, 874–884.

Smet, D. D., Kodeck, V., Dubruel, P., Peteghem, C. V., Schacht, E., & Saeger, S. D. (2011). Design of an imprinted clean-up method for mycophenolic acid in maize. *Journal of Chromatography A*, *1218*, 1122–1130.

Smit, M. H., & Rechnitz, G. A. (1993). Toxin detection using a tyrosinase-coupled oxygen electrode. *Analytical Chemistry*, *65*, 380–385.

Taylor, R. F., & Schultz, J. S. (1996). *Handbook of chemical and biological sensors*. IOP Publishing Ltd.

Terry, L. A., White, S. F., & Tigwell, L. J. (2005). The application of biosensors to fresh produce and the wider food industry. *Journal of Agricultural and Food Chemistry*, *53*, 1309–1316.

Torres, A. C., Ghica, M. E., & Brett, C. M. A. (2013). Design of a new hypoxanthine biosensor: Xanthine oxidase modified carbon film and multi-walled carbon nanotube/carbon film electrodes. *Analytical and Bioanalytical Chemistry*, *405*, 3813–3822.

Turdean, G. L. (2011). Design and development of biosensors for the detection of heavy metal toxicity. *International Journal of Electrochemistry*, *2011*, 343125.

Van Ginkel, S. W., Hassan, S. H. A., & Oh, S. E. (2010). Detecting endocrine disrupting compounds in water using sulfur-oxidizing bacteria. *Chemosphere*, *81*, 294–297.
Vlyskoiil, V., Labuda, J., & Barek, J. (2010). Voltammetric detection of damage to DNA caused by nitro derivatives of fluorene using an electrochemical DNA biosensor. *Analytical and Bioanalytical Chemistry*, *397*, 233–241.
Vyas, P., & O'Kane, A. A. (2011). Determination of vitamin B12 in fortified bovine milk-based infant formula powder, fortified soya-based infant formula powder, vitamin premix, and dietary supplements by surface plasmon resonance: Collaborative study. *Journal of AOAC International*, *94*, 1217–1226.
Vyas, P., O'Kane, A. A., & Dowell, D. (2012). Determination of vitamin B12 in infant formula and adult nutritionals by surface plasmon resonance: First action 2011.16 (Test kit method). *Journal of AOAC International*, *95*, 329–334.
Wan, Y., Zhang, D., & Hou, B. (2010). Determination of sulphate-reducing bacteria based on vancomycin-functionalised magnetic nanoparticles using a modification-free quartz crystal microbalance. *Biosensors & Bioelectronics*, *25*, 1847–1850.
Wang, J., & Chen, L. (1995). Hydrazine detection using a tyrosinase-based inhibition biosensor. *Analytical Chemistry*, *67*, 3824–3827.
Wang, J., Chen, L., Mulchandani, A., & Chen, W. (1999). Remote biosensor for in-situ monitoring of organophosphate nerve agents. *Electroanalysis*, *11*, 866–890.
Wang, J., Chicharro, M., Rivas, G., Cai, X., Dontha Percio, N., Farias, A. M., et al. (1996). DNA biosensor for the detection of hydrazines. *Analytical Chemistry*, *68*, 2251–2254.
Wang, G., Dewilde, A. H., Zhang, J., Pal, A., Vashist, M., Bello, D., et al. (2011). A living cell quartz crystal microbalance biosensor for continuous monitoring of cytotoxic responses of macrophages to single-walled carbon nanotubes. *Particle and Fibre Toxicology*, *8*, 4.
Wang, B., Jansson, I., Schenkman, J. B., & Rusling, J. F. (2005). Evaluating enzymes that generate genotoxic benzo[a]pyrene metabolites using sensor arrays. *Analytical Chemistry*, *77*, 1361–1367.
Wang, J., Rivas, G., Ozsoz, M., Grant, D. H., Cai, X., & Parrado, C. (1997). Microfabricated electrochemical sensor for the detection of radiation-induced DNA damage. *Analytical Chemistry*, *69*, 1457–1460.
Wang, B., & Rusling, J. F. (2003). Voltammetric sensor for chemical toxicity using [Ru(bpy)2-poly(4-vinylpyridine)10Cl]+ as catalyst in ultrathin films. DNA damage from methylating agents and an enzyme-generated epoxide. *Analytical Chemistry*, *75*, 4229–4235.
Wang, X. Y., Watanabe, H., & Uchiyama, S. (2008). Amperometric l-ascorbic acid biosensors equipped with enzyme micelle membrane. *Talanta*, *74*, 1681–1685.
Wang, Y., Ye, Z., Si, C., & Ying, Y. (2013). Monitoring of Escherichia coli O157:H7 in food samples using lectin based surface plasmon resonance biosensor. *Food Chemistry*, *136*, 1303–1308.
Wei, D., Oyarzabal, O. A., Huang, T.-S., Balasubramanian, S., Sista, S., & Simonian, A. L. (2007). Development of a surface plasmon resonance biosensor for the identification of Campylobacter jejuni. *Journal of Microbiological Methods*, *69*, 78–85.
Wen, Y., Xu, J., Liu, M., Li, D., & He, H. (2012). Amperometric vitamin C biosensor based on the immobilization of ascorbate oxidase into the biocompatible sandwich-type composite film. *Applied Biochemistry and Biotechnology*, *167*, 2023–2038.
Xia, Y., Zhang, J., & Jiang, L. (2011). A novel dendritic surfactant for enhanced microcystin-LR detection by double amplification in a quartz crystal microbalance biosensor. *Colloids and Surfaces B: Biointerfaces*, *86*, 81–86.
Xiulan, S., Yinzhi, Z., Jingdong, S., Liyan, S., He, Q., & Weijuan, Z. (2010). A quartz crystal microbalance-based immunosensor for shrimp allergen determination in food. *European Food Research and Technology*, *231*, 563–570.

Yeh, Y. P., & Ramsden, J. J. (2010). Quantification of the number of adsorbed bacteria on an optical waveguide. *Journal of Biological Physics and Chemistry*, *10*, 53–54.
Zhao, D., Jia, J., Yu, X., & Sun, X. (2011). Preparation and characterization of a molecularly imprinted polymer by grafting on silica supports: A selective sorbent for patulin toxin. *Analytical and Bioanalytical Chemistry*, *401*, 2259–2273.
Ziyatdinova, G., & Labuda, J. (2012). Biosensor with protective membrane for the detection of DNA damage and antioxidant properties of fruit juices. *Electroanalysis*, *24*, 2333–2340.