# *Chapter 3*
# Sensors and biosensors for endocrine disrupting chemicals: State-of-the-art and future trends

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## 3.1 INTRODUCTION

Endocrine systems control hormones and activity-related hormones in many living organisms including mammals, birds, and fish. The endocrine system consists of various glands located throughout the body, hormones produced by the glands, and receptors in various organs and tissues that recognize and respond to the hormones (USEPA, 2010a). There are some chemicals and compounds that cause interferences in the endocrine system and these substances are known as endocrine disrupting chemicals (EDCs). Wikipedia states that EDCs or "endocrine disruptors are exogenous substances that act like hormones in the endocrine system and disrupt the physiologic function of

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endogenous hormones. They are sometimes also referred to as hormonally active agents.'' EDCs can be man-made or natural. These compounds are found in plants (phytochemicals), grains, fruits and vegetables, and fungus. Alkyl-phenols found in detergents, bisphenol A used in PVC products, dioxins, various drugs, synthetic estrogens found in birth control pills, heavy metals (Pb, Hg, Cd), pesticides, pasticizers, and phenolic products are all examples of EDCs from a long list that is rapidly getting longer. It is suspected that EDCs could be harmful to living organisms, therefore, there is a concerted effort to detect and treat EDCs before they can cause harm to the ecosystem components.

In this chapter we discuss some of the EDC sensors and biosensors which have been developed over the last few years. The first part of the chapter is dedicated to EDC sensors and biosensors. We then include other sensors while discussing trends in sensors and biosensors keeping in mind that the technology used for the other sensors can be very well adapted for the fabrication of EDC sensors. The purpose of this chapter is to offer an opportunity to the readers to have a feel of the enormous possibilities that sensor and biosensor technologies hold for the detecting and quantifying of micro-pollutants in the environment. The chapter is based on a number of original and review papers which cited throughout the chapter.

## 3.2 SENSORS AND BIOSENSORS

### 3.2.1 The need for alternative methods

The most widely used methods for the determination of various EDCs are high-performance liquid chromatography (HPLC), liquid chromatography coupled with electrochemical detection (LC-ED), liquid chromatography coupled with mass spectrometry (LC-MS), capillary electrophoresis (CE), gas chromatography (GC), and gas chromatography coupled with mass spectrometry (GC–MS) (Nakata *et al.,* 2005; Petrovic *et al.,* 2005; Liu *et al.,* 2006a; Vieno *et al.,* 2006; Wen *et al.,* 2006; Gatidou *et al.,* 2007; Comerton *et al.,* 2009; Mottaleb *et al.,* 2009). These methods offer excellent selectivity and detection limits, however, they are not suitable for rapid processing of multiple samples and real-time detection. They involve highly trained operators, time-consuming detection processes, and complex pre-treatment steps. The instruments are sophisticated and expensive. Further, the methods are unsuitable for field studies and in-situ monitoring of samples (Rahman *et al.,* 2007; Rodrigues *et al.,* 2007; Huertas-Perez and Garcia-Campana, 2008; Saraji and Esteki, 2008; Blăzkova *et al.,* 2009; Le Blanc *et al.,* 2009; Suri *et al.,* 2009; Yin *et al.,* 2009). EDCs can also be detected using immunochemical techniques like enzyme-linked immunosorbent

assays (ELISA) (Marchesini *et al.,* 2005, 2007; Rodriguez-Monaz *et al.,* 2005; Kim *et al.,* 2007), however, these immunotechniques are less advantageous than chromatographic techniques because the stability of the biological materials used in assays is lower, and the assays involve complicated multistage steps that may involve expensive equipment. The specific antibodies or particular proteins must be obtained by recombinant techniques for assay fabrication (Le Blanc *et al.,* 2009; Yin *et al.,* 2009). ELISA-based methods are difficult to use for non-specialized laboratories and in the field. They involve labor intensive operations like repeated incubation and washing, and enzyme reaction for final signal generation (Blazkova *et al.,* 2009). Further, ELISA-based methods are specific for a single compound or, at the best, its structurally related compounds. They can not be used for multi-analyte detection and quantification. Unfortunately EDCs are structurally diverse and there is a continuous introduction of new EDCs into the environment due to market driven evolution of chemicals (Marchesini *et al.,* 2007).

There is a need for new, simple analytical techniques for EDCs with reliable and fast responses. High cost is a major hindrance for the introduction of new tools and equipment into existing laboratories. Lower capital, operation and maintenance costs will make such equipment very attractive. The equipment should be simple to operate, less time consuming, have high sensitivity, and suitability for real-time detection. Sensors of various kinds can be the alternative for expensive analytical methods (Yin *et al.,* 2009). The high number and structural diversity of EDCs calls for for the urgent development of sensors for monitoring activities (or measuring effects of the EDCs) rather than only the concentration of a single, or a set of, compounds (Le Blanc *et al.,* 2009).

### 3.2.2 Electrochemical sensors

Electrochemical sensors are cheap, simple to fabricate, and reusable. They have high stability and sensitivity. They can potentially be used for other species with the necessary modifications (Kamyabi and Aghajanloo, 2008; Yin *et al.,* 2009). Many phenolic compounds are successfully detected using electrochemical sensors as most sensors are oxidized at readily accessible potentials (Liu *et al.,* 2005a). Being able to decrease the redox potential needed for the electrochemical reaction makes the sensor more adaptable and sensitive to more EDCs. Chemically modified carbon paste electrodes have been prepared by Yin *et al.* (2009) for the detection of bisphenol A (BPA). Cobalt phthalocyanine modifier has been used in electrodes to help decrease the redox potential. Increased sensitivity and selectivity have been achieved for BPA in an aqueous medium. The detection limit was $1.0 \times 10^{-8}$ M (Yin *et al.,* 2009).

### 3.2.3 Biosensors

While chemicals and electrochemical strategies for determining contaminants are robust, they don't give us a complete picture of the ecological risks involved and impacts observed. Such information can be obtained only after proper interpretation by experts, however, combining both biological responses and chemical analyses may give us a better picture of the situation. We should be able to get results for the identification of toxic hotspots, toxic chemical characterization and estimation of ecological risks of the contaminants at relevant spatial scales. Such assessments call for rapid, inexpensive screening to characterize the extent of the contamination (Brack *et al.,* 2007; Farré *et al.,* 2007; Blasco and Picó, 2009; Fernandez *et al.,* 2009; USEPA, 2010b). Further, the use of biological tools will help in the quantification of an EDC or any other pollutant in terms of its eco-effects (Marchesini *et al.,* 2007). Different biological tools including biosensors have been extensively used in recent years. Biomonitoring is becoming an essential component in effective environmental monitoring (Grote *et al.,* 2005; Rodriguez-Mozaz *et al.,* 2005; Barcelo and Petrovic, 2006; Gonzalez-Doncel *et al.,* 2006; González-Martinez *et al.,* 2007; Tudorache and Bala, 2007; Blasco and Picó, 2009).

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as "a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals." Organelles include both mitochondria and chloroplasts (where photosynthesis takes place). Biosensors offer a number of advantages over conventional analytical techniques including portability, miniaturization and on-site monitoring. They are also capable of measuring pollutants in complex matrices and with minimal sample preparation. Even though biosensors can't yet measure analytes as accurately as conventional analytical methods they are very good tools for routine testing and screening (Rodriguez-Mozaz *et al.,* 2006a). Rodriguez-Mozaz *et al.* (2006a) have carried out an extensive review of biosensors for environmental analysis and monitoring. Their review covers biosensors for the measurement of pesticides, hormones, PCBs, dioxins, bisphenol A, antibiotics, phenols, and EDC effects. The monitoring process using conventional analytical methods involves the collection of water samples followed by laboratory-based instrumental analysis and such analyses only provide snapshots of the situation at the sampling site and time rather than more realistic information on spatio-temporal variations in water characteristics (Allan *et al.,* 2006; Rodriguez-Mozaz *et al.,* 2006a). Biosensors can be useful in situations when continuous and spatial data are needed. Biosensors have high

specificity and sensitivity. Further, a biosensor can not only determine chemicals of concern but can record their biological effects (toxicity, cytotoxicity, genotoxicity or endocrinedisrupting effects). Often, information on biological effects is more relevant than the chemical composition. A biosensor can provide an assessment of both the total and bioavailable/bioaccesible contaminants. However, the majority of the biosensor systems developed is still in the lab tables or in prototype stages and needs to be validated before mass production and use (Rodriguez-Mozaz *et al.,* 2006a; Farré *et al.,* 2009a)

Rodriguez-Mozaz *et al.* (2006a) further discussed specific biosensors developed for certain contaminants. Organophosphorous hydrolase (OPH) can be combined with optical or amperometric transducers to measure absorbance or oxidation reduction currents generated by hydrolysis byproduct of many pesticides (e.g., paraoxon, parathion) and chemical warfare agents (e.g., sarin and soman). OPH [or Phosphotriesterase (PTE)] enzyme can hydrolyze organophosphate pesticides to release p-nitrophenol which is electroactive and chromophoric and can, thus, be measured with an OPH biosensor (Rodriguez-Mozaz *et al.,* 2006a).

EDCs bind to a hormone receptor site or a transport protein and express their biological effects. This can interpreted as (1) mimicking or antagonizing the effects of the endogenous hormone; or (2) disrupting the synthesis or metabolism of endogenous hormones or hormone receptors. It is possible to use the same receptors or transport proteins targeted by the EDCs as their bio-recognition elements. A method like this allows us to monitor the endocrine disrupting potency of single or multiple chemicals in a sample based on their bio-effect(s) on the receptor (Marchesini *et al.,* 2007). For example, human estrogen receptor $\alpha$ (ER$\alpha$) group is capable of interacting with a large variety of chemicals (e.g., phytoestrogens, xenoestrogens, pesticides) that cause estrogenic effects *in-vivo*. The receptor family offers a variety of opportunities for use in tailor-made applications for EDCs. These include interaction between the ligand-binding domain (LBD) and its ligands or peptides derived from co-activator or co-repressor proteins (Fechner *et al.,* 2009), and the interaction between DNA-binding domains and certain DNA sequences (estrogen response elements) (Asano *et al.,* 2004; Le Blanc *et al.,* 2009). EDCs are chemicals that are able to interfere with interactions between ER$\alpha$ and these domains. Le Blanc *et al.* (2009) used this knowledge of the effects of EDCs on ER$\alpha$ receptors. They labeled ER$\alpha$ in an assay to determine the impact of EDCs. As compared to conventional methods, the new assays determine the total effect on the receptor instead of concentrations of single compounds. The signal obtained is the response of the organism which is exposed to EDCs. The detection limit was reported to be 0.139 nM of estradiol equivalents. While standard analytical techniques are

designed to find only known compounds the results of this assay incorporate all known and unknown EDCs (and possibly other compounds). These data are difficult to compare and validate. While validation will be a necessary step in the near future, the assay can be used now to monitor changes in the estrogenicity of environmental samples over time. Sanchez-Acevedo *et al.* (2009) recently reported the detection of picomolar concentrations of bisphenol A (BPA) in water using a carbon nanotube field-effect transistor (CNTFET). The CNTFET is functionalized with ERα where ERα serves as the recognition layer for the sensor. The sensor uses the molecular recognition principles. Single-walled carbon nanotubes (SWCNTs) have been used as transducers and ERα is adsorbed onto their surface. A blocking agent has been used in order to avoid non-specific adsorption on the SWCNT surface. BPA has been detected up to $2.19 \times 10^{-12}$ M in aqueous solution in 2 minutes. Fluoranthene, pentacloronitrobenzene and malathion present in the water didn't produce any interferences. Such a biosensor can be useful in a label-free platform for detecting other analytes by using an appropriate nuclear receptor (Sanchez-Acevedo *et al.,* 2009).

Marchesini *et al.* (2006) reported the use of a plasmon resonance (SPR)-based label-free biosensor manufactured by an US manufacturer. They used this in combination with a ready-to-use biosensor chip to screen bio-effect related molecules and predicted possible SPR biosensor uses for EDC bio-effect monitoring. While it is possible to use such biosensors for EDC detection, the exorbitant price of commercially available systems and the lack of portability for in-situ analysis are the major drawbacks of SPR-based biosensors. These are the major challenges that need to be overcome for SPR-based biosensors to be popular (Marchesini *et al.,* 2007). SPR-based sensors have been used for dioxins, polychlorinated biphenyl and atrazine (Farré *et al.,* 2009b) and the sensor needed 15 min for a single sample measurement. A portable SPR immunosensor for organophosphate pesticide chlorpyrifos (detection limit of 45–64 ng/L) as well as single and multi-analyte SPR assays for the simultaneous detection of cholinesterase-inhibiting pesticides have been reported (Mauriz *et al.,* 2006a, b; Farré *et al.,* 2009b). These sensors were made re-usable through the formation of alkanethiol self-assembled monolayers.

Bacterial and other cells are also used in sensors known as whole-cell sensors. During ongoing research to detect the estrogenic properties of commonly used chemicals, products, and their ingredients, researchers have developed many different live animal, whole cell, and *in-vitro* binding assays. ER-positive breast cancer cell lines show increased proliferation due to estrogenic activity. Many *in-vitro* assays can be used to detect estrogens. Hormone responsive reporter assays in human breast cancer cells and rat fibroblast cells are examples of this, however, they typically need complex equipment and reagents and are highly

sensitive to interferences (Gawrys *et al.,* 2009). Gawrys *et al.* (2009) have developed a simple detection system in which the ligand-binding domain of the estrogen receptor $\beta$ (ER$\beta$) has been incorporated into a larger allosteric reporter protein in *E. coli* cells. The reporter protein expresses itself by creating a hormone-dependent growth phenotype in thymidylate synthase deficient *E. coli* strains. If a knockout media is used then there will be a marked change in growth in the presence of various test compounds that can be detected by a simple measure of the turbidity. Estrogenic behavior in compounds found in consumer products was tested using this technique. The allosteric biosensor *E. coli* strain was used to evaluate estrogenicity of a variety of compounds and complex mixtures used in common consumer products. Perfumes, hand and body washes, deodorants, essential oil's and herbal supplements were included in the samples tested with 17 $\beta$-estradiol and two thyroid hormones (as controls). The system offered an additional advantage of detecting cytotoxicity of various compounds to the sensor strains. Cytotoxicity detection was based on the loss of viability of the cells in the presence of the test compound under nonselective conditions (Skretas and Wood 2005; Gawrys *et al.,* 2009).

A review by Farré *et al.* (2009) covered the developments in the whole-cell biosensor area. Amperometric biosensors based on genetically-engineered *Moraxella sp.* and *Pseudomonas putida* JS444 with surface-expressed OPH were used for the detection of organophosphorous pesticides (Lei *et al.,* 2005, 2007). The sensors measured up to 277 ng/L of fenitrothion. Liu *et al.* (2007) used horizontally aligned SWCNTs to fabricate biosensors for the real-time detection of organophosphate. SWCNT surface immobilized OPH triggers enzymatic hydrolysis of pesticides (e.g., paraoxon). The hydrolysis causes a detectable change in the conductance of the SWCNTs which is correlated to the organophosphorous pesticide concentration. Glass electrodes have been modified with genetically-engineered *E. coli* and organophosphorous pesticides degrading bacteria *Flavobacteium sp.* (Mulchandani *et al.,* 1998a, b; Berlein *et al.,* 2002).

The photosynthesis reaction mechanism (photosystem II or PS II) has also been used in biosensors (Giardi and Pace, 2005; Campàs *et al.,* 2008). The PSII-based biosensors can recognize analytes such as triazines, phenylurea, diazines, and phenolic compounds. In PSII, light is first absorbed by chlorophyll–protein complexes. The photochemically active reaction centre chlorophyll (P680) then becomes excited and donates electrons to the primary pheophytin acceptor. This charge separation is stabilized by the transfer of an electron to quinone $Q_A$ and subsequently to $Q_B$. $Q_A$, a firmly bound plastoquinone molecule is located in the D2 subunit while $Q_B$ is a mobile plastoquinone located in the D1 subunit of PSII. $Q_A$ and $Q_B$ are binding pockets. Many herbicides can bind reversibly to

the "herbicide-binding niche" which is the D1 subunit of PSII within its $Q_B$ binding pocket. Once bound to the niche, the herbicides displace the plastoquinone $Q_B$ and inhibit natural electron transfer. Once the electron flow is stopped, oxygen evolution also stops and the fluorescence properties of PSII change (Giardi and Pace, 2006; Chaplen *et al.,* 2007; Campàs *et al.,* 2008). While this is an exciting way of detecting herbicides, PSII based herbicide recognition is not very dependable as heavy metals may interfere (Chaplen *et al.,* 2007). Such interferences limit the use of PSII based biosensors (Giardi *et al.,* 2009). There are about 65 amino acids in a herbicide binding site. Giardi *et al.* (2009) hypothesized that modifying only one amino acid within the $Q_B$ binding pocket would change photosynthetic activity and herbicide-binding characteristics considerably. Also, depending on the position and type of amino acid substitution, different herbicides will show affinity for the site. They used unicellular green algae *Chlamydomonas reinhardtii* strains and modified the $Q_B$ pocket. The mutant algae cells were then used to fabricate a re-usable and portable optical biosensor with enhanced sensitivity toward different herbicide (e.g., atrazine, diuron, linuron). The detection limits ranged from $0.9\times10^{-11}$ to $3.0\times10^{-9}$ M (Giardi *et al.,* 2009).

### 3.2.4 New generation immunosensors

While conventional ELISA is considered inadequate for many contaminants, new generation of immunosensors are becoming increasingly popular. Electrochemical immunosensors can be used for real-time in-situ monitoring of EDCs like BPA. Immunosensors are widely used for the detection of an analyte where an enzyme is labeled with a specific antigen. Enzyme labeling is a time consuming and complicated procedure. However, label-free electrochemical immunosensors represent a very attractive technique to detect EDCs by monitoring changes in electronic properties due to immunocomplex formation on the electrode surface (Rahman *et al.,* 2007). Rahman *et al.* (2007) have fabricated a label-free impedimetric immunosensor for the direct detection of BPA. They prepared antigens through the conjugation of BHPVA with bovine serum albumin and then produced a specific polyclonal antibody. A covalent immobilization technique was used during sensor fabrication to attach a polyclonal antibody onto a carboxylic acid group which was functionalized on nanoparticle-based conducting polymer (Rahman *et al.,* 2005) coated onto a glassy carbon electrode. Ag/AgCl and Pt wires were used as reference and counter electrodes, respectively. The detection limit was determined to be 0.3 $\mu$g BPA/L (Rahman *et al.,* 2007).

Suri *et al.* (2009) provide an elaborate discussion of immunoanalytical techniques for pesticide analysis. Immunochemical techniques offer great potential for developing inexpensive, reliable sensors for effective field monitoring of many toxic molecules. Such a sensor can be based on the specificity of the antibody–antigen ($A_b$–$A_g$) reaction. Specific antibodies can be produced against pesticide molecules. As compared to other sensors, immunosensors can provide quantitative results with similar or even greater sensitivity, accuracy, and precision. Immunosensor data are comparable to standard chemical-based methods as well. Immunosensors are important tools because they complement existing analytical methods and provide low-cost confirmatory tests for many compounds, including pharmaceuticals and pesticides. A detectable signal is obtained from binding interactions between immobilized biomolecules ($A_b$ or $A_g$) and analytes ($A_g$ or $A_b$) of interest. Immobilization typically happens on the transducer surface. The sensor is based on the molecular recognition characteristics and, hence, the high selectivity of an $A_b$ can be achieved (Farré *et al.,* 2007; Suri *et al.,* 2009). $A_b$ binds reversibly with a specific $A_g$ in a solution to form an immunocomplex ($A_b - A_g$) (Suri *et al.,* 2009):

$$A_b + A_g \frac{K_a}{K_d} \leftrightarrow A_b - A_g \tag{3.1}$$

where $K_a$ = rate constants for association and $K_d$ = rate constants for dissociation. The equilibrium constant (or the affinity constant) of the reaction is:

$$K = \frac{K_a}{K_d} = \frac{[A_b - A_g]}{[A_b][A_g]} \tag{3.2}$$

An immunocomplex typically has a low $K_d$ value (in the range $10^{-6}$–$10^{-12}$) and displays a high $K$ value ($\sim$104). The equilibrium kinetics in solution suggest rapid association and dissociation while the direction of equilibrium depends on the overall affinity (Suri *et al.,* 2009). Immunosensors were initially used for clinical diagnostics. The development and applications of immunosensors for environmental pollutants (e.g., pesticides) are relatively new. The reasons for the time lag include difficulty in finding antibodies against pesticides (pesticides being low in molecular weight) (Suri *et al.,* 2009). $A_b$ affinity and specificity primarily determines the analytical capability of an immunosensor and, hence, the development of antibodies represents a key step in the sensor development (Farré *et al.,* 2007). Now, that antibodies can be produced against low-molecular mass pesticides, immunosensors are expected to become cost-effective devices for the on-site monitoring of

pesticides. However, many challenges remain. One of these challenges was the development of pesticide species-specific immunosensors. Pesticides are usually nonimmunogenic and it is, therefore, crucial to synthesize a suitable hapten molecule which can be coupled with a carrier protein to make a stable carrier-hapten complex. The carrier-hapten conjugate should mimic the structure of small pesticide molecules such that a suitable $A_b$ for a particular immunoassay for the specific target molecule can be generated (Suri *et al.,* 2009).

With recent developments, immunoassays have been used for the measurement of both single and multiple analytes. Organic pollutants like pesticides, polychlorinated biphenyls (PCBs), and surfactants can be rapidly and efficiently determined through immunochemistry (Farré *et al.,* 2007). Farré *et al.* (2009b) have discussed electrochemical immunosensors for environmental analysis (atrazine determination) which have used recombinant single-chain $A_b$ (sc$A_b$) fragments (Grennan *et al.,* 2003). Automated optical immunosensors have been used to detect many organic pollutants including estrone, progesterone, and testosterone in water samples. The detection limits were reported to be sub-ng/L (Taranova *et al.,* 2004). Labeled immunosensors have been used to detect hormones, enzymes, virus, tumor antigens, and bacterial antigens at concentrations around $10^{-12}$ to $10^{-9}$ mol/L (Campàs *et al.,* 2008; Wang *et al.,* 2008; Wang and Lin, 2008; Bojorge Ramírez *et al.,* 2009; He *et al.,* 2009)

Farré *et al.* (2007) list a number of limitations of the immunosensing approach including

(1) lengthy preparation time for immuno-reagents;
(2) lack of specificity as well as cross-reactivity;
(3) lack or limited response towards some groups of pollutants (e.g., perfluorinated compounds);
(4) poor stability under different thermal and pH conditions; and
(5) short life-times of biological components.

Farré *et al.* (2007) also enumerate key aspects that need to be addressed in future immunoassays which include

(1) development of more stable biological components;
(2) fabrication of more robust assays;
(3) assurance of better repeatability between different batches of production when disposable elements are involved; and
(4) integration of new technologies coupled to biosensors (e.g., the polymerase chain reaction).

González-Martinez *et al.* (2007) expect that immunosensors should be usable when

- a high number of samples need to be screened;
- on-line control is necessary;
- analysis is to be carried out in the field;
- different analytes need to be determined in a sample by different methods;
- data should be presented within minutes or in real time;
- samples need to be analyzed directly with none or hardly any pretreatment; and
- traditional methods do not work properly.

González-Martinez *et al.* (2007) described features of an ideal immunosensor as (1) very high sensitivity even to measure contaminants of interest in very diluted solutions; (2) high selectivity for compounds of interest without or with the minimum cross-reactivity problem; (3) applicable to the whole family of related compounds with generic immunoreagents; (4) high rapidity or speed without compromising sensitivity; (5) re-usable such that the device can work for a very long time (or large number of samples) without major maintenance; (6) capable of multiparameter determination (5–10 contaminants simultaneously); (7) versatile such that the sensor can be used for new analytes provided that the appropriate reagents are available; and (8) robust such that a sensor can be used under different conditions. Bojorge Ramírez *et al.* (2009) agree that there are a number of challenges to overcome for the mass production of immunosensors for a wide variety of compounds of interest. Antibodies to be used *in-vivo* need protein stability and antigen affinity. The industrial-scale mass production of antibodies is not yet possible without further advances in biochemical engineering technologies (Bojorge Ramírez *et al.,* 2009).

Fluorophores are now preferred over enzymes in immunosensors because they are more stable in solution than enzymes, also, the assay is shortened as the signal is displayed immediately (González-Martinez *et al.,* 2007). Wikipedia defines a fluorophore as a functional group of a molecule which becomes fluorescent under appropriate environmental conditions. Flurophores absorb the energy of a specific wavelength and re-emit it at a different, but specific, wavelength. The quantity and wavelength of the emitted energy depend on both the fluorophore and the chemical environment to which the fluorophore is exposed. Eu(III) chelate-dyed nanoparticles have been used as an antibody label

[*AQ*5]

in a fluoroimmunosensor for atrazine. The sensitivity (IC50) was reported to be ~1 μg $L^{-1}$ for this immunosensor (Cummins *et al.,* 2006).

Nanotechnology has made significant inroads into the immunosensor area too. Magnetic nanoparticles were functionalized with specific antibodies ($A_b$) and used in immunomagnetic electrochemical sensors (Andreescu *et al.,* 2009). The use of $A_b$-coated magnetic nanoparticles eliminates or at least reduces the need for regeneration of the sensing surface. Quantification of the formed immunocomplex is done through enzyme labeling. Quantification can also be achieved via electrochemical detection of the reaction products after the complex is exposed to the enzymatic substrates, or through fluorescent labeling (Andreescu *et al.,* 2009). Different environmental contaminants were detected in this way. The contaminants include PCBs (Centi *et al.,* 2005), 2, [AQ6] 4 D-herbicide, and atrazine (Helali *et al.,* 2006; Zacco *et al.,* 2006). Arochlor 1248 (a PCB) detection limits of 0.4 ng/mL using screen-printed electrode strips have been achieved as well as atrazine detection limits of 0.027 nmol $L^{-1}$ using anti-atrazine-specific antibody (Zacco *et al.,* 2006). Andreescu *et al.* (2009) reported in their review paper that paraoxon was measured at a low 12 μg/L level and a linear range within 24–1920 μg/L was achieved with an electrochemical immunosensor based on $A_b$-labeled gold nanoparticles on a glassy carbon electrode. Polymeric nanoparticles (e.g., 2-methacryloyloxyethyl phosphorylcholine and polysterene) coated with anti-bisphenol $A_b$ were used in a piezoelectric immunosensor for bisphenol A and an eight-fold sensitivity increase was achieved (Park *et al.,* 2006). In a reported work, [AQ7] Blăzkova *et al.* (2009) developed a simple and rapid immunochromatographic assay for a sensitive yet inexpensive monitoring of methiocarb in surface water using a binding inhibition format on a membrane strip. In the assay, the detection reagent consisted of anti-methiocarb $A_b$ and colloidal carbon-labeled secondary $A_b$. They used carbon nanoparticles to bind proteins noncovalently without changing their bioactivity. A detection limit of 0.5 ng/L was reported. The assay results (recovery 90–106%) were in a good agreement with those of ELISA (recovery 91–117%). The strips were stable for at least 2 months without any change in performance. The developed immunochromatographic assay has potential for on-site screening of environmental contaminants.

[AQ8] **Table 3.1** Examples of EDC sensors and biosensors (after Rodriguez-Mozaz *et al.,* 2006; Farré *et al.,* 2009a, 2007)

| Analyte | Transduction method | Limit of detection | Reference |
|---|---|---|---|
| Carbamates | Potentiometric | 15–25 μM | Ivanov *et al.,* 2000 |
| Dimethyl and diethyl dithiocarbamates | Amperometric detection | 20 μM | Pita *et al.,* 1997 |
| Bisphenol A | Potentiometric immunosensor | 0.6 ng/mL | Mita *et al.,* 2007 |
| Fenitrothion and ethyl p-nitrophenol | Organophosphates | 4 μg/L | Rajasekar *et al.,* 2000 |
| Progesterone | Amperometric detection | 0.43 ng/mL | Carralero *et al.,* 2007 |
| Parathion | Amperometric detection | 10 ng/mL | Sacks *et al.,* 2000 |
| 2,4-dichlorophen-oxyacetic acid | Amperometric immunosensor | 0.1 μg/L | Wilmer *et al.,* 1997 |
| Atrazine | Electrochemical amperometric | 0.03 nmol/L | Zacco *et al.,* 2006 |
| Chlorsulfuron | Electrochemical and amperometric | 0.01 ng/mL | Mallat *et al.,* 2001 [AQ9] |
| Estrogens | Total internal reflection fluorescence | 0.05–0.15 ng/mL | Rodriguez-Mozaz *et al.,* 2006b |
| Trifluralin | Optical wave light spectroscopy | 0.03 pg/mL | Szekacs *et al.,* 2003 [AQ10] |
| Sulphamethoxazole | Piezoelectric | 0.15 ng/mL | Melikhova *et al.,* 2006 |
| Isoproturon | Total internal reflection fluorescence | 0.01–0.14 μg/L | Blăzkova *et al.,* 2006 |
| Dioxins | Quartz crystal microbalances | 15 ng/L | Kurosawa *et al.,* 2005 |
| Paraoxon and carbofuran | Electrochemical (amperometric) | 0.2 μg/L | Bachmann and Schmid, 1999 |
| Phenols | Electrochemical | 0.8 μg/L | Nistor *et al.,* 2002 |
| Chlorophenols | Optical chemiluminescence | 1.4–1975 μg/L | Degiuli and Blum, 2000 |
| Nonylphenol | Electrochemical | 10 μg/L | Evtugyn *et al.,* 2006 |

## 3.3 TRENDS IN SENSORS AND BIOSENSORS

### 3.3.1 Screen printed sensors and biosensors

There have been efforts to develop and apply environmentally friendly analytical procedures for contaminant detection. Increasing concerns about the impact of chemical waste generated during conventional analytical procedures have accelerated the search for alternatives. "Green" analytical chemistry is especially relevant to the use of instruments in the field and in decentralized laboratories where treatments for toxic and hazardous wastes are not available. A range of environmentally friendly electrochemical sensors for water monitoring is available and a number of them are now in advanced prototype stages. Conventional electrochemical cells are now being replaced with screen printed electrodes (SPEs) connected to miniaturized potentiostats. SPEs are finding uses in major analytical laboratory equipment and as hand-held field devices. A number of SPEs are commercially available and it is possible to manufacture them in the laboratory for research applications since screen-printing technology is getting cheaper and more easily available (Rico *et al.,* 2009). Farré *et al.* (2009b) reviewed many recent papers and covered many topics including SPEs. Electrochemical DNA and protein sensors based on the catalytic activity of hydrazine have been developed as SPEs (Shiddiky *et al.,* 2008). Enzyme-based high sensitivity biosensors for 2, 4-D, atrazine, and ziram have also been reported [AQ11] (Kim *et al.,* 2008). Gold nanoparticles have been used on tyrosinase electrode for the measurement of pesticides in water (Kim *et al.,* 2008). Organophosphorous and carbamate pesticides (e.g., monocrotophos, malathion, metasystox, and lannate) were measured electrochemically using SPEs containing immobilized acetylcholine esterase (AChE) (Dutta *et al.,* 2008). The measured concentration ranged from 0 to 10 μg/L (Farré *et al.,* 2009b).

### 3.3.2 Nanotechnology applications

Nanomaterials are natural or engineered materials which have at least one dimension at the nanometer scale (≤100 nm). Nanomaterials possess completely new and enhanced properties as compared to the parent bulk materials. Examples of advanced nanomaterials include metallic, metal oxide, polymeric, semiconductor and ceramic nanoparticles, nanowires, nanotubes, quantum dots, nanorods, and composites of these materials. The unique properties of these materials are attributed to the extremely high surface area per unit weight, and their mechanical, electrical, optical, and catalytic properties. These properties offer a wide range of opportunities for the detection of environmental contaminants and toxins in addition to their remediation (Zhang, 2003; Li *et al.,* 2006;

Jimenez-Cadena *et al.,* 2007; Pillay and Ozoemena, 2007; Vaseashta *et al.,* 2007; Khan and Dhayal, 2008; Thompson and Bezbaruah, 2008; Bezbaruah *et al.,* 2009a,b). Nanotechnology incorporation into sensors (Trojanowicz, 2006; Ambrosi *et al.,* 2008; Gomez *et al.,* 2008; Guo and Dong, 2008; Kerman *et al.,* 2008; Wang and Lin, 2008; Algar *et al.,* 2009), miniaturization of electronics, and advancements in wireless communication technology have shown the emerging trend towards environmental sensor networks that continuously and remotely monitor environmental parameters (Huang *et al.,* 2001; Burda *et al.,* 2005; Liu *et al.,* 2005b; Jun *et al.,* 2006; Blasco and Picó, 2009; Zhang *et al.,* 2009).

Major research efforts are being targeted towards the development and application of nanomaterials in sensing (He and Toh, 2006; González-Martinez *et al.,* 2007). Nanomaterials are used in designing novel sensing systems and enhancing their performance (Farré *et al.,* 2009a). Satisfactory electrical communication between the active site of the enzyme and the electrode surface is a major challenge in amperometric enzyme electrodes. Aligned CNTs have been reported to improve electrical communication in such electrodes (Farré *et al.,* 2009a). Andreescu *et al.* (2009) have discussed environmental monitoring possibilities using nanomaterials. The paper cites a number of examples where nanotechnology has been successfully used in sensors. The use of nanotechnology in sensors and sensor hardware has resulted in the development of a number of miniaturized, rapid, ultrasensitive, and inexpensive methods for in-situ and field-based environmental monitoring. While these methods are not perfect and do not necessarily meet the general expectations, they are the harbingers of things to come. Nanoscale materials have been used for the construction of gas sensors (Gouma *et al.,* 2006; Jimenez-Cadena *et al.,* 2007; Milson *et al.,* 2007; Pillay and Ozoemena, 2007; Vaseashta *et al.,* 2007) enzyme sensors, immunosensors, and genosensors, for direct wiring of enzymes at electrode surfaces and for signal amplification (Liu and Lin, 2007; Pumera *et al.,* 2007). Metal oxide nanoparticles, which have excellent catalytic properties, are used for the construction of enzymeless electrochemical sensors (Hrbac *et al.,* 1997; Yao *et al.,* 2006; Hermanek *et al.,* 2007; Salimi *et al.,* 2007). Magnetic iron oxide nanostructures have potential for providing control for electrochemical processes (Wang *et al.,* 2005, 2006). Attachment of biological recognition elements to nanomaterial surfaces have led to the development of various catalytic and affinity biosensors (Andreescu *et al.,* 2009).

[*AQ*12]

Costa-Fernández *et al.* (2006) have published a review on the application of quantum dots (QDs) as nanoprobes in sensing and biosensing. Andreescu *et al.* (2009) also discussed the use of carbon nanotubes (CNTs) and QDs in sensing. The surface chemistry, high surface area, and electronic properties make the use of CNTs ideal for chemical and biochemical sensing. CNTs have the ability to enhance the binding of biomolecules and increase electrocatalytic activities.

With CNTs, the detection of several analytes (e.g., pesticides) is possible at a low-applied potential. There is no need to use electronic mediators, and, hence, interferences are reduced. Electrochemical biosensors were constructed by immobilizing enzymes like acetylcholinesterase (AChE) and organophosphate hydrolyse (OPH) (Deo *et al.,* 2005) within CNTs/hybrid composites (Arribas *et al.,* 2005, 2007; Sha *et al.,* 2006; Rivas *et al.,* 2007). A number of strategies for CNT functionalization and application in sensing and biosensing have been reported (Andreescu *et al.,* 2005, 2008). These sensors have superior sensitivity compared to macroscale material-based ones. CNT-based sensors have been used for the monitoring of organophosphorus pesticides (Deo *et al.,* 2005; Joshi *et al.,* 2005), phenolic compounds (Sha *et al.,* 2006), and herbicides (Arribas *et al.,* 2005, 2007). Gold, platinum, copper, and some other nanoparticles have been incorporated onto CNTs/polymeric composites to further enhance their characteristics (Andreescu *et al.,* 2009). Further, signal amplification has been achieved using gold nanoparticle in biosensors and in a variety of colorimetric and fluorescence assays (Andreescu *et al.,* 2009). QDs have been used as sensing probes for small metal ions (Costa-Fernández *et al.,* 2006; Somers *et al.,* 2007), pesticides (Ji *et al.,* 2005), phenols (Yuan *et al.,* 2008), and nitroaromatic explosives (Goldman *et al.,* 2005). QD-enzyme conjugates respond to enzyme substrates and inhibitors (Ji *et al.,* 2005), and antibodies (Goldman *et al.,* 2005). The conjugates approach has been used to fabricate QDs (Abad *et al.,* 2005; Ji *et al.,* 2005) for the detection of pesticides. Photoluminescence intensity of the QD bioconjugates changes in the presence of the analyte (e.g., pesticide paraoxon) and the changes in signal have been quantified and correlated to analyte concentrations (Ji *et al.,* 2005).

### 3.3.3 Molecular imprinted polymer sensors

A conventional biosensor selectively recognizes analytes and binds them into the specific binding layers provided. This binding creates different events such as optical, mass, thermal and electrochemical changes that produce their corresponding signals (Eggins, 2002). Lots of progress has been made on biological recognition, but there are still some complex compounds that can't be detected accurately by biosensors. Such compounds include antibodies and enzymes (Sellergren, 2001; Yan and Ramstrom, 2005). Molecularly imprinted polymers (MIPs) have drawn considerable attention in recent years. In the environmental area, they are being used for remediation and sensing. MIPs are synthesized using template (target) molecules which are cross-linked into a monomer. The cross linkers are specific to the template. The target-monomer complex is then polymerized and the template molecules are removed to leave the polymer matrix with "holes" specific to the target molecules (Haupt and

[*AQ*13]

Mosbach, 2000; Widstrand *et al.,* 2006). The MIP synthesis process is illustrated in Figure 3.1. The holes capture the target molecules from a sample even if they are present in small amounts. MIP materials have high recognition affinity to the target molecule. The holes are so specific that they allow only the target molecules to enter them and reject all others. MIPs are robust, cost effective, and easy to design. There are a number of advantages of MIP materials: (1) they are small in size; (2) they have increased number of accessible complementary cavities for the target molecule; (3) they have enhanced surface catalytic activity; and (4) they can establish equilibration with the target molecule very quickly due to the limited diffusional length (Nakao and Kaeriyama, 1989; Lu *et al.,* 1999). MIPs have been used in conjunction with optical and electrochemical techniques to detect amino acids, enzymes, antibodies, pesticides, proteins, and vitamins.

[AQ14]

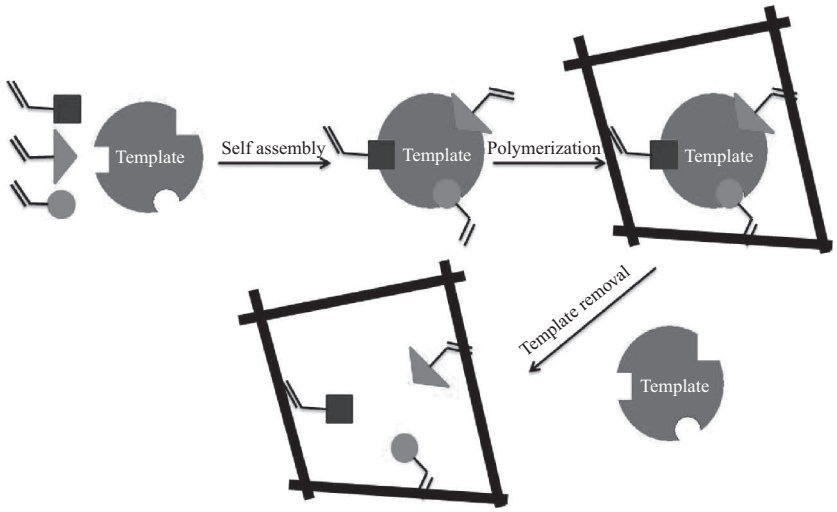


**Figure 3.1** Schematic representation of molecular imprinted polymer (after Shelke *et al.,* 2008)

[AQ15]

The efficiency of the MIP sensors depends on the interaction of the template molecule and the complementary functional monomer group (Whitcombe and Vulfson, 2001). Noncovalent and covalent interactions are observed between them. The noncovalent interactions that hold the template molecule and the functional monomer group together include hydrogen bonding, hydrophobic interaction, Van der Waals forces, and dipole-dipole interactions (Holthoff and Bright, 2007). However, if a functional group has strong covalent interactions,

[AQ16] nonconvalent interactions are suppressed (Graham *et al.,* 2002). Reversible covalent interactions can also bind the template molecule with the functional monomer group. Wulff *et al.* (1977) first introduced the concept of covalent interactions between the functional group and the template molecule and how the template molecule can be released by cleaving the bond. These types of interactions are favored if the functional monomer has diol, aldehyde, or amine. A MIP sensor selectively binds the analyte molecules and produces a transduction scheme to detect the analyte (Figure 3.2) (Lange *et al.,* 2008). A few examples of MIP-based sensors are listed in Table 3.2. The list includes the template molecules, transduction method, and detection limits for various analytes. Both organic and inorganic materials are used to synthesize the MIPs used in the sensors. Holthoff and Bright (2007) have discussed the use of polystyrene, polyacrylate, and inorganic polysiloxane to synthesize MIP-based sensors.

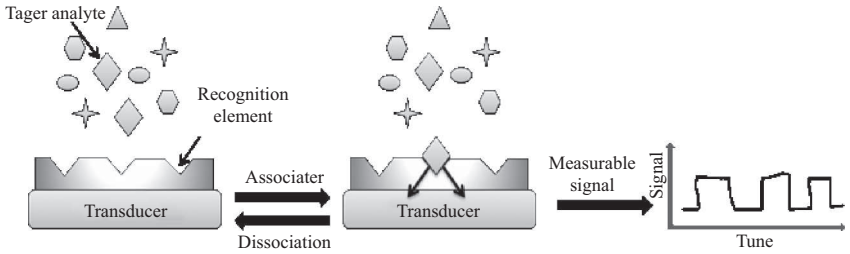


**Figure 3.2** Schematic Representation of an MIP-based biosensor and its response profile (after Holthoff and Bright 2007)

Conventional MIP-based sensors are typically designed for individual analytes. The use of nanotechnology in molecular imprinting holds a lot of promise to overcome this limitation. Micro- and nano-sensors are of interest to scientists and engineers as they can be used as arrays to analyze different molecules at the same time (Alexander *et al.,* 2006). MIP materials can be appropriately patterned on a chip surface and fabricated for multi-analyte sensing applications by interfacing with transducers. Various patterning techniques that are used to make these MIP-based micro- and nano-sensors include photo-lithography, soft-lithography, and microspotting. UV mask lithography has been used to make the micro- and nano-MIP sensors where MIP layer is applied onto a metallic electrode and then cured by UV irradiation (Huang *et al.,* 2004). For example, Pt electrode is used in acrylic molecularly imprinted photoresist (Du *et al.,* 2008; Gomez-Caballero *et al.,* 2008), and Au and Pt electrodes are used to fabricate albuterol (a bronchodilator) MIP microsensor (Huang *et al.,* 2007).

**Table 3.2** Examples of MIP-based sensors (after Holthoff and Bright 2007; Navarro-Villoslada *et al.,* 2007; Zhang *et al.,* 2008)

| Target analyte | Template | Transduction method | Detection limit | Reference |
|---|---|---|---|---|
| Atrazine | Atrazine | Electrochemical | 0.5 μM | Prasad *et al.,* 2007 |
| Cytidine | Cytidine | Electrochemical | Not reported | Whitcombe *et al.,* 1995 |
| Glutathione | Glutathione | Electrochemical | 1.25 μM | Yang *et al.,* 2005 |
| L-Histidine | LHistidine | Electrochemical | 25 nM | Zhang *et al.,* 2005 |
| Parathion | Parathion | Electrochemical | 1 nM | Li *et al.,* 2005 |
| L-Tryptophan | L-Tryptophan | Optical | Not reported | Liao *et al.,* 1999 |
| Adrenaline | Adrenaline | Optical | 5 μM | Matsui *et al.,* 2004 |
| 1,10-Phenanthroline | 1,10-Phenanthroline | Optical | Not reported | Lin and Yamada, 2001 |
| 9-Ethyladenine | 9-Ethyladenine | Optical | Not reported | Matsui *et al.,* 2000 |
| 9-Anthrol | 9,10-Anthracenediol | Optical | 0.3 μM | Shughart *et al.,* 2006 |
| 2,4-Dichlorophenoxy-acetic acid | 2,4-Dichlorophenoxy-acetic acid | Optical | Not reported | Leung *et al.,* 2001 |
| Penicillin G | Penicillin G | Optical | 1 ppm | Zhang *et al.,* 2008 |
| Zearalenone | Zearalenone | Optical | 25 μM | Navarro-Villoslada *et al.,* 2007 |

Microcontact printing is one of the emerging technologies for the production of patterned microstructures (Quist *et al.,* 2005; Lin *et al.,* 2006). MIP micro-patterns are created using this microcontact printing method. MIP microstructures can be synthesized using the poly(dimethyl-siloxiane) (PDMS) stamp technique (Yan and Kapua, 2001). However, incompatibility of the PDMS stamps to some organic solvents limits their applications (Vandevelde *et al.,* 2007). MIP based sensor for theophylline (a methylxanthine drug, also known as dimethylxanthine) has been synthesized using this technique and excellent selectivity for the template molecule was achieved. Similar results were reported for structurally similar caffeine (Voicu *et al.,* 2007). Micro-stereolithography technique is also used to synthesize MIP based sensors with 9-ethyl adenine as the template (Conrad *et al.,* 2003).

### 3.3.4 Conducting polymers

Conducting polymers have found increased applications in various industries. Some of the main classes of conducting polymers that are available for various applications include polyacetylene, polyaniline (PANI), polypyrrole (PPY), polythiophene (PTH), poly(paraphenylene), poly(paraphenylenevinylene), polyfluorene, polycarbazole, and polyindole (PI). Conducting polymers exhibit intrinsic conductivity when the conjugated backbone of the polymer is oxidized or reduced (Bredas, 1995). Apart from its conductivity, the change of electronic band in the conducting polymer affects the optical properties in the UV-visible and near IR region. The changes in conductivity and optical properties make them candidates for use as optical sensors. Chemical and electrochemical methods are used to inject charge (doping) into conducting polymers (Wallace 2003). The electrochemical method is preferred as it is easy to adjust the doping level by controlling the electrical potential.

Conducting polymers have been effectively used to detect metal ions. Polyindole and polycarbazole provide selective responses towards Cu(II) ions (Prakash *et al.,* 2002) and poly-3-octylthiophene (P3OTH) gives Nernstian responses to Ag(I) ions (Vazquez *et al.,* 2005). Extraction and stripping voltammetry method has also been used to detect Pb(II) and Hg(II) with conductive polymers (Heitzmann *et al.,* 2007). Further, ion selectivity of the sensors can be improved by introducing specific ligands (Migdalski *et al.,* 2003; Zanganeh and Amini, 2007; Mousavi *et al.,* 2008), ionophores (Cortina-Puig *et al.,* 2007) and monomers (Seol *et al.,* 2004; Heitzmann *et al.,* 2007) to the polymer backbone.

Organic molecules have an affinity towards the conducting polymer backbone, side group, and to the immobilized receptor group. This affinity is exploited to design conducting polymer sensors for organic molecules. Both biological and synthetic receptors can be used to selectively bind the organic molecule. Dopamine, ascorbic acid, and chlorpromazine sensing has been done by introducing $\gamma$-cyclodextrin receptor to poly(3-methylthiophene) (P3MTH) (Bouchta *et al.,* 2005) and $\beta$-cyclodextrin to PPY (Izaoumen *et al.,* 2005). A film of PANI and poly(3-aminophenylboronic acid) is used for the detection of saccharides by optical method (Pringsheim *et al.,* 1999). Syntheses of a variety of chemosensitive PANI and PPY conductive polymers to detect dicarboxylates, amino acids and ascorbic acid have also been reported (Volf *et al.,* 2002).

The electropolymerization method is also used to synthesize MIPs for the preparation of conductive chemosensitive film (Gomez-Caballero *et al.,* 2005; Yu *et al.,* 2005; Liu *et al.,* 2006). The electropolymerization method can control the thickness of the polymer film and this technique is compatible with the

combinatorial and high-throughput approach (Potyrailo and Mirsky, 2008). Conducting MIP film of PANI has been synthesized to detect ATP, ADP, and AMP (Sreenivasan, 2007).

Synthetic and biological receptors can be used to manipulate the sensitivity of a conducting polymer for different analytes (Adhikari and Majunder, 2004; Ahuja *et al.,* 2007). Some conducting polymers that have been modified with various receptors are listed in Table 3.3. To immobilize the receptor, it is bonded to the polymer matrix through covalent or noncovalent interaction. Physical adsorption (Lopéz *et al.,* 2006), the Langmuir-Blodgett technique (Sharma *et al.,* 2004), layer-by layer deposition technique (Portnov *et al.,* 2006), and mechanical embedding method (Kan *et al.,* 2004) are used to bind the receptor to the matrix through noncovalent bonding. Gerard *et al.* (2002) have discussed the advantages and limitations of these techniques.

**Table 3.3** Conducting polymer-based sensors and biosensors (after Lange *et al.,* 2008)

| Analyte | Receptor | Polymer | Transduction method | Reference |
|---|---|---|---|---|
| Uric acid | Uricase | PANI | Optical, Amperometric | Arora *et al.,* 2007; Kan *et al.,* 2004 |
| $H_2O_2$ | Horseradish peroxidase | PANI/ polyethylene terephthalate | Optical | Caramori and Fernandes, 2004; Borole *et al.,* 2005; Fernandes *et al.,* 2005 |
| Glucose | Glucose oxidase | 3-methylthio-phene/thio-phene-3-acetic acid copolymer | Amperometric | Kuwahara *et al.,* 2005 |
| Phenol | Tyrosinase | Polyethylene-dioxythiophene | Amperometric | Vedrine *et al.,* 2003 |
| Organipho-sphate pesticide | Acetylcholine-sterase | PANI | Amperometric | Law and Higson, 2005 |
| Cholesterol | Cholesterol esterase/ Cholesterol oxidase | PPY, PANI | Amperometric | Singh *et al.,* 2004; Singh *et al.,* 2006 |
| Glycoproteins | Boronic acid | Poly(aniline boronic acid) | Optical | Liu *et al.,* 2006 |

## 3.4 FUTURE OF SENSING

Sensors and biosensors have a number of disadvantages compared to standard chemical monitoring methods, however, they fulfill a number of requirements of current and emerging environmental pollution monitoring that chemical methods fail to address. Ongoing developments in material technology, computer technology, and microelectronics are expected to help sensor developers to overcome many of these problems. It is expected that progress in the development of tools and strategies to identify, record, store, and transmit parameter data will help in expanding the scope of the use of sensors on a broader scale (Blasco and Picó, 2009).

Additionally, the next generation of environmental sensors should operate as stand-alone outside the laboratory environment and with remote controls. New devices based on microelectronics and related (bio)-micro-electro-mechanical systems (MEMS) and (bio)-nano-electro-mechanical systems (NEMS) are expected to provide technological solutions. Miniaturized sensing devices, microfluidic delivery systems, and multiple sensors on one chip are needed. High reliability, potential for mass production, low cost of production, and low energy consumption are also expected and some progress has already been achieved in these areas (Farré *et al.,* 2007).

The recent developments in communication technology have not yet been fully exploited in the sensor area. New technologies like Bluetooth, WiFi and radio-frequency identification (RFID) can definitely be utilized to provide a network of distributed electronic devices in even very remote places. A wireless sensor network comprising spatially-distributed sensors or biosensors to monitor environmental conditions will contribute enormously towards continuous environmental monitoring especially in environments that are currently difficult to monitor such as coastal areas and open seas (Farré *et al.,* 2009a). Blasco and Picó (2009) expect that such a network can: (1) provide appropriate feedback during characterization or remediation of contaminated sites; (2) offer rapid warning in the case of sudden contamination; and (3) minimize the huge labor and analytical costs, as well as errors and delays, inherent to laboratory-based analyses. The laboratory-on-a-chip (LOC) is another concept that is going to impact future sensor technology. LOC involves microfabrication to achieve miniaturization and/or minimization of components of the analytical process (sample preparation, hardware, reaction time and detection) (Farré *et al.,* 2007). It has been suggested that nanoscale and ultra-miniaturized sensors could dominate the production lines in the next generation of biotechnology-based industries (Farré *et al.,* 2007).

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