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# Recent advances of biosensors in biomedical sciences

Ruchi Bhadoria[1*] and Hotam Singh Chaudhary[1]

*Corresponding author:

Ruchi Bhadoria

1. Dept of Biotechnology, Madhav Institute of Technology & Science, Gwalior, India

## Abstract

This review discusses recent advances in biosensor technology which draw on the disciplines of physics, chemistry, biochemistry and electronics. We first show that a biosensor consists of three components, a biological detection system, a transducer and an output system. Biological receptors are reviewed, followed by a detailed discussion of transducers, optical, electrochemical, piezoelectric, and others which involve interesting physics and show particular promise for commercial biosensors, are discussed thoroughly. New developments in biosensor design are appearing at a high rate as these devices play increasingly important roles in daily life. We describe a new technology, the Ibis T5000, for the identification of pathogens in clinical and environmental samples; a novel ion channel switch biosensor (ICSB), offers a rapid and sensitive immunodiagnostic for viral detection at point-of care; Chip-NMR, new diagnostic platforms have been developed to measure biomolecule abundance with high sensitivity; Graphene has attracted strong scientific and technological interest in recent years. Finally, future trends in biosensor development are discussed. In this context, bioelectronics, miniaturization, and especially biotechnology seem to be growing areas that will have a marked influence on the development of new biosensing strategies in the next future.

Keywords: Biosensor; transducers; ion channel; Chip-NMR; Graphene

## Introduction

There is a great need for a tool that can quickly, reliably, and accurately identify contaminating bioagents in the atmosphere. Biosensors can fundamentally serve as low-cost and highly well-organized devices for this purpose in addition to being used in other day-to-day applications. (1)

A biosensor is an integrated device that converts a molecular recognition event to a detectable physico-chemical signal. In general, the biosensor consists of a recognition element for the target (bio) chemical species and a transducer that is coupled to offer the output signal (2-4).

The history of biosensors started in 1962 with the development of enzyme electrodes by Leland C. Clark. Since then, research communities from various fields such as very large scale integration (VLSI), physics, chemistry, and material science have come together to build up more sophisticated, reliable, and mature biosensing devices (1). Biosensors are defined as analytical devices incorporating a biological material, a biologically derived material (enzyme, antibody, receptor or microorganism), integrated within a physicochemical transducer or transducing microsystem (analytical device) (5). In contrast to chemical sensors, which are derived from synthetic compounds, most biosensors are constructed by using biomacromolecules, such as nucleic acids (DNA or RNA) or proteins, as the recognition platform (6-8). In particular, proteins characterize one of the most sophisticated biomacromolecules and play crucial roles in numerous biological processes to mediate and regulate a range of chemical reactions within cells. Biosensors are known as immunosensors, optrodes, chemical canaries, resonant mirrors, glucometers, biochips, and biocomputers. Two commonly cited definitions by S. P. J. Higson and D. M. Frazer, respectively, are "a biosensor is a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter,"and "a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event)."(1)

Biosensors should be distinguished from a bioassay or a bioanalytical system, which require additional processing steps, such as reagent addition (9) and where the assay design is permanently fixed in the construction of the device.

The name biosensor signifies that the device is a combination of two parts: a bioelement and a sensor element. The basic

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concepts of a biosensor's operation can be illustrated with the help of Fig. 1. A specific bioelement, such as an enzyme, recognizes a specific analyte and the sensor element transduces the change in the biomolecule into an electrical signal. The bioelement is very specific to the analyte to which it is sensitive. It does not recognize other analytes (1).

Biosensors are being developed for different applications, including environmental and bioprocess control, quality control of food, military, medical applications and, particularly in bioterrorism detection and prevention. In fact, most of the commercially available biosensor systems are applied in the clinical and pharmaceutical markets. In addition, biosensor technology has been recently reviewed from the perspectives of agricultural monitoring (10), ground water screening (11), ocean monitoring (12) and global environmental monitoring (13).

Accordingly, most research and development has been devoted to this area. In the food industry, the detection of contaminants, verification of product content, monitoring of raw materials conversion, and product freshness (14) are areas of potential biosensor application. The beer industry has already identified ways for improving and controlling their products through the use of biosensors (15). Biosensors can be also a defense tool through the early detection of hazardous materials such as germs.

Biosensors have diversity in biomedical, industry, and military applications, as shown in Fig. 2. biosensors have tremendous potential for commercialization in other fields of application such as biosensor-based instruments in food and beverage production, environmental sampling, and noninvasive instruments or clinical analysis.(1)

However, commercial adoption has been slow because of several technological difficulties. For example, due to the presence of biomolecules along with semiconductor materials, biosensor contamination is a major issue. consists of a bioelement and a sensor element. The bioelement may be an enzyme, antibody, living cells, or tissue. The sensing element can be electric current, electric potential, and so on. Different combinations of bioelements and sensor-elements constitute. The intention of this article is to converse recent advances and trends in the use of biosensors and related bioanalytical assays for monitoring applications. The trends and areas of advancement for various biorecognition elements are summarized in Table 1

## Basic concepts

The bio and the sensor elements can be coupled as one of the four possible ways demonstrated as in: membrane entrapment, physical adsorption, matrix entrapment, and covalent bonding. In the membrane entrapment scheme, a semipermeable membrane separates the analyte and the bioelement; the sensor is attached to the bioelement. The physical adsorption scheme is dependent on a combination of vander Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor. In the case of the covalent bonding, the sensor surface is treated as a reactive group to which the biological materials can bind. The typically used bioelement enzyme is a large protein molecule that acts as a catalyst in chemical reactions but remains unchanged at the end of the reaction. (1)

## Biosensor structure

A sensor can be defined as a device which responds to a physical stimulus producing a response which can be used for measurement, interpretation or control. The sensor is comprised of three essential components: the detector, which make out the physical stimulus; the transducer, which converts the stimulus to a useful, invariably electronic, output; and the output system itself, which involves amplification, display etc in an appropriate format (see figure 2). The term 'biosensor' is now generally applied to those devices which employ a biological/biochemical detection system. So, for example, a conventional pH meter operating in a fermentation process is not classified as a biosensor, whereas the detection of a potassium ion concentration by the antibiotic, valinomycin, is biosensing. Because the biological detection system or receptor, as it is commonly known, is the definitive component of the biosensor, it is essential to devote some discussion to receptors in this review, even if it falls outside the normal physics fare. This is done in the following section. The final component of the biosensor, the output system, is the domain of information technology, and while valuable of considerable effort as far as a finished product is concerned, is beyond the scope of this review (71).

The second component of a biosensor, the transduction process is very much the domain of the physicist, but it is also important to consider the interface between the detector and the transducer, which often, in fact, constitutes the major hurdle in the development of a practical device (71).

Biosensors can be classified according to three schemes: (i) the detector type, e.g. an immunosensor, (ii) the physics of the transduction process, e.g. an amperometric sensor, or (iii) the application, e.g. a medical biosensor. Each scheme can be useful in a particular context, though (ii) and (iii) are most commonly used (71).

## Receptors (detector)

We have already acknowledged that the receptor, the biorecognition element, is the definitive component of the biosensor. It is also the most crucial, being responsible for the selective recognition of the analyte, generating the physicochemical signal monitored on the transducer and, in the end, the sensitivity of the device (72-73). Receptors can be categorized into two distinct types, catalytic, as typified by enzymes, and irreversible or affinitive, of which antibodies are the best-known example. A third group, amplified or hybrid configurations of the catalytic and affinitive types, can also be distinguished (71).

*Catalytic receptors:* Among this class are single-enzyme systems, multiple enzymes, organelles, whole cells or organisms, and

slices of animal or plant tissue, the latter typically containing numerous enzymes and various cofactors with which they function more efficiently. Recently 20 different amino acids which are so structured as to grant a remarkable ability for catalysing specific reactions. This similar structure also limits their functional stability.

*Affinity receptors:* As opposed to catalytic receptors, the affinity classes are even more specific in the nature of the binding, with binding constants of 109–1012, but do not show catalytic activity. This fact has consequences for sensing applications. Affinity receptors are more suited to 'one-shot' detection rather than monitoring applications, since the binding is mostly irreversible. It is possible to break the binding complex, usually by changing the pH to 2, but this tends to decrease the affinity and specificity of the receptor. The high binding constant also favours the selective detection of very small analyte quantities (72)

*Hybrid receptors:* Lowe *et al* (1990) have suggested that amplified receptor systems, involving a high-affinity recognition step followed by an amplification, cycling or cascade step, and therefore having characteristics of both the catalytic and irreversible receptors, will be capable of monitoring concentrations in the 10-12–10-15 range (72).

## Transduction techniques

The second component of a biosensor, the transduction process is very much the domain of the physicist, but it is also important to consider the interface between the detector and the transducer, which often, in fact, constitutes the major problem in the development of a practical device (71). It is also the most crucial, being responsible for the selective recognition of the analyte, generating the physicochemical signal monitored on the transducer and, in the end, the sensitivity of the device (72-73).
The use of piezoelectric transducers in biosensors was foreshadowed in the work of Sauerbrey (1959) who not only pioneered the use of the QCM but thoroughly analysed the physics of the device. The concern in this work was purely in determining the thickness of thin layers adhering to a surface by microweighing. One of the major advantages of optical sensors is their ability to probe surfaces and films in a non-destructive manner. Additionally, they offer advantages in speed, safety, sensitivity and robustness, as well as permitting *in situ* sensing and realtime measurements. An optical immunosensor consists of either an antibody or antigen immobilized on a suitable surface. The primary function of this type of sensor is to produce a measurable signal upon interaction with a biospecific immunological component.

## Common types of biosensors

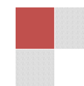
In resonant biosensors, an acoustic wave transducer is coupled with an antibody, or bioelement. When the analyte molecule, or antigen, gets attached to the membrane, the mass of the membrane changes. The resulting change in the mass subsequently changes the resonant frequency of the transducer. This frequency change is then measured. There are many types of biosensors, few biosensors are classified here in detail on the basis of receptors and transducer (74).

## On the basis of Bioreceptors some Biosensors are classified as follows:

## Biomolecule immobilization

A biosensor is an analytical device containing an immobilized biological sensitive material (enzyme, antibody, antigen, organelles, DNA, cells, tissues or organic molecules) in contact with or integrated within a transducer (piezoelectric, acoustic, optical, calorimetric or electrochemical), which finally converts a biological signal into a quantitatively measurable electrical signal. The usual aim is to produce a thin film of immobilized biologically active material on or near the transducer surface which responds only to the presence of one or a group of materials or substances requiring detection. Despite the initial loss in activity, recent advances in biosensor immobilization technologies have resulted in biosensors being stored for periods up to a year without further loss in sensitivity. Since the immobilization technique used to attach the biological material to the sensor surface is crucial to the operational behaviour of the biosensor, realistic strategies for the development of immobilization techniques are essential for practically useful biosensor.
There are a number of requirements that the immobilization technique must satisfy if biosensors are to be of practical use: (i) the biological component must retain substantial biological activity when attached to the sensor surface; (ii) the biological film must remain tightly associated with the sensor surface whilst retaining its structure and function; (iii) the immobilized biological film needs to have long-term stability and durability; and (iv) the biological material needs to have a high degree of specificity to a particular biological component. These conditions must be satisfied for an efficient sensing device.
Therefore, an important aspect of biosensor development, especially affinity based biosensors, is the ability of the immobilization technique used to afford the desired distribution and/or orientation of biological molecules once immobilized (75).
The incorporation of biological components in membrane structures was first described by Clark and Lyons (1962). Since that pioneering work, in which an enzyme-based glucose sensor was developed by the entrapment of glucose oxidase in a membrane enclosed sandwich, there have been a number of methods which have been described for the immobilization of enzymes and proteins on surfaces and within various matrices (76). The principal methods of immobilization are:
(1) physical or chemical adsorption at a solid surface;
(2) covalent binding to a surface;
(3) entrapment within a membrane, surfactant matrix, polymer or microcapsule;
(4) cross-linking between molecules.





In addition to these conventional methods, more recently the methods of sol–gel entrapment, Langmuir–Blodgett (LB) deposition and electropolymerization have all been extensively used to immobilize biological components. The immobilization method employed will depend on a number of factors, but in general the method needs to be compatible with the biomolecule being immobilized, the sensor surface or matrix on which immobilization is to proceed, and, ultimately, the end use of the sensor. Adsorption of biomolecules from solution onto solid surfaces can proceed via either physical or chemical interactions. Physical adsorption involves van der Waals forces, ionic binding or hydrophobic forces, whereas in chemisorption there is a sharing or transfer of electrons to form a chemical bond. In early work, immobilization by adsorption was used successfully to couple proteins to various solid substrates, including derivatized glass, plastics and silicone rubber (77). More recent work has focused on the binding of biomolecules onto metal surfaces for biosensing applications.

## Enzyme-based biosensors

A wide range of biomolecular recognition elements have been used for biosensors for potential environmental applications. These can be organized by structural (e.g, enzyme, antibodies or microorganisms) or functional (e.g, catalytic, complex cellular functions) characteristics. There are numerous advantages for enzyme biosensors. These include a stable source of material (primarily through biorenewable sources), the ability to modify the catalytic properties or substrate specificity by means of genetic engineering, and catalytic amplification of the biosensor response by modulation of the enzyme activity with respect to the target analyte. There are also some limitations for enzyme-based biosensors with respect to environmental applications. These include the limited number of substrates for which enzymes have been evolved, the limited interaction between environmental pollutants and specific enzymes, and in the case of inhibitor formats, the lack of specificity in differentiating among compounds of similar classes such as nerve agents as well as organophosphate (OP) and carbamate pesticides.

Recent progress with respect to enzyme biosensors for environmental applications has been reported in several areas. These areas include the following; genetic modification of enzymes to increase assay sensitivity, stability and shelf life; improved electrochemical interfaces and mediators for more efficient operation; and introduction of sampling schemes consistent with potential environmental applications (78).

## Antibody-based biosensors

Antibody-based biosensors (immunosensors) are inherently more versatile than enzyme-based biosensors in that antibodies have been generated which specifically bind to individual compounds or groups of structurally related compounds with a wide range of affinities. There are, however, several limitations in the use of antibody-based biosensors for monitoring applications. These limitations include the complexity of assay formats and the number of specialized reagents (e.g, antibodies, antigens, tracers, etc.) that must be developed and characterized for each compound and the limited number of compounds typically determined in an individual assay as compared to the multiple compounds that contaminate environmental samples (78).

Recent advances reported for antibody-based biosensors for environmental applications have primarily been focused toward these limitations. For example, the simultaneous detection of six hazardous bacteria and protein toxins was demonstrated on a planar waveguide array biosensor (79). The biohazards included ricin, cholera toxin, *F. tularensis, B. adortus, B. anthrasis* and eneterotoxin B from *S. aureus* in the presence of environmental contaminants such as sand, clay, pollen and smoke. The instrumentation was automated and the assay was compatible for development as a field assay.

## Cell-based biosensors

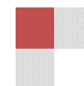
Cell-based biosensors for environmental applications can be organized according to cell type. For example, bacteria, yeast, algae and tissue culture cells. Although there are numerous examples of genetic modification to these cell types, genetically engineered bacteria (GEMs) are most often reported in cell-based biosensors (80). Bacteria have been genetically engineered to construct gene fusions typically composed of a regulatory system (i.e., native promoter) linked to a reporter(s) genes. For these genetically modified microorganisms often referred to as 'biosensors' or 'bioreporters', the presence of an effector (nonspecific stressor or biochemically active compound or toxin) results in a cascade of events that produces some measurable response. Effectors for which bioreporters have been constructed include: non-specific stressors such as DNA damage, gamma radiation, heat shock, and oxidative stress; toxic metals such as cadmium, chromate, cobalt, copper, iron, lead, mercury, nickel and zinc; organic environmental pollutants such as chlorinated aromatics, benzene derivatives, organic peroxides, trichloroethylene and PCBs; and compounds of biological importance such as nitrate, ammonia and antibiotics (81).

Genetically engineered microbial and cell-based biosensors show several advantages and limitations with respect to potential environmental applications. Limitations primarily involve the maintenance of their environment (i.e, nutrients, O2, pH, ionic strength, etc.) and the time required for a response. Recent advances in reporter microorganisms have involved novel fusions of a wide range of promoters with conveniently measured reporters, as well as the construction of unique sensing platforms that can be used to study individual organisms as well as population responses (78).

## DNA biosensors

Due to their wide range of physical, chemical and biological activities, nucleic acids have been incorporated into a wide range of biosensors and bioanalytical assays, many of which show the potential for adaptation (82). In addition to these genetically unrelated uses of DNA for biosensors, a number of biosensors





and bioassays have been reported for the detection of chemically-induced DNA damage. There is also an ongoing attempt in the area of biosensor technology for measuring DNA hybridization prerequisite for genetic identification of pathogenic microorganisms. The measurement of DNA damage using electrochemical biosensors has been demonstrated using the direct measurement of oxidation–reduction properties of the bases (83, 84) or indirectly using electrochemical probes (85). These biosensors have been used to measure toxic aromatic amines (84), oxidative damage (85, 86), and bioactivated benzo(a)pyrene (87). DNA damage can also been measured using fluorescence-based biosensors and bioanalytical techniques. In recent report, DNA adducts of benzo(a)pvrene were measured using low temperature fluorescence on a gold biosensor chip (88). This rapid screening assay was sensitive to a variety of forms of DNA damage, including strand breaks, crosslinks and adduct formation. DNA hybridization microarrays have been recommended as a platform for the parallel detection of multiple pathogenic microorganisms relevant to both biodefense and environmental contamination applications (89). The technology required for this type of application would require that biosensors be rapid, sensitive, and compatible with profitable development. Examples of this approach have been demonstrated in food industry. DNA microarray techniques have been reported for the simultaneous detection of virulence factors in their food borne pathogens (90). Advances in the progress of hybridization biosensors have also included a visual DNA chip for detection of hepatitis virus (91), lead labeled oligonucleotides detected hybridization is used by anodic stripping voltammetry (92), hybridization using gold nanoparticles by optical detection (93), and DNA hybridization using silver precipitation on gold nanoparticle-labeled oligonucleotides by electrochemical detection (94,95). Although these biosensors and array techniques were not specially developed for environmental applications, this type of technology is prerequisite for development of DNA-based biosensors for environmental applications.

## On the basis of Transducers some Biosensors are Classified as follows :

### Electrochemical biosensors

These devices are mainly based on the observation of current or potential changes due to interactions occurring at the sensor-sample matrix interface. This device was applied to the fast glucose assay in blood samples from diabetics. At present, there are many proposed and already commercialized devices based on the biosensor principle including those for pathogens and toxins, some even based on a multi-channel configuration (96, 97). The most typical part of electrochemical biosensors is the presence of a suitable enzyme in the biorecognition layer providing electroactive substances for detection by the physico-chemical transducer providing the measurable signal. A rather limited number of enzymes processed in biotechnology were chosen for the monitoring of clinical metabolites and, especially from the group of oxidoreductases: glucose oxidase (98) and glucose dehydrogenase (99) for glucose assays and cholesterol oxidase co-immobilized with cholesterol esterase for the cholesterol assay (100). Peroxidase and alkaline phosphatase are the most common enzyme labels for electrochemical affinity biosensors (101). Compared to optical methods, electrochemistry allows the analyst to work with turbid samples, and the capital cost of equipment is much lower. On the other hand, electrochemical methods present slightly more limited selectivity and sensitivity than their optical counterparts. Techniques are generally classified according to the observed parameter: current (amperometric), potential (potentiometric) or impedance (impedimetric).

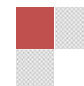
### Amperometric methods

This is perhaps the most common electrochemical detection method used in biosensors. It works on the grounds of an existing linear relationship between analyte concentration and current. The sensor potential is set at a value where the analyte, directly or indirectly, produces a current at the electrode. In the case of biosensors, where direct electron exchange between the electrode and either the analyte or the biomolecule is not permitted, redox mediators are required (102). Redox mediators are small size compounds able to reversibly exchange electrons between both the sensor and the enzyme of choice (e.g., ferricyanide, osmium or ruthenium complexes, dyes, etc.). Many different combinations and strategies to build biosensors are possible. The actual choice depends on constraints imposed by sample matrix, analyte, or usability. Bacterial biosensors do not differ much from more conventional biosensors (103). In this work, *E. coli* is detected in 30 min and between 100 and 600 cellsmL-1 using a flow-through immunofiltration method coupled to amperometry. (104)

### Potentiometric methods

These are the least common of all biosensors, but different strategies may be found nonetheless (105). For example, they may consist of an ion selective membrane and some bioactive material, e.g., an enzyme. The enzyme catalysed reaction consumes or generates a substance which is detected by the ion-selective electrode. Since potentiometry yields a logarithmic concentration response, the technique enables the detection of extremely small concentration changes. Another approach involves the use of suitably modified ion selective field effect transistors (ISFETs) (106) which utilise the semiconductor field-effect to detect biological recognition events. ISFETs use an electric field to create regions of excess charge in a semiconductor substrate in order to enhance or decrease local conductivity. They consist of a p-type silicon substrate with two n-doped regions known as source and drain, separated by a short distance (gate) covered by a layer of insulator. The gate insulator is typically $SiO_2$ and it is covered by an ion selective membrane





which is selectively permeable to a certain ion, e.g., K+, Ca2+, F-, as described in (107). The application of these devices in the area of biosensors is reasonably new (105) and their use is not spreading as quickly as other electrochemical techniques due to, amongst others (i) problems related to production which include incompatibility of most biomolecule immobilization methods with the ISFET fabrication technology and difficult packaging and encapsulation at wafer level, (ii) poor detection limits, linear range and reproducibility and (iii) inadequate device stability (104).

## Electrochemical impedance spectroscopy (EIS)

Impedance spectroscopy represents a powerful method for the study of conducting materials and interfaces. In this technique, a cyclic function of small amplitude and variable frequency is applied to a transducer, and the resulting current is used to calculate the impedance (108) at each of the frequencies probed. The most rigorous approach involves solving the system of partial differential equations governing the system. The second way, which is often preferred because of its relative simplicity, consists in the interpretation of the data in terms of equivalent circuits. The latter are made up of a combination of capacitors and resistors suitably arranged. Although this methodology is widely accepted because of ease of use, extreme care must be taken to ensure that the equivalent circuit obtained makes physical sense. An advantage of EIS compared to amperometry or potentiometry is that labels are no longer necessary, thus simplifying sensor preparation. Along these lines, Alocilja et al. reported a conductimetric method using polyclonal antibodies against *E. coli* (109). Although impedimetric techniques are very promising, a lot of work is still needed in order to bring the technique up to a competitive level. Even the fundamental understanding of the phenomena involved in this type of immunosensors is largely to be developed. For instance, studies of the effect of electrode size and their separation distance has not been found in the recent literature, but it is not entirely unreasonable to believe that using the appropriate electrode configuration and sample pre-treatment steps, detection limits below 103 CFUmL-1 could be achieved (104).

## Optical Biosensor

These are probably the most popular in bioanalysis, due to their selectivity and sensitivity. Optical biosensors have been developed for rapid detection of contaminants, toxins or drugs and even pathogen bacteria. Recently, fluorescence and surface plasmon resonance, SPR, based methods have gained momentum because of their sensitivity (104).

## Fluorescence detection

Fluorescence occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy. When the electron returns to its original ground state it emits a photon at lower energy. Another important feature of fluorescence is the little thermal loss and rapid (<10 ns) light emission taking place after absorption. The emitted light is at a longer wavelength than the absorbed light since some of the energy is lost due to vibrations, this energy gap is termed Stoke's shift, and it should be large enough to avoid cross talk between excitation and emission signals. Antibodies may be conjugated to fluorescent compounds, the most common of which is fluorescein isothiocyanate (FITC) (110). There are, however, other fluorescent markers. The use of lanthanides as sources of fluorescence in luminescent assays has very recently been reviewed (111). Although lanthanides pose several important advantages (good stability, low background luminescence under normal light conditions and large Stoke's shift) compared to more traditional fluorophores, their use is very restricted due to safety reasons. Fluorescence detection, in contrast to SPR, is also used in combination with established techniques such as PCR and ELISA. Such is the case of a hand-held real-time thermal cycler recently developed (112). This analyser measures fluorescence at 490 and 525 nm, which enables the simultaneous detection of more than one microorganism. Although this work claims detection times of 30 min, it should be pointed that overnight culturing is required to achieve best results. Fluorescence resonance energy transfer (FRET) biosensors are based on the transfer of energy from a donor fluorophore to an acceptor fluorophore (104).

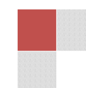
## Surface plasmon resonance

SPR biosensors (113) measure changes in refractive index caused by structural alterations in the vicinity of a thin film metal surface. Current instruments operate as follows. A glass plate covered by a gold thin film is irradiated from the backside by p-polarised light (from a laser) via a hemispherical prism, and the reflectivity is measured as a function of the angle of incidence, . The resulting plot is a curve showing a narrow dip. This peak is known as the SPR minimum. The angle position of this minimum is determined by the properties of the gold-solution interface. Hence, adsorption phenomena and even antigen–antibody reaction kinetics can be monitored using this sensitive technique (as a matter of fact, SPR is used to determine antigen–antibody affinity constants). The main drawbacks of this powerful technique lay in its complexity (specialized staff is required), high cost of equipment and large size of most currently available instruments (although portable SPR kits are also available commercially, as is the case of Texas Instruments' Spreeta system). SPR has successfully been applied to the detection of pathogen bacteria by means of immunoreactions (114, 115).

## Piezoelectric biosensors (mass based)

According to the modern definition, a biosensor is an analytical device comprising a biological or biologically derived sensing element either integrated within or intimately associated with a





physicochemical transducer (116). In affinity sensors a stoichiometric binding event takes place and the associated physicochemical changes are detected by an appropriate transducer (117). The transducers which are mainly used in the development of affinity biosensors are piezoelectric- and optical-based techniques. Together with piezoelectric transduction, some optical approaches have the advantage of not requiring the use of labels such as radioactive or fluorescent tags, and of recording in real-time the affinity reaction allowing also kinetic studies (118). The term "piezoelectric" derived from the Greek word piezen meaning "to press." The first investigation on the piezoelectricity was performed in 1880 by Jacques and Pierre Curie (119), who observed that a mechanical stress applied to the surfaces of various crystals, caused a corresponding electrical potential across the crystal, whose magnitude was proportional to the applied stress. Application of an alternating electric field across the crystal substrate results in an alternating strain field. This causes a vibrational, or oscillatory, motion in the crystal, resulting in the generation of acoustic standing waves. Recently, however, the interest in the application of piezoelectric devices in the field of analysis has increased, since it was realized that many opportunities for molecular sensing can be opened up once a suitable recognition layer or molecule is coated on the crystal. In particular, piezoelectric biosensors have found a wide range of applications in food, environmental and clinical analysis (120).

## DNA piezoelectric biosensors

The biorecognition elements that are normally employed in the realization of an affinity biosensor are antibodies, receptors or nucleic acids. In recent years, nucleic acids have received increasing interest as bioreceptors for biosensors and biochips for specific sequence detection (120). The biorecognition mechanism, generally, involves hybridization of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The complementory of adenine:thymine (A:T) and cytosine:guanine (C:G) pairing in DNA forms the basis for the specificity of biorecognition in DNA biosensors. These biosensors are often based on the immobilization of a fragment of DNA with a specific sequence (probe) and on the monitoring and recording the variation of the transducer signal when the complementary fragment (target) in solution hybridizes with the probe.The first report on the direct detection of nucleic acid interactions based on the use of acoustic wave devices was provided by Fawcett et al. (121). A large amount of work in this field has been produced by Thompson and colleagues (122) who investigated RNA–protein, DNA–DNA (123), and RNA–RNA (124) interactions.

## Immobilization of nucleic acids

Probe immobilization is a fundamental step in DNA based piezoelectric biosensor development. Often, the detection limits and, in general, the analytical performances of the biosensor can be improved by optimizing the immobilization procedure on the quartz surface (125,126). Actually, the limitation of QCM devices is nonspecific adsorption of molecules present in real matrices, since QCM is a mass sensor and any molecule able to bind or to be adsorbed on the surface is a potential interference. Moreover, receptors, in this case DNA, must be attached to the solid support retaining native conformation and binding activity. Moreover, many published papers showed that immobilization techniques based on direct adsorption or on protein coating, resulted in appropriate sensor signals, but only crosslinker procedures using thiols or the interaction between avidin and biotinylated molecules, provided a long sensor lifetime and an increased stability against degradation during the regeneration process (127). Moreover, the hybridization reaction between the immobilized probe and the target sequence in solution is also described and specific applications are presented. The examined immobilization methods allow also the reuse of the sensor, by dissociation of the double stranded DNA, maintaining the probe bound to the sensor surface, ready for a new measurement cycle.

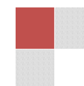
## Other Biosensors

## ESI–MS Based biosensor

Recently, Ibis T5000 biosenor used for the identification of pathogens in clinical and environmental samples. The Ibis T5000 couples nucleic acid amplification to high-performance electrospray ionization mass spectrometry and base-composition analysis. The system enables the identification and quantification of a broad set of pathogens, including all known bacteria, all major groups of pathogenic fungi and the major families of viruses that cause disease in humans and animals, along with the detection of virulence factors and antibiotic resistance markers. The Ibis T5000 is a universal biosensor. The mass spectrometer component of the Ibis T5000 enables more information to be extracted from PCR reactions than can be obtained with standard individual probes. This enriched information extraction occurs in two dimensions simultaneously. First, a large number of PCR amplicons can be analysed. This enables the use of PCR primers that amplify groups of organisms in mixed populations, rather than single species.

For example, primers for viruses can be designed to encompass entire viral families that comprise hundreds of characterized species, and primers for bacteria can be designed that encompass the entire bacterial domain of life. Second, a large amount of information is obtained from each individual amplicon by mass spectrometry. The mass spectrometer weighs each amplicon with sufficient accuracy that the composition of nucleotides (As, Gs, Cs and Ts) can be unambiguously determined. Although not as information rich as the sequence (the linking order is not determined using ESI–MS), for many diagnostic purposes, the nucleotide composition of a nucleic acid can have the same practical value. For example, when a small set of primers is strategically chosen, approximately six PCR reactions can yield sufficient information to identify the bacteria that are present to the species level2. For viruses, primers can be





designed to encompass broad genera, such as Alphaviruses3 or Mastadenoviruses4, or even whole virus
families, such as the Orthomyxoviridae5 or Coronaviridae6. When primers are designed to amplify all known members within a target group, previously uncharacterized members are also detected. This is a crucial advantage of the Ibis T5000 technology relative to probe based molecular methods, for which anticipation of the target nucleic acid sequence is required to design the probe (128).

## Ion Channel Switch Biosensor

Cornell (2002) and Cornell et al. (1997) recently developed a novel ion channel switch biosensor (ICSB) which offers a rapid and sensitive immunodiagnostic for viral detection at point-of care (129,130). The ICSB uses a simple electrical reader for objective measurement, unlike commercially available optically read kits that require subjective interpretation of the test results. The ICSB offers the additional advantage that it does not require the time-consuming steps of specimen extraction, washing and incubation which are essential in currently used approaches such as enzyme linked immunosorbent assays (ELISA) (131) or the polymerase chain reaction (PCR) test (132, 133). The ICSB employs a bilayer lipid membrane (BLM) and the ion channel molecule gramicidin A, tethered via disulphide groups to a gold surface. The membrane is separated from the gold surface by ethylene glycol spacer molecules which provides a reservoir for ions to pass through the gramicidin ion channel. Target specific antibody Fab fragments are attached to the mobile gramicidin molecules in the outer monolayer leaflet and to other non-ion channel sites tethered to the electrode surface. The arrival of the target analyte crosslinks the antibodies attached to the mobile outer layer channels, to those attached to other fixed sites on the membrane surface. Due to the low density of tethered channels within the inner membrane leaflet, the cross-linking of the antibodies by the target molecules causes the outer layer channels to be anchored away from their tethered inner layer partners. Gramicidin dimer alignment is thus prevented, disrupting the current across the membrane (134).

## Chip–NMR biosensor

A number of new diagnostic platforms have been developed to measure biomolecule abundance with high sensitivity (135), enable early disease detection and gain valuable insights into biology at the systems level. Some examples include nuclear magnetic resonance (NMR) with hyperpolarized gas, nanowire and nanoparticle sensors, surface plasmon resonance devices and mass spectrometry. Many of these devices and techniques, however, requiring time-consuming purification of samples typically followed by a set of amplification strategies, may lack the ability for the multiplexed measurements that are desirable in identifying complex diseases (135,136) or may not be amenable for easy point-of care translation. Here we report a chip-based diagnostic magnetic resonance (DMR) system for rapid, quantitative and multichanneled detection of biological targets. Using readily available magnetic nano- and microparticles as a proximity sensor to amplify molecular interactions, the DMR system can perform highly sensitive and selective measurements on small volumes of unprocessed biological samples. When a few magnetic nanoparticles bind their intended molecular target through affinity ligands, they form soluble nanoscale clusters, which leads to a corresponding decrease in the bulk spin-spin relaxation time (T2) of surrounding water molecules. These advantages render the proximity assay ideal for fast, simple and high-throughput sensing operations, especially in miniaturized device format. To date, however, measurements have relied primarily on clinical or bench top NMR systems requiring large sample volumes and complex data acquisition. Miniaturizing an entire NMR system, including the source of external magnetic fields, was technically challenging, mainly owing to the low NMR signal level intensity coming from the small sample volume and low magnetic field strength. We have overcome these limits by optimizing the design of the NMR system and by introducing microfluidics onto the NMR chip (137).

## Graphene Based Electrochemical Biosensor

Graphene has attracted strong scientific and technological interest in recent years (138-143). It has shown great promise in many applications, such as electronics, energy storage and conversion (supercapacitors, batteries, fuel cells, solar cells), and bioscience/biotechnologies because of its unique physicochemical properties: high surface area (theoretically 2630 m2/g for single-layer graphene) (138, 142), excellent thermal conductivity and electric conductivity, and strong mechanical strength. Many methods have been developed to produce grapheme. In 2004, Geim and coworkers (144) first reported graphene sheets prepared by mechanical exfoliation (repeated peeling) of highly oriented pyrolytic graphite. This method, which is called scotch-tape method (141,142), is still widely used in many laboratories to obtain pristine perfectstructured graphene layer(s) for basic scientific research and for making proof-of-concept devices. Most of graphene used in electrochemistry are produced with the last method of GO reduction. Graphene from GO reduction, which is also called functionalized graphene sheets or chemically reduced graphene oxide, usually has abundant structural defects and functional groups which are advantageous for electrochemical applications (145).

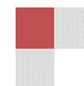
### *Graphene-Based Electrochemical Sensors*

The excellent electrochemical behaviors of graphene indicate graphene is a promising electrode material in electroanalysis (146,147). Several electrochemical sensors based on grapheme and graphene composites for bioanalysis and environmental analysis have been developed (145).

### *Graphene-Based Enzyme Biosensors*

On the basis of the high electrocatalytic activity of graphene toward $H_2O_2$ and the excellent performance for direct electrochemistry of GOD, graphene could be an excellent electrode material for oxidase biosensors. Zhou et al. reported a





glucose biosensor based on chemically reduced graphene oxide (CR-GO). Graphene (CR-GO)-based biosensor exhibits substantially enhanced amperometric signals for sensing glucose: wide linear range (0.01 – 10 mM), high sensitivity and low detection limit of 2.00 mM. The linear range for glucose detection is wider than that on other carbon materials-based electrodes, such as carbon nanotubes (148) and carbon nanofibers. The response atthe GOD/CR-GO/GC electrode to glucose is very fast and highly stable (91% signal retention for 5 h), which makes GOD/CR-GO/GC electrode a potential fast and highly stable biosensor to continuously measure the plasma glucose level for the diagnosis of diabetes. Graphene/metal nanoparticles (NP) based biosensors have also been developed. Shan et al. reports a graphene/AuNPs/chitosan composites film based biosensor which exhibited good electrocatalytical activity toward $H_2O_2$ and $O_2$. Wu et al. (149) reports GOD/graphene/ PtNPs/chitosan based glucose biosensor with a detection limit of 0.6 mM glucose. These enhanced performance were attributed to the large surface area and good electrical conductivity of graphene, and the synergistic effect of graphene and metal nanoparticles (145).

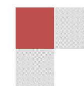
### *Graphene-Based Electrochemical DNA Biosensors*

Electrochemical DNA sensors offer high sensitivity, high selectivity and low cost for the detection of selected DNA sequences or mutated genes associated with human disease, and promise to provide a simple, accurate and inexpensivie platform of patient diagnosis (150). Electrochemical DNA sensors also allow device miniaturization for samples with a very small volume. Among all kinds of electrochemical DNA sensors, the one based on the direct oxidation of DNA is the simplest (145).

## Future Prospects and Challenges

Biosensors for potential environmental applications continue to show advances in areas such as genetic modification of enzymes and microorganisms, improvement of recognition element immobilization and sensor interfaces, and introduction of improved operational formats and unique environmental applications. The use of genetically modified AChE in biosensors has significantly increased their sensitivity to inhibition by OP pesticides (151-153). Furthermore, genetic modification shows the potential for selection of enzyme variants that are specific for a range of individual compounds. Novel gene fusions have resulted in more sensitive and versatile reporters such as GFP and show the potential for construction of a battery of organisms that respond to a wide range of physical and chemical stressors using a single detection platform. One area where continued progress could yield significant advances for environmental applications would be to better characterize bioreporter organisms as surrogates for human exposure. Better methods for immobilization of enzymes and antibodies to sensor surfaces continue to increase the robustness and improve prospects for commercialization of biosensors for environmental applications. Future advances in immobilization will likely focus on directing biorecognition elements to addressable locations on micro or nano-sensor arrays (154). One of the challenges that must be met for this type of system would be the development of parallel computational methods to convert electronic responses for each analyte into important concentration data. In this respect progress has been reported for automated sensor systems that operate in environment (155). A biosensor approach toward measuring genetic damage has involved the detection of chemically-induced damage to DNA (156). One of the challenges for this area will be the development of environmental applications associated to ecosystem and human exposure to genotoxins. This would require the isolation and analysis of DNA, genotoxic substance after exposing with the organism. This type of application would also require both, extent of DNA damage with high sensitivity and the amount of DNA required for the analysis. The development of biosensors receptive to biochemical responses are also a trend where important advances are likely to be made in the future. For example, a range of receptor-based biosensors that are receptive to estrogenic and endocrine disrupting compounds have been reported (157– 159). The challenge in this case will be to calibrate the biosensor response to the risk of adverse biological effect rather than simply receptor binding affinity. Biosensor techniques for potential environmental applications have continued to show sustained advances in a wide range of areas. It is also likely that these advances will play an important role in the development of biosensor systems for the environmental market. Nevertheless, until biosensors achieve operational characteristics similar to the simple pH electrode in terms of durability, selectivity, extended concentration range, and resistance to biofouling, they will likely continue to experience significant obstacles to widespread acceptance and use for environmental monitoring. Nevertheless, there are a number of areas where the unique capabilities of biosensors might be exploited to meet the requirements of environmental monitoring. Advances in areas such as toxicity, bioavailability-, and multi-pollutant-screening, could widen the potential market and allow these techniques to be more competitive. Miniaturization, reversibility and continuous operation may allow biosensor techniques to be incorporated as detectors in chromatographic systems. Because in many cases the transduction technology is well established, most of the research is focused on improving immobilization techniques of the biological element to increase sensitivity, selectivity, and stability. While critical, the latter has received relatively little attention probably in part because there is a tendency to design disposable devices that are most useful in quality assurance laboratories but do not allow on-line implementation for process control. Another dynamic area of research is miniaturization of sensors and flow systems. Development of these technologies is mainly driven by the need for in vivo applications for medical diagnosis and may not find immediate use in the agricultural and food industries. After almost 40 yr of research in biosensors, a wide gap between research and application is evident. The lack of validation, standardization, and certification of biosensors has resulted in a very slow transfer of technology. With faster computers and automated systems this process should accelerate in the future.





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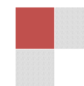

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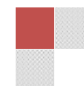


**Fig 1.**

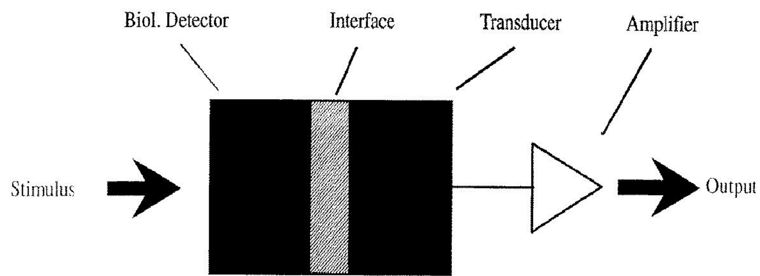



**Figure 2.** Schematic of a biosensor.

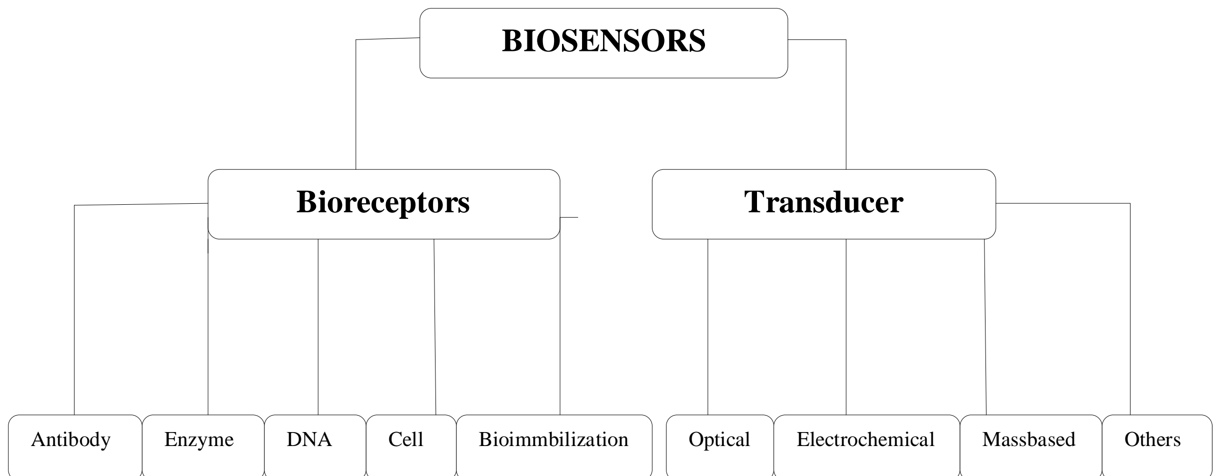
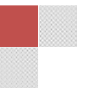
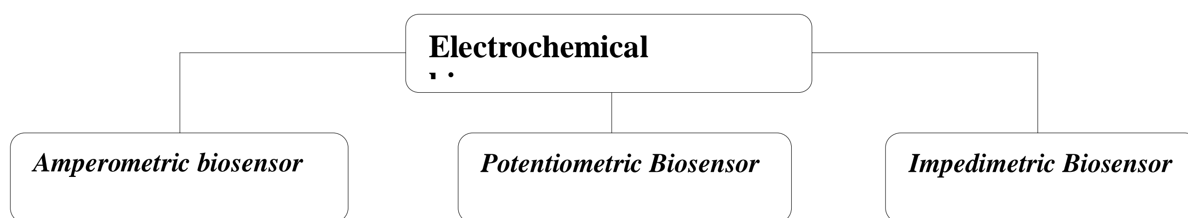



**Fig 3.** Schematic of Biosensor Classification Schemes








Fig

**Table 1:**
**Areas of advancement for biorecognition elements**

| Biorecognition element | Area of advancement | Reference |
|---|---|---|
| **Enzyme** | Genetic modification for improvement of sensitivity and stability. | [16-18] |
| | Improvement of sensor interface. | [19–24] |
| | Improvement of operational format and unique environmental applications. | [25–30] |
| **Antibody** | Multi-analyte detection. | [31-34] |
| | Automation and demonstrated environmental applications. | [33,34] |
| | Reversible binding and simplified or improved format. | [35–39] |
| **Cellbased** | Novel gene fusions responsive to specific compound classes and assay format improvements. | [40–42,43,44] |
| | Novel gene fusions responsive to toxic or genotoxic stressors. | [45–46] |
| | Native organisms responsive to specific compounds, toxic stressors and biological oxygen demand. | [47,48–53] |
| **DNA** | DNA damage. | [54–58] |
| | DNA hybridization and detection of pathogens. | [59–62] |
| **Receptors** | Estrogenic and endocrine disrupting compounds | [63–70] |