

Immunochemical techniques for environmental analysis

I. Immunosensors

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Immunoassays are analytical techniques based on the avidity and specificity of the antigen-antibody reaction. However, new immunochemical techniques have emerged recently as a consequence of the incorporation of scientific advances and knowledge from other areas such as molecular biology, microelectronics and chemistry. The principles of these techniques and their application to environmental analysis are presented.

1. Introduction

Immunochemical techniques are based on the interaction of antibodies (Ab) with antigens (Ag). Antibodies are polymers containing hundreds of individual amino acids arranged in a highly ordered sequence. These polypeptides are produced by immune system cells (B lymphocytes) when exposed to antigenic substances or molecules. Abs contain in their structure recognition/binding sites for specific molecular structures of the Ag. According to the 'key-lock' model an Ab interacts on a highly specific way with its unique Ag. The interaction is reversible, as determined by the law of mass action, and is based on electrostatic forces, hydrogen bonding, and hydrophobic and Van der Waals interactions. This feature constitutes the key to the immunochemical techniques.

Immunoassay technology originated in the late 1950s when Yalow and Berson (Nobel Prize awarded) published on the development of a quantitative immunological assay which could detect human insulin at the picogram level in small samples of body fluid. In the following years this technology found wide application in biochemistry,

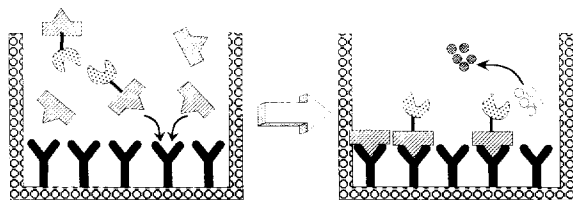
endocrinology and clinical chemistry. In 1980 Hammock and Mumma [1] pointed out the potential of this powerful technology for pesticide analysis and prompted its acceptance by environmental chemists. Since then, the interest of many academic, governmental and industrial laboratories has grown enormously. The impact of immunochemical techniques on the environmental field is evident [2] in the extensive variety of immunochemical protocols which are available for the detection of trace contaminants, including pesticides, industrial residues, and their degradation products (detailed information can be found in current reviews [5-9]). Reasons for these developments include the selectivity and sensitivity exhibited by the antibodies and the simplicity of performing the immunoassays. Recently other immunochemical techniques have emerged which are gaining acceptance by environmental chemists. Our aim is to present an overview of these new techniques, whose principles will be briefly illustrated.

2. Immunoassays

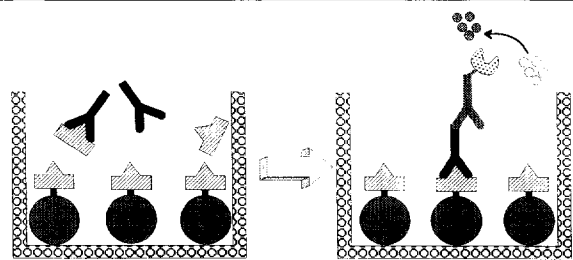
Immunoassays (IAs) are based on the use of labels to detect the immunological reaction. Although fluorescent and chemiluminescent labels have been gaining popularity in the last few years, enzyme labels such as horseradish peroxidase or alkaline phosphatase are still the most popular non-isotopic labels, alongside the use of radioisotopes in radioimmunoassay (RIA) formats. Among the enzyme immunoassays (EIA), those based on heterogeneous conditions are most commonly employed and are referred to as enzyme-linked immunosorbent assays (ELISAs). For ELISAs, either Abs or Ags are immobilized on a solid phase to facilitate the separation of free and bound fractions. The most usual configurations are shown in Fig. 1 and are briefly described below. However, readers are referred to other publications for more extensive descriptions of the many types of immunoassay formats (e.g. [10])

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Direct Competitive ELISA



Indirect Competitive ELISA



Sandwich ELISA

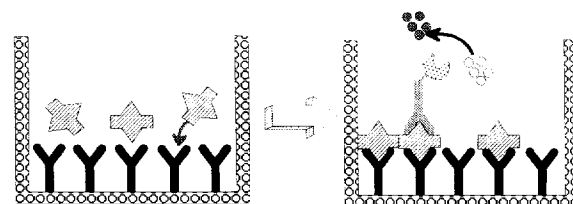


Fig. 1. Scheme of the heterogeneous IA configurations most commonly employed (see text for description).

2.1. Direct competitive ELISA

Ab coating format. An equilibrium is established between the Ab bound to the solid surface (either directly or through orientating reagents such as anti-IgG or protein A), the analyte, and the analyte–enzyme tracer which are in solution. After the main incubation step the unbound reagents are washed away and the amount of enzyme bound to the solid phase by the Ab is measured. A reduction in enzyme activity is directly proportional to the amount of analyte present.

Ag coating format. This is based on the competition between the immobilized Ag (or surface-derivatized analyte) and the analyte for a fixed small amount of labeled Ab.

2.2. Indirect competitive ELISA

This works on the same principle as the Ag coating format, but in this case the concentration of the

analyte is measured indirectly by the quantitation of bound Ab with a second Ab which is covalently labeled with an enzyme or other marker.

2.3. Sandwich ELISA

In this case, an excess of labeled Ab is used to detect the analyte captured by another Ab bound to the solid surface. This configuration is restricted by the fact that the analyte must have multiple Ab binding sites.

3. Flow injection immunoanalysis

Flow injection immunoanalysis (FIIA) methods provide attractive alternatives for the automation of IAs and also of immunosensors (discussed below). The sample is incorporated in a carrier stream which enters a reactor chamber where the immunological reaction takes place. FIIA methods have been developed based on a variety of detection principles which have been reviewed [11]. As an example from the environmental field, FIIA has been used for the detection of triazines, reaching very low detection limits [12–14]. An interesting possibility is the real-time monitoring of effluents with FIIA systems, which would fill a major need associated with many analytical processes.

4. Immunoaffinity chromatography

Immunoaffinity chromatography (IAC) uses the reaction Ab–Ag to extract an analyte selectively from complex environmental matrices. The Ab is attached covalently to a rigid or semi-rigid support which is then packed dry, or as a slurry, into small columns. A variety of support materials conveniently activated, is now commercially available. However, whenever possible it is desirable to use those which orientate Ab binding sites by linking the Ab through its Fc region (i.e., protein A, anti-IgG, hydrazide derivatized surfaces, etc.). Detailed information on the principles and perspectives of IAC can be found in recent reviews [15–17], the operation protocol is shown schematically in Fig. 2. The desorption technique, the flow-rate (0.2–4 ml/min), and operating pressure (approx. $0.34 \cdot 10^6$ Pa) should be optimized on each system, and have a strong influence on the column lifetime. For lipophilic molecules a gradient of organic co-solvent (such as acetonitrile or

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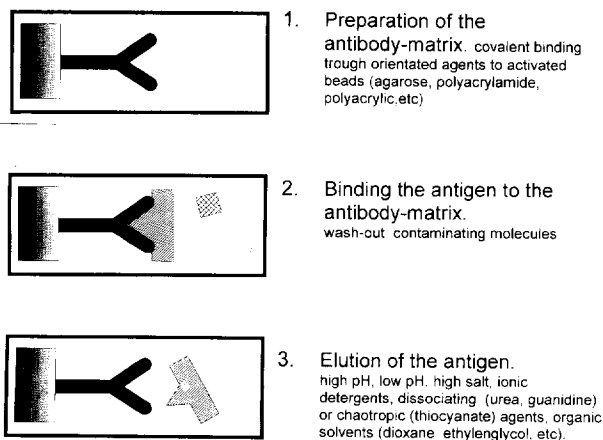


Fig. 2. Main steps involved in immunoaffinity chromatography.

methanol) can be used to dissociate the analyte and regenerate the column. Essentially, we can distinguish between the two kinds of immunoaffinity applications given below.

4.1. Immunoaffinity clean up

The application of the immunoaffinity principle for the extraction of pesticides from environmental samples was suggested ten years ago when specific antibodies were used to extract paraquat from macerated glassfiber filters [18]. Although there have been many reports on the application of this principle very few describe its application in the environmental field. Ab-coated microtiter plates have been used to extract triazine metabolites from urine samples [19]. Recently, several immunosorbents have been evaluated for the selective enrichment of phenylurea and triazine herbicides [20] prior to HPLC analysis. The use has also been described of imazethapyr class-specific antibodies on immunoaffinity columns to extract these compounds efficiently from animal tissues, body fluids, corn plants, grain, and wheat plant extracts [3].

4.2. High-performance immunoaffinity analysis

The direct application of IAC, although simple in theory, does not yet have broad use. Elution of the analytes from the column frequently does not yield sharp peaks. Narrowing of the peaks is thus usually accomplished by coupling the immunoaffinity column to a reversed-phase column. Automated column-switching systems which link IAC

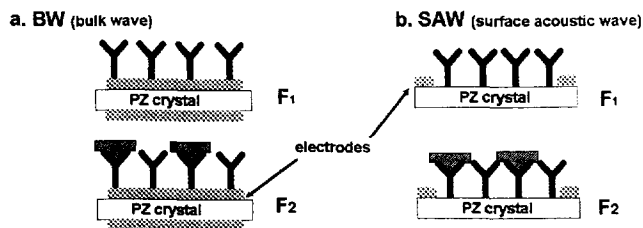
with a C_{18} HPLC column or a gas chromatograph have been reported to extract a variety of small analytes from biological fluids [21–23]. In the environmental field this approach has been used for the isolation of carbofuran [24,25] and for the concentration and subsequent separation of triazines [25].

5. Immunosensors

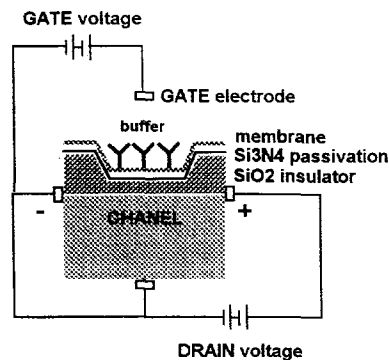
Much effort has been made in the last decade to develop biosensors (for general reviews see [26–29]). A biosensor is an analytical device which consists of an immobilized biological component in intimate contact with a transduction device that converts a signal from the biological element into a quantifiable electrical signal (Fig. 3). When biological molecules interact specifically, changes in physicochemical parameters are generated and are sensed electronically. Usually the term biosensor implies reversibility or real-time readout. For an immunosensor or immunoprobe, either Ab or Ag constitutes the bio-specific component. An immunosensor (for reviews see [30–32]) can work under homogeneous conditions, although heterogeneous formats are more developed for any of the IA configurations mentioned above. Table 1 shows some of the benefits and limitations of the immunochemistry applied to biosensor development. Remarkable features include the larger range of substrate specificities, and the possibility of varying the properties, such as the specificity or avidity of the antibodies, by judicious design of the immunogen, careful screening of the antibodies elicited, and site-directed mutagenesis.

Unlike conventional IAs an immunosensor should be capable of detecting the analyte *continuously, reversibly* and *selectively* (see Table 2). However, one of the major drawbacks is that Ab–Ag interactions are not readily reversible, because of the high values of the affinity constants. With enzyme-based biosensors there is a catalytic event. Thus, combined in the initial binding are amplification and transduction systems. Antibody-based biosensors present a more difficult problem. Since there is no catalytic event other systems are needed for complete transduction to a physical response and amplification. Recent studies of the catalytic properties of antibodies may overcome this obstacle. Catalytic antibodies not only bind but also chemically transform the target molecule. At present, the only example reported uses antibodies

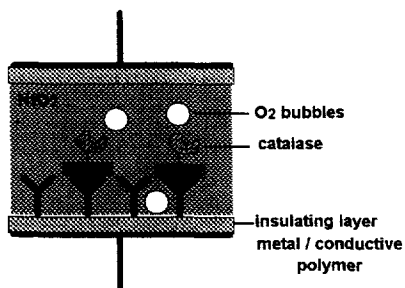
A. Piezoelectric



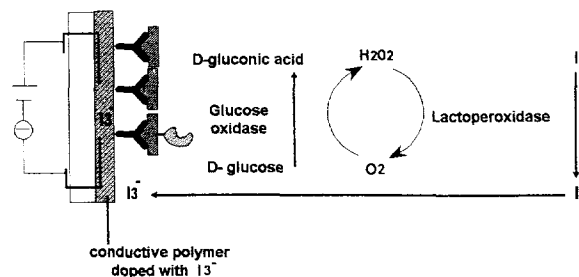
B. Potentiometric (FET)



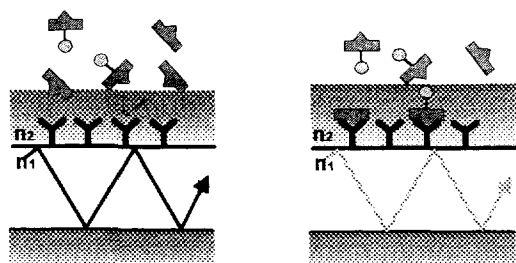
C. Capacitive



D. Conductimetric



E. Optic: EW



F. Optic: SPR

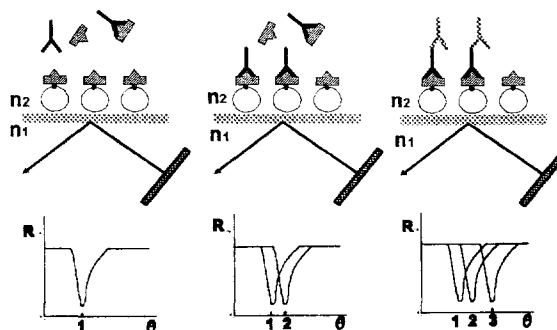


Fig. 3. Schemes of the main types of immunosensor transducers employed for environmental analysis (see text for details).

which catalyze the hydrolysis of phenyl acetate as the sensing element to detect this analyte [33]. However, many researchers are concentrating on single-use immunoprobes to side-step the problem of irreversibility. Another inconvenience is that the physicochemical changes resulting from the Ag-

Ab interaction are often insufficient to provide detection limits comparable to those of conventional IA. We will see in the next section how, as a consequence, indirect systems are being developed which rely on the use of enzyme- or fluorescence-tagged reagents.

Table 1
Antibody-based immunosensors: benefits and limitations

Benefits
<ul style="list-style-type: none"> ▶ Potentially, antibodies can be obtained to any kind of chemical structure ▶ Antibody properties such as affinity and selectivity can be modulated by: <ul style="list-style-type: none"> ● adequate hapten design, ● careful screening of the antibodies elicited and ● alteration of the amino acid sequence by site directed mutagenesis. ▶ Immunochemical techniques have been shown to provide high sensitivity ▶ Antibodies offer homogeneity regarding chemical structure and properties, and allow for standardization of procedures such as immobilization, stabilization, calibration, storage, etc. ▶ Antibody molecules are frequently more stable than enzymes: longer lifetime of the biosensor could be expected
Limitations
<ul style="list-style-type: none"> ▶ High affinity constants. Strong interaction of antigen–antibody. Regeneration of the sensing layer may be troublesome. ▶ Direct electrochemical detection occurring after molecular recognition is difficult. ▶ Labeling of the immunoreactants with enzymes, fluorescent chemicals or electrochemically active substances is often necessary to produce the desired detection signal. ▶ Production and screening of the antibodies is expensive and time consuming.

Immunosensors can be divided into four classes, depending on the transducer technology employed: piezoelectric, electrochemical, optical, and thermometric. In this section we will introduce the fundamental features of the first three types because of their major prospects in environmental research.

5.1. Piezoelectric sensors

Piezoelectrics are materials (usually quartz crystals) that may be brought into resonance by appli-

Table 2
Desirable features of an immunosensor

<ul style="list-style-type: none"> ▶ Targeted specificity ▶ Short response times. Fast measurements ▶ Direct detection of the immunoreaction, so <ul style="list-style-type: none"> ▶ no need of sequential addition of immunoreagents. ▶ sample preparation not necessary when analyzing complex samples (blood, food, waste water, etc.). ▶ Possibility of developing continuous flow devices, allowing <ul style="list-style-type: none"> ▶ possibility of taking immediate remedial actions in fields such as environmental monitoring, medical diagnosis and industrial processing/process control. ▶ reversibility of the immunological reaction ▶ Miniaturization possible, giving <ul style="list-style-type: none"> ▶ compact designs for field use, ▶ in vivo diagnosis, ▶ measurement of multiple analytes in small samples. ▶ Electronic processing allows: ▶ integration into other devices that could allow actions to be taken automatically in response to immunosensor measurements, ▶ data can be processed, stored, exported and displayed in many different ways.

cation of an external alternating electric field. The frequency of the resulting oscillation is determined by the mass of the crystal (for reviews see [34–37]). Piezo-immunosensors may, in principle, be used for the direct detection of the Ag–Ab reaction. There are two main ways of using the piezoelectric properties of some materials.

Bulk acoustic (BA) devices. Adsorption of the analytes occurs on the surface, which is typically connected to an oscillator circuit, but resonance occurs on the entire mass of the crystal (see Fig. 3A). Thus, if such a crystal is coated with a selectively-binding substance such as an Ab, and placed in an atmosphere containing the selected analyte, the mass of the crystal will increase and the resonant frequency will decrease in accordance with the Sauerbrey equation: $\Delta f = -2.3 \cdot 10^6 f^2 (\Delta m/A)$ (where f is the oscillation frequency in Hz, Δm is the mass of adsorbed material in g, and A is the sensing area in cm^2)

Surface acoustic wave (SAW) devices. An acoustic wave moves just at the surface of the crystal. Mass-loading on the acoustic path between two sets of electrodes will alter the phase-wave velocity and cause a shift in the frequency (see Fig. 3A).

Possible limitations of this technology include the lack of specificity and interferences from the liquid media where the analysis takes place. However, some applications have appeared recently for the detection of pesticides such as atrazine [36], 2,4-dichlorophenoxyacetic acid (2,4-D) [38,39] or parathion [37] (see Table 3 for performance of these devices).

Table 3
Features of reported immunosensig devices for pesticides

Transducer type, (Device), Company	Analyte	Competitive IA yes/no	IA configuration	D/I ^a	Flow mode	Meas. time (min)	Detection limit	Ref.
Piezoelectric	Parathion	No	—	D	Yes (air)	2–5	n.r. ($I_{50} = 35 \mu\text{g/l}$)	[37]
	Atrazine	No	—	D	No	15	0.03 $\mu\text{g/l}$	[36]
	2,4-D	Yes	Direct/Ag coating	D	Yes	30	5 $\mu\text{g/l}$	[38]
Potentiometric (ISFET) ^b	2,4-D	Yes	Direct/Ab coating	I	No	40	1 $\mu\text{g/l}$	[43]
	Phenylacetate ^c	No	—	D	Yes	30	5 μM	[33]
Capacitive (CASIS), Biotronic	Glucose ^d	Yes	Concanavalin coating	I	—	n.r.	0.1 $\mu\text{g/l}$	[47]
Systems								
Corporation								
Conductimetric, Ohmicron Corporation	Atrazine	Yes	Direct/Ab coating	D	No	15	< 0.025 $\mu\text{g/l}$	[49]
Optic (FOB) EW	BPT	No	Homogeneous IA	D	Yes	60	1 fmol	[51]
	Parathion	Yes	Indirect	D				[56]
FOBIA, Ciba-Geigy	Imazethapyr	Yes	Direct/Ab coating	D		4–6	0.3 $\mu\text{g/l}$	[53]
	Atrazine	Yes	Direct/Ab coating	D	No	10	0.5 nM	[54]
	Atrazine	Yes	Direct/Ab coating	D	Yes	15–30	5 nM	[55]
SPR	Atrazine ^e	Yes	Indirect	D	No	15	0.05 $\mu\text{g/l}$	[39]

^aD = direct immunosensing; I = indirect immunosensing, the product of an enzyme reaction causes the physicochemical change.

^bIon-sensitive field-effect transistor.

^cBased on a catalytic antibody system.

^dIncluded as a model of the CASIS prototype, although not based on an immunological reaction.

^eUtilizes the commercial device BiaCore™ from Pharmacia Biosensors, n.r. = not reported.

5.2. Electrochemical sensors

Detection by electrochemical sensors can be inexpensive, and when coupled to chemical amplification systems, such as enzymes, it may achieve very low detection limits. A true *immuno-electrode* would detect the binding of immunoreagents directly, for example as changes in surface potential. However, to date this has not provided sufficient sensitivity. Instead, there have emerged what are known as *electrochemical IAs*, where an enzyme is usually involved. For general considerations for this class of immunosensor, see [26]. Of the numerous techniques reported we will focus on those which look most promising for environmental analysis.

Potentiometric immunosensors. Potentiometric immunosensors make use of a change in potential that occurs when either an Ab or Ag is immobilized on an electrode and its specific binding partner binds to it (for a review see [40]). These IAs are based on the premise that proteins in aqueous solution are polyelectrolytes and that because an Ab is a protein its electrical charge will be affected on

binding an Ag. The potential difference between an electrode onto which an Ab has been immobilized and a reference electrode will depend on the concentration of the free Ag. Direct detection is often accompanied by only small changes in the measured potential (1–5 mV) leading to poor sensitivity and low precision in assays. Additionally, these potentiometric electrodes are rather prone to non-specific binding. In this group of biosensors are included the field-effect transistor (FET) devices. In these, the conductivity of the channel region (measured by the application of a voltage between source and drain electrodes) is controlled by the strength of the electrical field generated by the gate, providing amplification. ImmunoFET devices have one of the immunoreactants placed on the gate electrode (see Fig. 3B). The membrane between the sensing layer and the semiconductor surface must be fully insulating and thin enough to allow redistribution of small charge changes which occur as a result of the immunoreaction and lead to variations in the electrical field. Although not much development has occurred with this type of immunosensor, the possibility of min-

iaturation on a chip and integration into a complete automated system is attractive. The application of this technology to food analysis has been reported [41,42].

Potentiometric electrode linked immunoassays (indirect detection). Potentiometric IAs use as the method of detection a conventional potentiometric electrode, e.g., an ion selective electrode, a CO₂ electrode, ammonia electrode or pH electrode. Their major disadvantage is the necessity of using enzyme labels, such as urease, and separation steps to differentiate free and bound labeled species as in the normal IA protocols. An immunoprobe based on a competitive IA has been developed for the herbicide 2,4-D [43]. In this case, the signal is amplified by the product of the enzyme label (HRP) which is detected on a pH-sensitive FET. To this group also belongs the biosensor mentioned above which is based on catalytic antibodies which hydrolyze phenyl acetate [33] (see Table 3 for characteristics of these immunosensors).

Amperometric immunosensors. Amperometric assays measure the current generated when electroactive species are either oxidized or reduced at the electrode. The current observed has a linear relationship with the concentration of the electroactive species (for the general concepts see [41],[42]). The enzymes catalyzing these reactions are typically oxidoreductases, but hydrolytic enzymes such as alkaline phosphatase can be used if they produce electroactive species. The electrode sensing element is usually constructed of platinum, but gold or various forms of carbon have also been used. Immediately adjacent to the electrode is the enzyme layer which is formed by entrapment of the enzyme within a gel, or by other immobilization procedures. Although this technology looks very promising in view of the development of enzyme-based biosensors (see [42]) its application to immunoprobes is still very limited, and no applications have been reported in the environmental area. Such probes usually rely on the IA configurations mentioned above, and labeled species and washing steps are introduced. Schram et al.[44] have developed an interesting dual-Ab system for the detection of progesterone which overcomes these inconveniences A liposome-based IA which contains encapsulated electroactive species has been proposed by Durst et al. for detection of polychlorinated biphenyls (PCBs) and alachlor in the field [45].

Capacitive sensors. The concept of capacitive affinity sensors is based on the fact that the

capacitance of electrolytic capacitors depends on the thickness and dielectric behavior of a dielectric layer placed on the surface of a metal plate. Changes in the dielectric constant which result from Ab-Ag interactions are thus measured. Any variation in the surface potential leads to a shift of the capacitance versus voltage curve. The dielectric constant is an intrinsic property of substances and is a measure of their ability to store energy in response to electric fields. Bresler et al. [47] of Biotronic System Corporation have developed a prototype called CASIS (capacitive affinity sensor instrumentation system) that is based on the biochemical generation of bubbles. Oxygen bubbles are generated by catalase reacting with H₂O₂ as a label agent of an ELISA system and result in a large change in capacitance. This system has been applied for glucose determination using concanavalin A as a capture reactant and glucosamine-catalase conjugate as competitor (see Table 3), but it could soon be applicable to other small analytes, by coating different antibodies on the surface of this prototype (see Fig. 3C).

Conductimetric sensors. With these sensors, as with capacitive devices, disturbances of a sensing layer placed between two electrodes are determined. In this case, changes in the conductivity are measured as variations in the measured current. At the moment two kinds of conductimetric devices are being developed.

- *Bilayer sensors*, are bilayer lipid membranes which mimic a natural membrane cell containing the Ab. The selective interaction with the analyte results in a sudden measurable current [48]. Although these systems could allow direct detection one of their main problems is their inherent mechanical instability.
- *Chemiresistors*, are based on the measurement of changes in electrical conductivity of certain polymers containing appropriate dopant agents. Sandberg et al.[49] have developed a device to measure atrazine (see Table 3). A suitably chosen electroconductive polymer can simultaneously serve as the solid phase to support immobilized antibodies and, through conductivity modulation by the dopant (such as iodine), function as the measuring device. The conductivity-reading cycle is initiated when lactoperoxidase and glucose in a buffer are added to the sensor. The H₂O₂, generated by the oxidation of glucose is used by the second enzyme to reduce I⁻ to I₃⁻, which is incorporated into the mem-

brane and produces a change in the conductivity (see Fig. 3D).

5.3. Optical sensors

Optical sensors are based on the measure the absorption or emission of a given wavelength of light by the immunoreactants. These sensors take advantage of the use of optical fibers.

Fiber optic biosensors (FOBs) consist of a fiber-optic strand with appropriate indicating-chemistry placed at the far, or distal, tip of the fiber. Light is introduced into the proximal end, and travels to the distal tip by total internal reflection. The indicating layer interacts with the analyte of interest and alters the light in proportion to the analyte concentration. Changes in the absorbance, luminescence, polarization, or refractive index can all be used as optical transduction mechanisms (for a review see [50]). The main advantages of these types of sensors include the feasibility of remote analysis, the light transmittance is bidirectional, there is a well established silica-derivatization chemistry, and they have low cost. The simplest approach to an optical detection system uses the measurement of the fluorescence emission from one of the immunoreactants involved in the reaction. For example Sepaniak et al. [51] describe the measurement of benzo[*a*]pyrene which itself has fluorescence. The benzopyrene is trapped by an antibody solution placed on the tip of the optical fiber and is protected from the external medium by a membrane. However, competitive- and sandwich-IA protocols are most commonly employed. In this context, an application for continuous monitoring has been reported. This uses a homogeneous optical immunosensor which is based on delivery systems constructed with polymers that can sustain a continuous release of immunoreactants for long periods of time [52]. It has also been suggested constructing a single-fiber element having several individual fibers which contain selectively indicating sensing elements to provide an array with a wide variety of multi-analyte detection.

Evanescent wave (EW) biosensors. When light is propagated through a waveguide (n_1) by multiple total internal reflections an electromagnetic wave called an evanescent wave is generated in the optically rarer external medium (n_2 , with $n_1 > n_2$). Since the distance of penetration of the evanescent wave is only ca. 200 nm, only surface-bound molecules will interact with the light (see Fig. 3E). The principal advantage of this system is that possible interferences from the bulk media are

avoided. Two methods of measurement are possible, as follows.

- *Attenuated total reflection (ATR)*, the energy absorbed by the molecules bound to the surface is monitored as an attenuation of the internally reflected beam. However, the analyte must have a characteristic absorption band and the absorbed light is usually a very small percentage of the total transmitted light, leading to poor sensitivity in assays.
- *Total internal reflection fluorescence (TIRF)*, the absorption of evanescent photons by surface-bound molecules is the first of a two-step process whereby the photons are re-emitted at a longer wavelength as fluorescence. Examples can be found on the application of this principle to the analysis of pesticides such as imazetaphyr [53], triazines [54,55], and parathion [56] (see Table 3). A fully automated prototype called FOBIA (fiber optic biospecific interaction analysis) has been developed by Ciba-Geigy [55] to detect atrazine, taking advantage of the favorable properties of evanescent-wave fiber-optic fluoroimmunosensor technology combined with the precise fluid handling offered by FIA. Water samples and soil extracts that were centrifuged and directly injected, did not interfere with the assay. Although soil extracts showed a brown-yellow color with a strong emission band in the spectral region where the label fluorescence was collected, no detectable contributions were observed to the luminescence signals in the device.

5.4. Surface plasmon resonance (SPR)

A surface plasmon is an evanescent electromagnetic field generated at the surface of a metal conductor (usually Ag or Au) excited by the impact of light of appropriate wavelength at a particular angle (θ_p). For a given wavelength of light a surface plasmon effect is observed as a sharp minimum in light reflectance when the angle of incidence is varied. This critical angle is very sensitive to the dielectric constant of the medium adjacent to the metal surface and is therefore affected by analytes binding to that surface (see Fig. 3F). This principle allows the direct detection of the biological interaction. A commercial apparatus, BIAcore™ from Pharmacia, available for coating with the desired Ab or Ags was used by Minunni and Mascini to develop an SPR-based immunosen-

sor for atrazine detection [39]. These devices look promising for environmental analysis.

Other optical principles have been reported as the detection systems of immunosensors with environmental applications—such as *grating couplers* for atrazine [57,58] and *scanning densitometry* for the detection of PCBs [45,46]

6. Summary

We have shown the potential of the immunochemical approach in emerging techniques for environmental analysis. Some of the technologies are still in the early stages of development and their reduction to practice may be slow. However, this will be justifiable because of the importance of validating them between among different laboratories. Although with catalytic biosensors the electrochemical transduction has proved good for many applications the future of immunosensors seems to lie with optically-based systems. Future biosensor research will depend on advances in many other areas of science because of the interdisciplinary character of this field. However, an important area which will be discussed in a later paper relates to the obtention of antibodies or other receptor molecules. In this context, recent progress on the obtention of recombinant antibodies will surely influence further development of this field [4]. The availability of the devices in the near future also depends on manufacturing costs which relies on the possibility of mass production of the immunosensors. The field of medical diagnosis field offers the best opportunities for major applicability [59–61], although environmental monitoring and the food industry may be important growing markets [62].

Acknowledgements

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