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Biochemical Sensors: The State of the Art

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Abstract. The basic components of a (bio)chemical sensor and the main concepts involved in the (bio)chemical sensor methodology are considered in order to depict the state of the art of the development of research in this field, paying special attention to the evolution of the published scientific literature in analytical chemistry.

Key words: biochemical sensors, biosensors, chemical sensors, flow injection analysis, review.

The main challenges in analytical chemistry today are obviously related to bioanalytical chemistry and concern the fast, specific and simultaneous detection of several compounds in clinical, food and environmental samples. For this purpose, (bio)chemical sensors are one of the most promising tools for improving detection and quantification of chemical and biological parameters. (Bio)chemical sensors are devices which incorporate a biologically active element in contact with a signal processor (see Fig. 1), which allow us to measure directly, in different media, including in vivo, a series of parameters of great analytical interest. Research in the field of (bio)chemical sensors, which at present is well developed, had to come a long way to make the tremendous jump from classical chemistry to the development of integrated devices for measuring biochemical parameters.

Historical Development

When in 1900 Sørensen [1] proposed the concept of pH, he was unable to predict that, on the basis of this parameter, the first sensors would be developed for the potentiometric determination of $[H^+]$ in biological samples, such as lysed red blood cells, tissue samples and, today, single cells, thus showing the important influence of pH on the rate of enzymatic hydrolysis and on respiration and metabolic processes.

In the early stages, the development of (bio)chemical sensors was associated with that of modern electrochemistry and for this reason the contribution of Heyrovsky [2] was extremely important and also the application of potentiometric and polarographic techniques to the measurement of oxygen in biological fluids. The development of the first oxygen microelectrode by Davies and Brink in 1942 [3] is another of the landmarks in the pioneering studies on biosensors and with the Clark

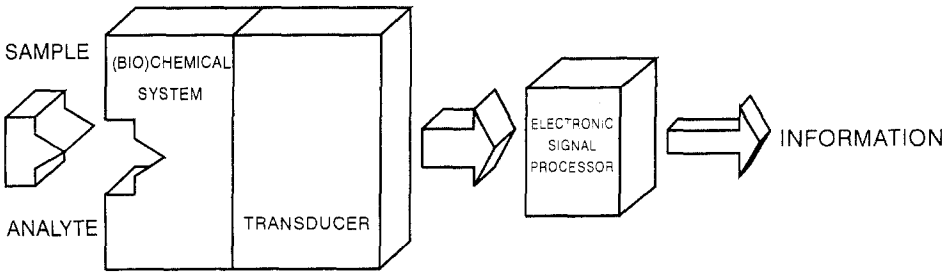

Fig. 1. Schematic diagram of a (bio)chemical sensor

membrane-covered O_2 electrode [4, 5] the application of biosensors in medical and biological fields was generalized.

On the other hand, studies developed by Clark for the indirect determination of glucose in blood, by using the oxygen electrode for the measurement of the O_2 consumption in the reaction with glucose oxidase, can be considered as the origin of the first biosensor in which the direct spatial combination of a matrix-bound biologically active receptor and an electronic device make possible the use of a single enzyme preparation for the analysis of several samples.

The entrapment of glucose oxidase in a polyacrylamide gel improved the operational stability of the enzyme and simplified the sensor operation [6]. These studies opened the door to the development of a series of enzyme electrodes and biochemical analyzers developed in the seventies and eighties. In 1975 Janata assembled a direct immunoelectrode [7] and in 1983 Lowe and Goldfinch used, for the first time, optical indication in an enzymatic sensor [8].

To the above mentioned highlights of the historical development of (bio)chemical sensors, indicated in Fig. 2, we must add the many efforts made in the fields of microelectronics and also in the development of immobilization strategies, in order

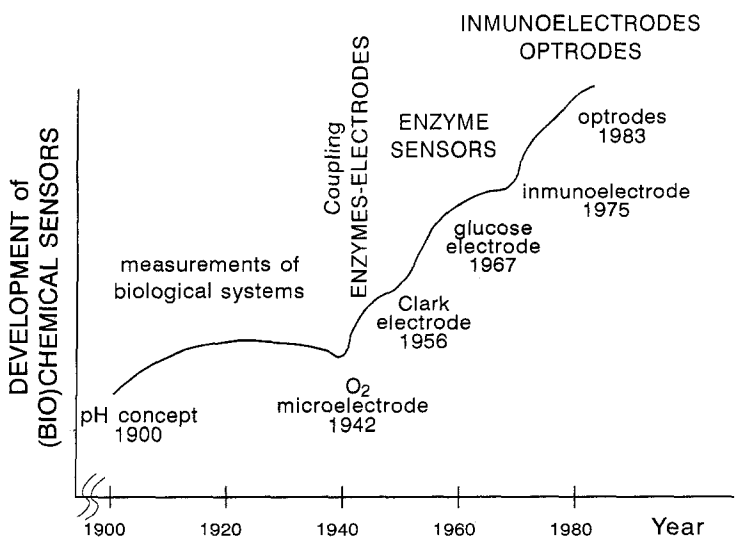

Fig. 2. Highlights in the historical development of (bio)chemical sensors

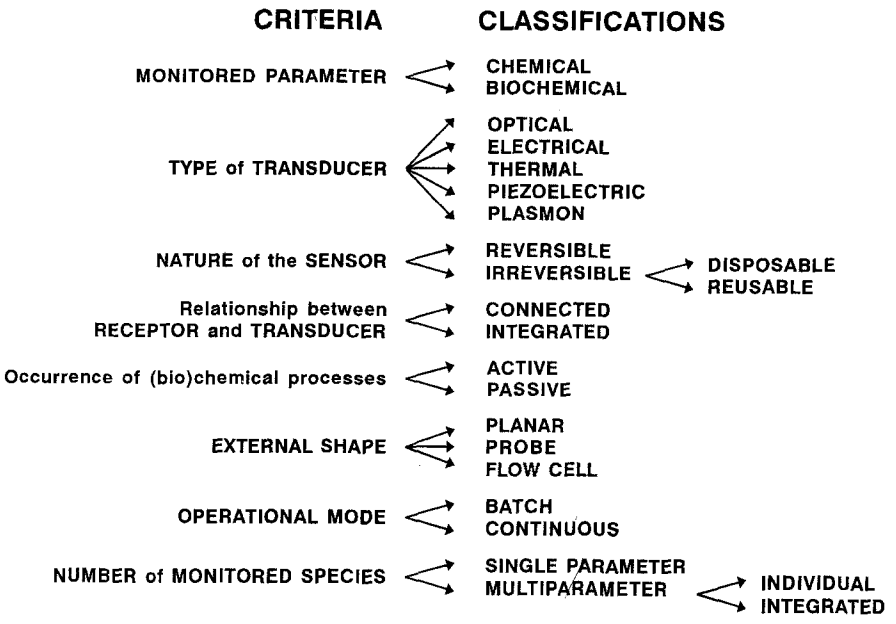

Fig. 3. Classification of (bio)chemical sensors

to provide reliable and selective sensors for monitoring chemical and biological parameters in different types of matrices.

Structure of a Biosensor

Having defined a (bio)chemical sensor as a device incorporating a receptor [9], which is, in general, a biocomponent which performs the molecular recognition of the element to be determined [10], a transducer, and an electronic signal processor (see Fig. 1), (bio)chemical sensors can be classified in different ways, taking into account different criteria. Thus, as Valcárcel and Luque de Castro have suggested [11], on considering the monitored parameter, sensors can be classified as chemical or biochemical, but considering their nature, they can be classified as reversible, irreversible, disposable or re-usable, and from the external shape they can be considered planar, probes or flow cells. Other types are found from other criteria, as summarized in Fig. 3.

The Electronic Signal Processor

The electronic signal processor is probably the part of a (bio)chemical sensor which is least dependent on the type of sensor and is only a function of the fast development in this field. Research in increasingly sophisticated and low-cost microcomputers favour real-time data acquisition and processing, thus improving the response of the chemical and (bio)chemical sensors when applied in a process stream.

Modern electronics computers have provided the basis for a range of biosensors in which silicon strain gauges have been integrated with silicon diaphragms and signal processing circuitry on the same chip, providing fully integrated sensors with very small dimensions. On the other hand, the charge-coupled device cameras in use

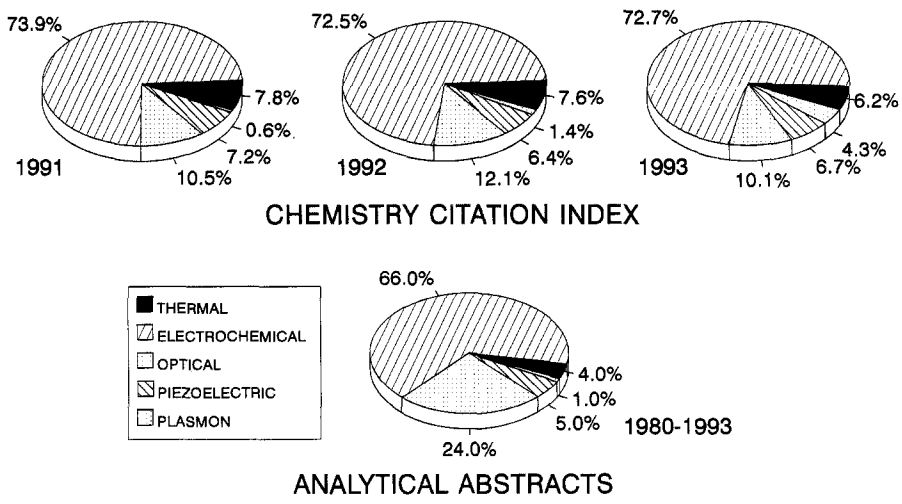

Fig. 4. Comparison between data found in two data bases for the relative importance of the most common transducers employed in the biosensors literature

for automatic visual inspection are smaller and more suitable for digital signal processing than their predecessors, the television tubes being replaced by solid-state silicon imaging arrays [12]. In the field of ion-selective electrode devices, the main development in recent years concerns miniaturization, which favours portability and in vivo use and also reduces construction costs [13].

The Transducer

The physicochemical change involved in the reaction between the biologically active material and the analyte must be converted into electrical output before its processing, and for this purpose a series of transducers may be employed.

As can be seen in Fig. 4, in which the relative numbers of published papers on the use of the currently most employed transducer are depicted, it can be confirmed that electrochemical sensors are the most popular [14], including both potentiometric and amperometric electrodes [15] and also some conductometry measurements [16]. This information has been taken from data found in the *Analytical Abstracts* data base for the period between 1980 and 1993.

Electrochemical transducers are employed in approximately 66% of the published literature on biosensors in the field of analytical chemistry, optical transducers being employed in only 24% and the rest corresponding to other sensors, e.g. thermal, piezoelectric or plasmon. The relative importance of the most common transducers in biosensor technology is due to the fast development of electrodes in the biological field and also to the specific applications of these in analytical chemistry .

Due to the easy miniaturization of electrochemical devices (see Fig. 5, which depicts the evolution from the single cathode sensor to the multiple cathode and the multiple sensor) and to the development of basic studies on electrochemical processes, electrode-base transducers continue to be the most important type and, on comparing the above-mentioned data with those found from another data base, the

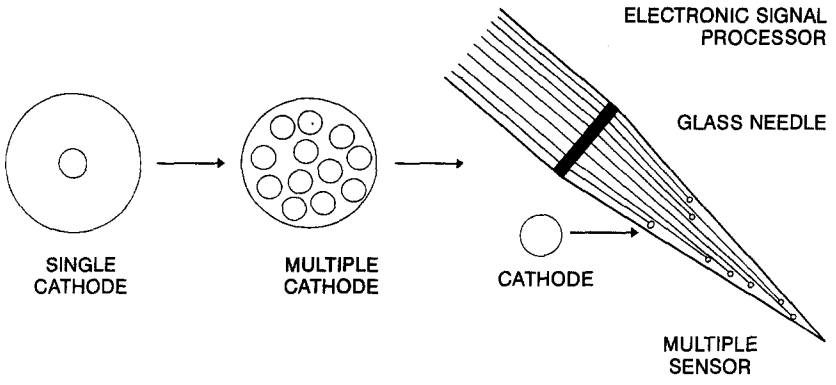

Fig. 5. The challenge of miniaturization. The development from single cathode sensors to multiple cathode and multiple sensor systems

Chemistry Citation Index, for the years 1991, 1992 and 1993, it can be concluded that the relative importance of these transducers continues unchanged in the most recent literature (see Fig. 4).

The basic type of optrode sensor is based on the use of fibre optics as light conducting systems combined with absorbance, luminescence or reflectance measurements and also on the appropriate selection of an immobilized reagent which changes the optical properties upon interaction with an analyte [17–19]. This kind of device does not require a reference signal or the use of electrical shielding, and is very appropriate for continuous monitoring of flow systems. Among the advantages offered we can also mention the fact that samples remain chemically unchanged and that the sensitivity of the measurements can be very good, specially when fluorometric measurements are employed. However, in this type of (bio)chemical sensor, suitable selection of the immobilized reagent is of prime importance in order to provide reversible measurements or easily regenerable and re-usable systems.

Thermal transducers are based on the measurement of temperature changes provided by the enthalpy change related to a (bio)chemical reaction and their universal applicability in the field of enzyme sensors is the reason for their importance. This kind of transducer is well-established in research applications and measurements have improved from the preliminary studies, in which the enzymes were attached directly to the thermistor and dipped in the sample solutions, to the use of flow-through microcalorimeters and integrated-circuit temperature- sensitive structures modified with enzymes [20, 21].

Piezoelectric transducers can be used to detect small changes in mass that occur on their surfaces. The piezoelectric microbalance incorporates a synthetic quartz crystal, with one of the sides covered by gold, aluminium or nickel inserted into a radio frequency amplifier. Measurements are carried out in differential mode by using crystals with and without a biologically active film [22].

Based on the piezoelectric phenomena, but incorporating electrodes on the same side of the crystal, rather than on opposite sides, surface acoustic wave transducers have been developed in which one electrode induces the piezoelectric vibration and creates a Rayleigh surface acoustic wave which travels to the other electrode,

operating in the gigahertz range [23]. With this type of transducer, several gases, such as H_2, NH_3, NO_2, H_2S and SO_2, can be measured, and also the humidity level.

The plasmon is a specific type of optical transducer based on the surface plasmon resonance phenomenon. It is a relatively new type of transducer in which the measurement of changes in the refractive index of a solution in contact with a film of Au, Pt or Ag surface-modified with a reagent to produce a matrix on which macromolecules can be covalently immobilized [24]. This system provides an alternative to the use of labelled molecules, and the changes in the surface plasmon resonance angle cause changes in the resonance signal which are linearly related to the concentration of the analyte in the solution.

The Biochemical Receptor

The receptor is the biochemical system which introduces analytical selectivity into the signal processing of the sensor. Selective molecular recognition of the target analyte is the main goal of (bio)chemical sensor technology and can be achieved by the use of affinity systems, such as enzymes for substrates, antibodies for antigens, lectin for sugars and nucleic acid sequences for their complementary sequences. Thus, a series of purified molecules, or in some cases tailored molecules, can be employed as biochemical receptors. However, in some cases more complex systems can be used, including hormones, bacteria, yeasts, whole cells or subcellular fractions, microorganisms, organelles or tissue sections [25, 26], but proteins are the most widely used molecular recognition systems.

From the 3589 references to papers on sensors and biosensors found in the 178661 references in the *Analytical Abstracts* data base for the period between January 1980 and December 1993, 740 are related to enzyme sensors and there are only 103 on antibody-based (bio)chemical sensors. Thus these two types of bioreceptor correspond to 20.6 and 2.9%, respectively, of the published literature. These data are consistent with those obtained from *Chemistry Citation Index* for the years 1991, 1992 and 1993, in which the percentage of the literature concerning biosensors in which enzymes are used is 20.8, 20.2 and 23.0%, respectively, with antibody-assembly contributing only 3.1, 2.4 and 3.4%. Figure 5 depicts the relative importance of enzymes and antibodies in the literature on biosensors and Fig. 6 shows, from the *Analytical Abstracts* data base, the evolution of the published literature on enzyme-based sensors and antibody sensors as a function of time.

The increasing number of published papers on both enzyme and antibody (bio)chemical sensors (Fig. 7) and the parallelism between the evolution of the literature on both fields show that the research is far from saturation. The more important role of enzymes can be explained by their greater versatility and easier availability, and on the reversibility of the enzyme-substrate interactions, in contrast to the permanent and essentially irreversible immuno antigen-antibody complex.

Immobilization Strategies

For the repeated use of biological receptors in analytical devices it is necessary to fix them in an appropriate material in order to incorporate them in the biosensor [27]. There are many techniques for immobilizing biologically active agents, but the most

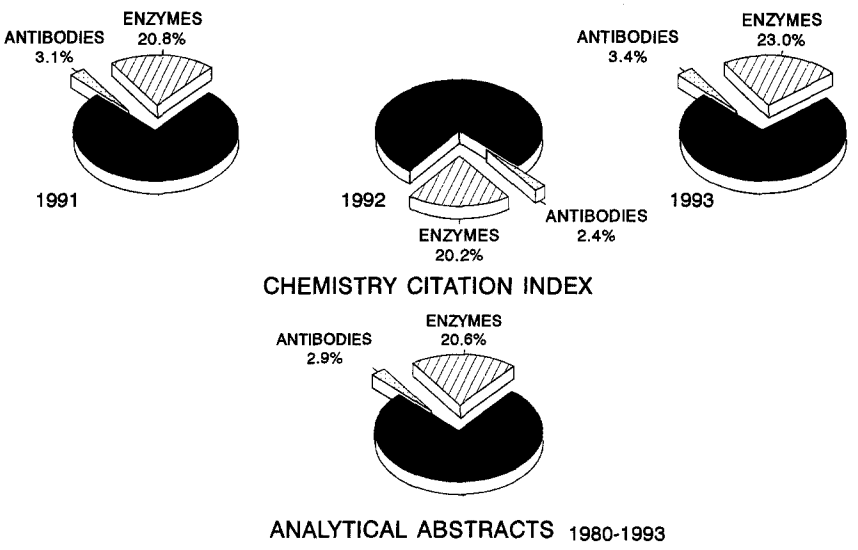

Fig. 6. Relative importance of enzymes and antibodies in the development of sensors

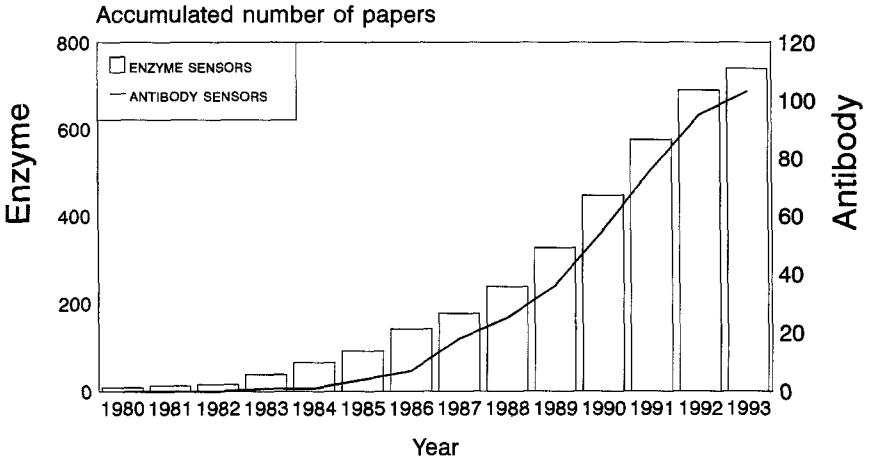

Fig. 7. Development of enzyme-based and antibody-based (bio)chemical sensors

common are covalent coupling, crosslinking, gel entrapment and simple physical adsorption; all these methods have numerous variants, and it is necessary to select the most appropriate technique for the different individual problems.

Covalent binding of the biological receptors involves a previous activation of the support, the coupling of the biomolecule and the removal of the adsorbed molecules. In general the support must have active chemical groups such as -OH, $-NH_2$, -COOH and -SH which can be functionalized for covalent attachment, but it is of prime importance that the active sites in the biomolecules are not affected by the immobilization process and so avoid losses of activity [28].

Crosslinking is a simple procedure in which a biopolymer may be intermolecularly crosslinked by bi- or multi-functional reagents. In general, proteins can be crosslinked with each other or with another inert protein. This method can be

applied after adsorption or gel entrapment of the bioreceptor and provides a strong chemical binding of the biomolecules. Gel entrapment of bioreceptors, using gelatin, polyacrylamide, cellulose triacetate, collagen or other reagents, prevents the biomolecules from diffusing from the reaction mixture, providing an intermediate situation between covalent binding and adsorption.

Adsorption is the simplest technique and offers several possibilities based on charge-charge interaction, hydrogen bonds or hydrophobic adsorption and in some cases, such as in enzymatic flow analysis determinations in non-aqueous media [29], it provides a good way because the activity of biomolecule is not affected by the immobilization procedure and the yield of immobilization is 100%.

The use of immobilized biomolecules in (bio)chemical sensor methodology is common practice. However, in order to evaluate the cost of the use of biosensors it would be convenient if authors indicated the yield of the immobilization procedures and the total amount of bioreceptor necessary to achieve a certain number of determinations, in order to demonstrate clearly the feasibility of the devices in terms of costs and possibilities for real analysis.

The Ideal Characteristics of (Bio)Chemical Sensors

There is common agreement on the desirable properties of an ideal (bio)chemical sensor. In addition to the main features of all analytical methods, such as accuracy, precision, sensitivity and selectivity, it is necessary that biosensors should have some additional properties concerning the absence of hysteresis, short-term and long term stability, features concerning the calibration curves and the calibration procedure and additional properties related to the ruggedness and cost of the manifolds.

On the other hand, and to provide good applicability of the biosensors in analytical work, it is desirable for biosensors to be portable and biocompatible in order to be able to carry out in situ and in vivo measurements and also that they provide a real-time response which could ensure the continuous measurement of the parameters, because one of the main challenges of research in the field of (bio)chemical sensors is the development of methodologies which could facilitate the application of academic developments to solve real problems.

Specifically, the ideal (bio)chemical sensor is one which can be operated in a reversible way or alternatively be re-usable and easily regenerable or suitable for a single use, in the case of irreversible, non-regenerable systems (see Fig. 8).

The Use of Biosensors in Real Analysis

During the last ten years the development of basic research concerning (bio)chemical sensors has provided exciting new ideas but a challenge remains: the practical application to real analysis with relatively low cost but with all the guarantees concerning accuracy and precision of results. Therefore, a big effort must be made in order for the use of biosensors to be suitable in current analysis.

Four major fields have attracted the use of (bio)chemical sensors, namely the food industry, medical and clinical analysis, environmental control, and pharmaceutical and biotechnology laboratories, with very important applications also for military defence.

Main properties	Additional properties	Application properties	Specific properties
Accuracy	No hysteresis	Portability	Reversibility
	stability	Real-time response	Re-usability
		Biocompatibility	Regenerability
Precision	Low background signal	Usability in direct real sample analysis	Suitability
Sensitivity	Good limit of detection		
	High dynamic range		
	Easy calibration		
Selectivity	Linearity		
	Simplicity of construction and operation		
	Ruggedness		
	Low cost		
	Long life-time		

Fig. 8. Analytical features of (bio)chemical sensors

Most of the biological receptors have been taken from biological systems in the human body and, therefore, it is not surprising that the development of (bio)chemical sensors in clinical chemistry is of special importance. A series of methods, from the use of test strips to the development of automated enzymatic analysis, are used to determine metabolites in blood and urine for diagnostics, in the micro- and millimolar ranges, and to monitor parameters involved in a series of diseases, such as the control of victims of heart attacks and strokes, and the screening and treatment of diabetes. They are also used to control parameters during surgical operations. A special type of application obviously concerns *in vivo* determinations, which will discussed in more detail below.

The application of biosensors in the food industry is of interest for the quality assessment of foods and specially for the on-line control of fermentation processes. However, in this field the presence of large amounts of potentially interfering compounds [30] involves the need for careful sample treatment and study of the most convenient experimental conditions for the use of biosensors.

Environmental protection requires the use of fast, accurate and reliable analytical methods for the determinations of toxic gases, organic pollutants and heavy metals in air, water and soil samples, and for this purpose (bio)chemical sensors provide very good tools. Both enzymatic electrodes and portable immunoassay-based devices can be used for these purposes [31]. On the other hand, in some countries, such as Japan, microbial sensors are routinely used for the analysis of effluent water [32], providing a parameter similar to the biological oxygen demand.

Pharmaceutical industries and biotechnology laboratories are involved in areas of high added-value product manufacturing and, therefore, the automation of process control by means of (bio)chemical sensors is of a great interest [33]. It can be expected that in the next few years these companies will develop research extensively in both, the basic and applied aspects.

The detection of warfare agents, nerve gases and other toxic agents is a very interesting aspect for the defence industry and confidential research is carried out on this aspect in many national laboratories [13].

The in vivo Use of (Bio)chemical Sensors

A specific application of biosensors in the clinical chemistry is the in vivo monitoring of chemicals in the human body in order to continuously detect changes in real time, which could help to elucidate patterns in the disease state and so help to correct harmful perturbations. Various analytes, like gases, ions, metabolites, hormones or drugs, may be continuously measured by the use of (bio)chemical devices. However their use in vivo is severely limited by the fact that sensors must be operated in reversible conditions in order to assure long term use [34, 35].

In this field, in addition to the typical problems related to the analytical features of (bio)chemical sensors, we have to take into account the problems arising from the implantation of the devices and the biocompatibility of the material employed, especially the outer membrane which must be in permanent contact with the organism. Intracerebral and intravascular implantation introduces serious risks and tissue monitoring involves additional difficulties coming from local reactions. In addition, as suggested by Turner [36], we must take into account the impact on quality of life which is related to the psychosocial effects of the use of biosensors on both the personal activities and the psychological perception of the parient.

The biocompatibility of the biosensor devices must be ensured in order to avoid adverse reactions in the organism but also to maintain chemical communication with the tissues, instead of the defence mechanisms which can be developed in the biological samples, such as cell precipitation, blood coagulation or inflammatory responses in the tissues.

Biosensors and Flow Analysis

The development of flow analysis methodologies [37, 38] offers a series of advantages in the application of (bio)chemical sensors. Biosensors and flow manifolds are totally compatible and the use of several sensors for monitoring a series of reactions can be carried out by using flow cells or flow-through biosensors. Additionally, flow analysis methodology offers a fast and low-cost way for the continuous regeneration of irreversible (bio)chemical sensors in order to ensure their re-usability [39].

As can be seen in Fig. 9, the scientific literature on sensors and flow injection analysis (FIA) has increased tremendously since 1980, especially in the last five years, in which the growth rate of the literature has been of the order of 37 papers per year, thus showing the advantages offered by the synergistic combination between FIA and biosensors.

A recent development in the field of flow analysis is the development of flow-through (bio)chemical sensors [11], which integrate separation, reaction and detection and can reduce dispersion of samples, increasing sensitivity and selectivity of the determinations and also the sample throughput, offering new possibilities for the implementation of the regeneration steps. Other developments concern the use of (bio)chemical receptors [29] and enzyme electrodes [40, 41] in organic media.

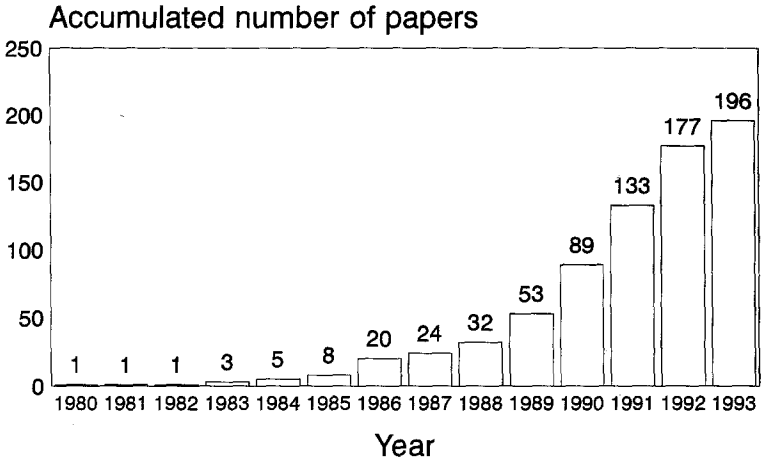

Fig. 9. Evolution of the FIA literature on (bio)chemical sensors

To sum up, the use of biosensors in flow analysis dramatically increases the versatility of the devices and reduces sample and reagent consumption, providing important benefits in practical applications.

The Present State and Future Developments

The tremendous effort in recent years in both biochemical engineering and microelectronics has produced an interesting and very original background from which the basis of the methodologies for (bio)chemical sensor development can be well established. Some new ideas, such as the scanning electrochemical microscope, the nanofabrication of devices and multichannel flow-through systems will be of special importance in the next few years. The scanning electrochemical microscope, developed by Bard and Conorkers [42], has been applied to enzyme kinetic measurements and also provides spatially resolved electrochemical information about samples ex vivo and in vivo [43]. Massive nanofabrication of biosensors, based on computeraided design and microlithography will be very important for the miniaturization of biosensors. The development of multichannel flow-through systems [44] will provide new tools for exploring matter in order to obtain chemical information.

A special field of research, which will be increasingly developed in the future, is the use of (bio)chemical sensors for metal ions determination. A good example in this sense is the development of optrodes based on the use of natural molecules, such as pioverdine. Pioverdine is a natural siderophore, synthesized by the pseudomonas fluorescens, which can react with Fe(III) selectively causing a strong quenching of the fluorescence of the immobilized molecule [45], thus providing a sensitive method for the determination of Fe in environmental and clinical samples.

For the determination of heavy metals in the environment, the development of metallothionein-based (bio)chemical sensors will be very important in a near future for the selective analysis of metals. Methallothionein is a low relative molecular mass (7000 D) protein with 30% amino-acid residues of cysteine, each with a thiol group [46, 47]. This protein has six molecular isomers [48], and it is enriched in kidney

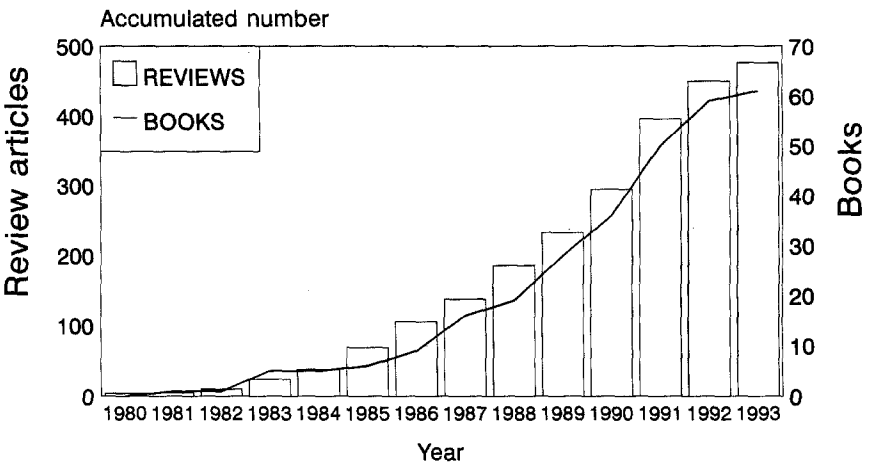

Fig. 10. Development of the basic literature on (bio)chemical sensors

under heavy metal exposure. Thus it can be used as an indicator of environmental pollution, specially as recent studies by Ribas and coworkers [49] have shown a certain selectivity of the different isomers of metallothionein to different metals, such as Ni, Zn, Cu, Cd, Pb and Hg, thus offering a good way for the development of specific biosensors.

A look back into the past, taking into consideration only the most relevant literature published in the last few years, shows that the field of (bio)chemical sensors is still a very active live research area and, as can be seen in Fig. 10, the number of books and reviews published in the last ten years provides a good indication of the activity in this field.

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