Ursula E. Spichiger-Keller

Chemical Sensors and Biosensors for Medical and Biological Applications

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Preface

Chemical sensors are intended to solve analytical problems complementary to that provided by standard analytical instruments. In order to become commercially viable, chemical sensors have to be combined with an appropriate sampling device and electronics in such a way that the overall dimensions of the final device, the price and ease in handling, are acceptable. These parameters determine the profile of sensing devices in the vast range of applications in industrial and bio-process control, in environmental monitoring and in monitoring of toxic effluents (e.g. cyanide), in food technology, in field measurements, in emergency-care analysis, and point-of-care testing (POCT) in medicine. An unexplored area is the use of chemical sensors in toxicology. In order to cope with various fields of applications, the brand "the Lab in the Bag" was coined specifying the trend of further developments.

Several comprehensive volumes on chemical sensors had been published. However most of them are more focused on the development of the physical part, the transducers. This volume intends to provide an overview on the variety of chemical sensors focusing on analytical-chemical aspects generally, and on biological applications specifically. The field of chemical sensors could be depicted as a space which is spread by 3 coordinates: the biological or life sciences along one axis, physical-chemistry and chemistry along another, and mathematics and statistics along the third axis. This "space" reflects the complexity of the field. This volume tries to take sufficient account of each axis and gives an overview of the field with special focus on the developments in the goup of Prof. W. Simon, Laboratory for Organic Chemistry, involving the habilitation thesis of the author, and on developments in the Centre for Chemical Sensors/Biosensors and bioAnalytical Chemistry at ETH Zürich-Technopark. Each chapter is devoted to a separate theme. So the references have been inserted after each thematic block or chapter, beginning with chapter 1. Each thematic block or section is closed by conclusions.

In the first chapter, the question as to whether chemical sensors and biosensors have to be differenciated is discussed. In the course of this chapter, chemical sensors are defined and related to particular areas in analytical chemistry. A brief history of the field is given describing the development of chemical sensors. This is followed by a discussion of market trends and comments on possible future developments of the general situation in analytical laboratories.

The second and third chapter sets out to give an overview on the chemical and physico-chemical principles underlying the preparation of chemical and biochemical sensors. These chapters cope with the modelling of interactions, the investigation of interactions, and the basic theories underlying a reversible response which enables continuous monitoring. An understanding of these principles is assumed in chapter five and six, where some sensors developed and tested by the author's own research group are presented. In many cases, only a brief description is given, but this is compensated for by the provision of extensive references. A major subject of the author's research has been the investigation of the influence of the medium, the bulk of the sensing layer, incorporating the active compounds (chapter 4), and the development of the magnesium-selective electrode so that it can be routinely used in plasma and whole blood. Major efforts were devoted to the synthesis of the magnesium-selective ionophore

ETH 5506 in order to make this ligand accessible as ETH^T 5506 to industrial production (appendix 10; ETH^T means ETH-Technopark).

The seventh chapter discusses the problems of reliability and interpretability of results. In all fields of analytical chemistry, these are at least as important as the development of new methods and procedures. Several sections focus on decision and discrimination problems analogous to analytical data treatment in medicine, in order to solve decision problems in general analytical chemistry. The author's experience with quality control and discrimination analysis is referred to.

In the interests of completing this book, it has not been possible to go into great detail about the experimental conditions and fundamental explanations for all results presented. However, many of these can be found in the references provided. In selecting topics, I was governed by a desire to cover those which fill a gap in the existing comprehensive volumes of other authors. In addition, these topics provide insights into the actions of specific sensors, which illustrate their characteristics in detail, and which show the differences of basic concepts.

I would like to dedicate this book first to the memory of the late Prof. Wilhelm Simon in recognition of his outstanding contribution to the field. It was in his laboratory that I realised that productive research is, among other things, the reflection of personal and scientific discipline, the unguarded exchange of ideas and daily critical discussions. In writing the Habilitation thesis, I missed his critical comments and suggestions, and his sometimes strange, but always stimulating ideas.

Secondly, I dedicate this book to those students and colleagues who are new to the field of chemical sensors and who will, I hope, find it a useful reference work. The appendices, in particular, are intended to be helpful for those involved in the development and in practical applications of chemical sensors. The appendices, specifically appendix 9, contain much information not easily available elsewhere.

Ursula E. Spichiger, August, 1997

Contents

1 Introduction

1.1 Chemical Sensors as Alternative Analytical Tools

The technical potential of analytical chemistry has continued to grow over the past 30 years. It has evolved from being a field little more scientific than alchemy to becoming an exact science with almost no limits to its applications.

Analytical chemistry is the chemist's way of answering the question: "What's in it?". The chemical components of a substance are determined through chemical analysis [1]. The number of chemical components which are identified by the analytical method depends on its resolution or detection limit. For example, with detection limits in the range 10^{-9}–10^{-12} moles L^{-1}, the number of components in drinking water increases exponentially. In practice, therefore, analytical chemistry is mostly concerned with determining *characteristic* components of a substance in order to answer specific questions and to yield specific information. These characteristic components are known as "analytes" or "laboratory parameters" when subjected to the analytical procedure and when listed in a report [2]. In what follows, the originally chosen, unchanged material is called the "specimen", whereas an appropriately representative portion of a substance, which is fed to the analytical instrument after adequate pretreatment, is called the "sample" [2]. For each specimen there are certain typical chemical components or analytes about which the analytical chemist seeks chemical information.

In analytical chemistry today, it appears that the most important decision is to select an appropriate, highly capable instrument, together with the necessary hard- and software, and to adapt the chemical procedure recommended by the standardization authorities. Ideally, the results flowing from the analyzer merely need to be collected and reported. Although this may seem extremely direct, the time spent on collecting, transporting, and pretreating specimens causes a bottleneck in most analytical processes; in many cases this is not seriously addressed, and needs to be reduced. Moreover, unstable analytes and analytes present at very low levels (ppb) are best analyzed *on-site*. As a result, the laboratory has to move closer to the source of the specimen, which means developing more user-friendly analytical instruments. Along with innovations in analytical chemistry, social pressures from the environmental movement, and economic pressures arising from health care reform, have been responsible for many new trends.

Choice of the analytical instrument is important as it is the central element of the analytical procedure and is routinely handled by analytical chemists and chemists. The chemical and instrumental analysis is more likely to be limited by the chemistry of the specimen and its characteristic components than by the instrumental procedure. For example the biological matrix, specifically the protein content, has a crucial influence on the analytical procedure, owing to interactions with reagents and adsorption on surfaces. Special efforts are required to get rid of the effects of these interaction and to ensure that high-quality information is obtained by an appropriate analytical procedure. The term "high-quality information" is not restricted to the uncertainty of results and aspects of quality assessment, but is primarily concerned with getting
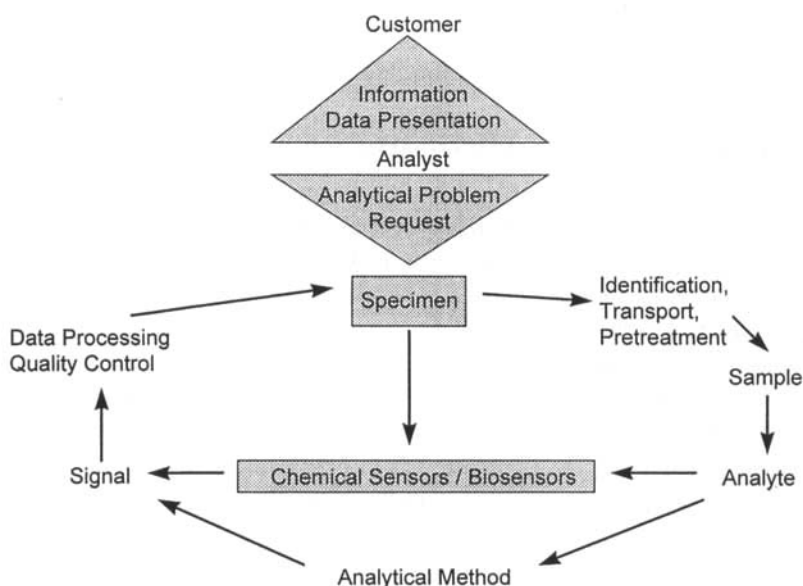
Figure 1-1. The analytical procedure and data evaluation process in a general analytical system and in a dedicated system, such as a chemical or biochemical sensor

the most essential and meaningful information. This information answers the question: which specific component or species involves the most useful and most relevant information in view of a decision about the quality of a substance, e.g., its biological activity or toxicity? Such a component might be one typical species, e.g., the fraction of the free, active electrolyte rather than the total concentration, or the free fraction of a single enantiomer rather than the racemate. Some very basic problems in standardizing quantitative information for typical species, and providing quality control specimens (e.g., to do with ion activity measurements) are currently still unsolved.

Given this situation, an eclectic approach to chemical and instrumental specimen analysis seems most appropriate. Used well, it should: (a) allow immediate *on-site* measurements; (b) eliminate matrix effects; (c) achieve high selectivity and user-friendly handling; (d) allow screening based on certified cut-off limits; (e) expand the application area; (f) allow modification of the methods or instruments. A novel concept in analytical chemistry needs to be governed by a strategy which involves and defines the *necessary* steps and procedures not the best possible. In addition, such a concept may not be oriented to increasing throughput, but, rather to increasing the efficacy of the analytical process (see below). Necessarily, such a concept must evolve not only from a profound and global insight into analytical processes, but also from a thorough understanding of the underlying chemical and physicochemical processes, and may be supplemented by chemometric approaches.

In *conventional analytical chemistry*, determining an analyte involves various steps (see also Figure 1-1 and [3]):
1. Define the problem; 2. collect the specimen; 3. identify the specimen; 4. transport the specimen to the laboratory; 5. select an appropriate method; 6. pretreat the specimen and prepare the sample; 7. perform the measurements; 8. compare with reference and quality control specimens; 9. calculate statistical parameters; 10. decide on the performance and reliability of the analysis; 11. transform data to give an interpretable value; and, 12. present the data.

The complete procedure is challenging for the analytical chemist as it normally requires considerable skill and a feeling for automation and robotics. Often it is necessary to use several different techniques and instruments in solving an analytical problem. In between identifying the analyte and presenting the results, additional steps may be necessary, e.g., choosing and evaluating sophisticated additional separation steps or chromatographic columns, connecting specific detectors, specifying a flow-cell, or eliminating interfering solutes and solvents.

Despite the skill involved in carrying out the complete analytical process, more and more analytical tests can now be carried out *on-site* rather than in a central laboratory. Such front-line analysis has ecological and economic advantages, such as:

- Eliminating the need to transport specimens, which is particularly problematic with unstable analytes
- Reducing the effort required to identify the analyte, and to interpret and transmit the results
- Providing immediate answers to a problem
- Avoiding queues in the high-tech, central laboratory
- Stimulating thinking in terms of *efficacy*, which may be defined either the number of true positive results per total number of analyzed specimens or samples (positive efficacy) or the number of true negative results per total number of analyzed specimens or samples (negative efficacy), in contrast to *efficiency*, which is the number of correctly allocated specimens per total number of analyzed samples, and *throughput* (total number of analyzed specimens per unit time) (see section 7.2.4 and Appendix 2)

In order to tackle an analytical problem, the customer and analyst must agree on the information needed, and the specimen and sampling required to obtain it. It is then up to the analyst to decide which procedure will be appropriate for dealing with the special properties of the specimen. The two triangles in Figure 1-1 denote communication between the customer and the analytical laboratory which is catalyzed by the analytical chemist in the middle of the sandwich. When applying biochemical or chemical sensors, the analytical process is straight-line, since sensor technology allows the analyte in a specimen to be quantified directly. Thus, the analyst can avoid having to transport the specimen, pretreat it, and prepare a sample (at least steps 4 to 6 above). By using sensor technology, the steps in the first part of the data evaluation process can be reduced in principle to just one, namely, the interaction between the sensing element or sensor surface and the specimen. However, in many cases pH- and/or ionic strength buffering of the specimen (*conditioning*) is recommended in order to improve accuracy, which means making use of a continuous flow system. Instead of having to choose the chemical procedure and the detecting system, the required selectivity and detection limit have to be estimated, and the limiting operational conditions need to be considered when defining the

analytical problem. The more selective the sensor, the more dedicated the system, and, as a result, the cheaper and easier it is to use. If the technology applies a couple of poorly selective sensing elements arranged in a sensor array, it may be necessary to resort to sophisticated exterior data processing in order to interpret the measured values and to ensure that the sensing system was accurate. Sensor technology can be particularly useful and preferable to well-established analytical procedures in testing situations where:

- Continuous or periodical testing by laypeople is necessary
- There is a shortage of skilled manpower and/or natural resources
- Working with chemical reagents is avoided
- The analytes are not stable and quick answers are required
- Front-line screening saves on resources for economic and/or ecological reasons

In order to improve the analytical methods involving sensor technology and the evaluation of their results, it will be necessary, in the future, to find ways of: (a) ensuring adequate sensitivity and accuracy; (b) validating the results reliably; (c) providing matrix independence and ruggedness of the analytical procedure; and (d) making the procedures user-friendly. Technological advances will involve: optimizing the performance over time (long-term stability), combining sensor techniques, designing modular and multidimensional sensing systems, and facilitating specific applications. Sensor technology is particularly appropriate in life sciences (biotechnology), in novel cultivation techniques, in the medical field, and in process monitoring, but there is considerable room for improving applications in these areas.

Chemical and biochemical sensors have attracted considerable attention because they can provide information about the active molality of the free fraction of an analyte. No other analytical techniques can do this. It is likely that, with future sensor technology, elemental analysis will be refined or replaced by the speciation of specific chemical fractions. This has already happened to some extent with a few inorganic and organic analytes. Unfortunately, there is still a tendency to evaluate total concentrations solely, especially in biology and medicine, although the activity of a metal ion is at least as relevant as the total concentration, and is, presumably, the most relevant fraction in toxicological studies. It is essential that the correlations between sensor outputs and the toxicity of a species should be investigated in tests using animals so that animal-free toxicology tests can be performed subsequently (see next section). There is also a growing need to determine active fractions for medical purposes, e.g., for electrolytes such as calcium and magnesium ions where the complexed fraction amounts to around 50% of the total concentration. Standard techniques in general analytical chemistry have very limited ways of dealing with the problem of direct, selective detection of a defined fraction of an analyte in the specimen or sample.

Biosensors

Another very direct detecting system is the living organism. In response to the *Toxic Substances Control Act* (TSCA), the U.S. Environmental Protection Agency (EPA) was charged with the

responsibility of assessing the hazards particular chemicals posed for human health [4]. For this purpose, whole-organism bioassays and physiological studies were used effectively in identifying potentially common modes of action, common analytical approaches, and in developing a knowledge base for an expert system designed to predict toxic mechanisms from the structure. A variety of organisms have been used in testing the toxicity of xenobiotics. Among these are protozoa, especially ciliates such as *Tetrahymena pyriformis*, and various fish, in particular the rainbow trout. The assessment of the *fish acute toxicity syndrome* (FATS) has been investigated through careful examination of the behavioral responses of trout, and associated variations in some commonly used diagnostic parameters which are correlated to the respiratory-cardiovascular toxic effects of xenobiotics dissolved in water [4].

Although using living organisms may seem a very simple and inexpensive technique, special care must be taken to ensure that the biochemical environment is controlled, usually by monitoring it electronically. The reliability of biological monitoring is sometimes impaired by individual variations in the inbuilt repair mechanisms of damage associated with resistance to different agents. However, the most relevant parameter in toxicological risk assessment is the lipophilicity of a xenobiotic and, therefore, the partition of the free species between water and the living organism. This suggests that at least some of the *in vivo* tests could be replaced by *in vitro* tests using chemical sensors. Some typical bulk membrane sensors, where the active component is incorporated into an apolar solvent polymeric layer, respond preferably to the lipophilicity of a target compound (see section 3.1). Currently one of the most interesting questions is whether the physicochemical activity of a xenobiotic correlates with its biological activity. Analytical experiments may help to answer this question for both charged and uncharged species.

A living organism is a complex and sophisticated biosensing system. Some chemical senses in animal species as well as in plants are so exquisitely developed that communication can take place through "biochemical" reactions. Biosensor research has sought to mimic such natural processes in the laboratory by fixing and connecting isolated cells and organs to a transducing and/or detecting system, usually an electrochemical receiver and amplifier [5, 6]. In one such study, the latent potential of living "bioreceptors", in this case the olfactory organs or the antennules of Hawaiian crabs, were treated so as to create an intact neuronal chemoreceptor-based biosensor called a receptrode [7]. This project involved confronting new aspects of detection and data processing. The various neurons of the antennular receptrodes generated action potentials with different amplitudes. The complex multiunit data were analyzed by employing an amplitude sorting program similar to that used in clinical encephalography. The amplitudes were associated with the selectivity of the olfactory organ, incorporating a multitude of different receptors, whereas the frequency of the depolarization and voltage change corresponded to the intensity of the stimulus by volatile amines (e.g., trimethylamine oxide).

A chemoreceptor-based biosensor or receptrode like the one described above has some very desirable characteristics, such as: high specificity, extremely low detection limit, large dynamic range, and very short response time [7]. The major problem with using the antennules of Hawaiian crabs was they only had a short life-time of 48 h for the following reasons:

1. Autolytic processes destroy parts of the tissue
2. Neurons needed to be continually supplied with nutrients, electrolytes, and oxygen

These conditions are difficult to reproduce in the laboratory, especially within the confines of a sensor tip.

Other papers on receptrodes have looked at such things as the use of fish scales in optical devices [8] or of the taste receptor cells of the larval tiger salamander as electrochemical sensors [9]. Even if, so far, these studies have only resulted in rather unreliable devices, it is still essential to discover the fundamental properties of such sensors in order to create promising novel devices [10].

In 1991, a very critical review on biosensors was published by G.A. Rechnitz [11]. Since this time, several excellent overviews and books have appeared, the latest ones were edited by F.W.S. Scheller et al. [12], R.F. Taylor and J.S. Schultz [13]. Lüdi et al. [14] have discussed possible applications of sensors in industry.

Since both living organisms and isolated organs are selectively sensitive to agents and irritations, attempts have been made to develop artificial systems with comparable sensitivity. In these, enzymes incorporated in "biosensors" have been mainly used to mimic the recognition process [12c, 15, 16]. In 1991 Schultz defined biosensors as: *"Raffinierte moderne Pendants zu den Kanarienvögeln in Kohlebergwerken, deren Verhalten Hauer und Steiger vor gefährlichen Ansammlungen von Grubengas warnte, basieren auf pflanzlichen oder tierischen Molekülbausteinen"* (they are refined modern equivalents to the caged canary used in coal mines to warn miners of dangerous collections of methane (mine gas) and are based on vegetable or animal molecular building blocks).

Biosensors and chemical sensors differ in that they employ different recognition processes. In biosensors, natural materials are coupled to physical transducers. Excellent transducing elements are generally available, although the molecular recognition component is rarely satisfactory, owing to its short lifetime or the complexity of the signal. In chemical sensors, the recognition component is, in some cases, a fully synthetic, specially tailored molecule. The most successful chemical sensor involves incorporating valinomycin into a synthetic membrane. Since valinomycin is essentially a natural peptide, it is open to debate as to whether this may be considered to be a fully synthetic recognition model.

1.2   The Concept of Chemical and Biochemical Sensors

It is not easy to distinguish clearly between a sensor and a complex analytical system. Integrated gas chromatographs, infrared and mass spectrometers may be called chemical sensors. However, a chemical sensor is typically more versatile and cheaper than traditional instrumentation. Some definitions of "chemical sensor" are given by ANSI, DIN, VDI/VDE, ICE-Draft a.q. [17]. However confusing the range of definitions may be to the layperson, it is quite clear to experts what is meant. This is why only a rough and rather arbitrary definition is given here [18].

Janata stresses that a chemical sensor must provide "a real time insight into the chemical composition of the system" and couple "recognition and amplification" with a resulting electrical signal. One definition supported by the IUPAC commission in a provisional draft is [19a]:

> Analytical chemical sensors are miniaturized transducers that selectively
> and reversibly respond to chemical compounds or ions and yield electrical
> signals which depend on the concentration.

If this is interpreted strictly, reversibility must mean that successive concentration changes in both directions can be continuously monitored. As a consequence, sensors integrating antibodies which are regenerated by a washout process cannot be considered as chemical sensors according to this definition [20].

In another IUPAC paper, devices such as indicator tubes and test strips, which do not provide continuous signals, are considered to be dosimeters rather than sensors [21a]. However, the IUPAC definition does not take into account the fact that the signal yield is closely related to the molality of the free analyte in the sample, which might differ from the concentration. In addition to the very restricted definition, chemical sensors involve a broad spectrum of *transducing process* performed optically, gravimetrically, calorimetrically, or in various other ways as shown in Figure 1-2. Remarkably, the transducing process, including coupling the chemical recognition element to the physical part of the sensor, may have a profound effect on chemical selectivity and analytical performance (see chapter 4). Those aspects were taken into account in a new draft by the IUPAC Commission on General Aspects of Analytical Chemistry. This draft provides a more detailed and more general definition and a broad discussion of chemical sensors which states in the first phrase[19b]:

> A chemical sensor is a device that transforms chemical information, ranging
> from the concentration of a specific sample component to total composition
> analysis, into an analytically useful signal.

At present, the final signal in a chemical sensor is still always electrical, but this may change with the development of optical computers.

According to Figure 1-2, biosensors constitute a subgroup of chemical sensors where biological host molecules, such as natural or artificial antibodies, enzymes or receptors or their hybrids, are equivalent to synthetic ligands and are integrated into the chemical recognition process.

Selectivity is related to *specificity*. Selectivity means that an interfering species responds with the same type of signal, e.g., with the same wavelength or working potential, but with an intensity different from that of the target analyte. High selectivity means that the contribution of an interfering species to the signal relative to the primary analyte is minimal, although the active molality of both covers the same range (see chapters 3 and 4). Specificity, on the other hand, characterizes the unique property of a bioreceptor, e.g., an enzyme, which, in responding to a specific target substrate, generates a specific product. Therefore biosensors, in responding to that specific substrate or product, generate a specific signal or signal change. In the case where an enzyme shows cross-reactivity to an interfering substrate, it is assumed that it produces a different product which results in a sensor response clearly different from that of the primary substrate (e.g., different wavelength, different working potential). In practice, only two classes of
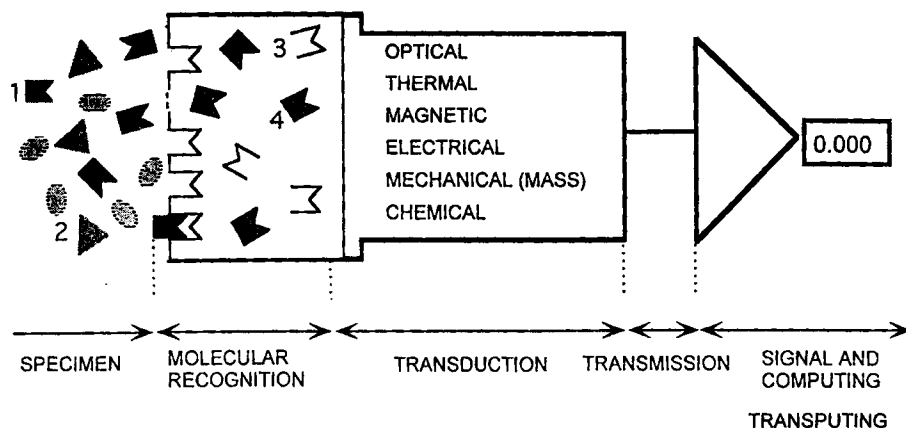
Figure 1-2. General model for chemical sensors, differentiating between molecular recognition, transduction and data processing

enzymes are used in sensor technology, namely oxidases and dehydrogenases, which produce products such as hydrogen peroxide or NADH (nicotinamide adenine dinucleotide, reduced form) which are detected in a wide range of biosensors. Therefore the term "selectivity" has been used to describe the discriminative power of a biosensor in the same way as for other chemical sensors [22]. Generally, the selectivity of a biosensor allows for a mixed response to both the target analyte and the interfering species. Therefore, characterizing the selectivity coefficient for a typical application may be more relevant to the operation of such biosensors than relying on its specificity [22].

Biosensors as a subgroup of chemical sensors are defined as operating with either high specificity or an exceptionally high natural selectivity, but with considerably restricted stability and lifetime in many cases. As a consequence, the lifetime of the sensor has to be sacrified in favour of the natural selectivity or specificity.

The quantity detected is always a measure of the active molality of the analyte, whose calibration is strongly correlated with quantities such as: the active molality of the interfering species, the pH and temperature of the sample, and the ionic strength and osmolality relevant for both charged and uncharged analytes (see chapter 3). For biotechnological as well as medical applications, the analyte activity delivers only the biologically relevant information when measured in the specimen directly, preferably by a "realtime" approach. The most important features of chemical and biochemical sensors are shown in Table 1-1.

Thermodynamic reversibility is important in ensuring continuous monitoring with chemical sensors. Individual sensor reactions include: thermodynamically reversible reactions, steady-state reactions and non-reversible reactions in disposable sensors. Thermodynamics is central in understanding the principles involved in operating the individual devices. Chapter 3 is devoted to these reaction mechanisms.

The key to the design of a chemical or biochemical sensor is the recognition process of an organic or inorganic substrate by a receptor–molecule generating a host–guest product (see

Table 1-1. Features and benefits of chemical and biochemical sensors

Features	Benefits
targeted specificity, selectivity	versatility, dedicated systems
selective assay in complex samples	ease of use, front-line analysis, reagent-free or reagent-poor operation
short response time	fast measurements and high sample throughput
electronic processing and electronic control of calibration	consumer friendliness, ensuring safety of the assay
reversibility	continuous measurements, low waste, no consumption of the analyte
enzymatic steady-state	enzymatic turn-over of the analyte/substrate
availability, low cost	disposable or exchangeable elements

chapter 2). The sensing schemes of the molecular recognition element are based on bulk or surface interactions, on mechanisms where the analyte is adsorbed or where it partitions between the sample and the bulk phase (see section 2.1). The target analyte or substrate may be any organic or inorganic ion or any uncharged molecular species. In order for a sensor to detect an analyte successfully in a complex sample matrix containing some analogously reacting species, high selectivity is required.

Selectivity may be achieved by using various designs of optical as well as electrochemical sensors furnished with synthetic carriers (see section 2.2), enzymes [23–25], or antibodies [26–28], or by using sensors based on competitive binding [29]. Enzymes are defined as reacting reversibly. In fact, what really happens is that they reach a stable steady state, assuming a constant mass transfer of substrates and products. In contrast, antigen–antibody reactions exhibiting high specificity are, in most cases, not reversible with a reasonable rate constant, owing to the exceptionally high affinity for their substrates associated with low detection limits. On reviewing the literature, it seems that an artificial recognition process can overcome the severe limitations of natural compounds [7, 8].

One of the most outstanding recent developments has been the design of artificial enzymes or catalytic antibodies [30–32]. The molecular recognition principles based on synthetic host compounds are more modest than those of artificial enzymes. When modelling host–guest

interactions (see section 2.2), the shape of the analyte has to *match* the site of the host species. A broad range of electrodes specified for various anions and cations are available, and are routinely used with reasonable analytical performance in diagnostic instruments, in clinical analyzers [33, 34], and in environmental analysis. In clinical chemistry, highthroughput analyzers, preferably based on optical assays, produce 5000–15 000 results per hour. Furthermore, the use of ion-selective electrodes (ISEs) in discrete analytical systems has increased throughput considerably. In the best case, the resident time of a sample in the ISE–module, which analyzes at least four parameters in series equivalent to four different typical ions, is 6 s.

Although ligands and ligand cocktails are currently used worldwide, the approach developed by Simon for recognizing and sensing ions is not mentioned in any of the volumes on sensors [35, 36]. The design of ligands for molecular recognition has been extended to include the recognition of uncharged species, such as: humidity (H_2O) [37, 38], ethanol [39, 40], glucose [41], creatinine [42], gases such as CO_2 [43], HSO_3^- and SO_2 [44], and NH_3 [45, 46]. It offers exciting prospects for the optical translation and transduction of reversible host–guest interactions.

The sensing system can, to a certain extent, be adapted and tailored to fit its applications. The detection limit, the selectivity, and the dynamic range may be shifted by modifying the ligand or the optical transducer and the surrounding bulk medium according to chemical or physicochemical principles (see chapters 4–6). Applications in various fields as different as medical analysis and biotechnology have been undertaken successfully [47–49]. A survey evaluating optical assays is given in [50].

Strong competition in the field of sensor technology over the past 10 years has led to an increase in the number of models available. Nevertheless, only a few types of chemical and biochemical sensors appear to be viewed as reliable tools for analytical chemistry and to be used widely in this growing market sector. Physical sensors, on the other hand, have become well-established in a competitive market and are regularly used in different monitoring systems and devices. The concept of the chemical sensor is, however, not new. A brief history focusing on the development of chemical sensors, especially on aspects of commercial use, will be presented in the following section.

1.3   Recognition Processes and Sensor Technology: Milestones

The technology of sensors and actuators has a long history. Wilhelm von Siemens built one of the first sensors in 1860. He made use of the temperature dependence of a resistor made of copper wire to measure temperature [51]. The fundamental principles behind physical sensors and transducers largely apply to chemical and biochemical sensors. The history of the development of *chemical sensors* for medical applications is summarized in Appendix 1 [52, 53]. The first really significant event from the commercial point of view occurred around 1932, when Arnold Beckman developed the modern glass electrode [54]. In 1937, Kolthoff and Sanders [55] published a paper made use of solid-state electrodes, such as the silver halide and fluoride-selective electrodes (for an account of the development of solid-state and glass-electrodes, see Frant [54]).

The key feature of carrier-based chemical sensors involves the recognition of the analyte by using a ligand tailored for the purpose. The sensing element is that critical part of the sensor where the primary transduction occurs and, as such, is vitally important in the operation of the whole sensor. The basic concept of chemical sensors owes much to the investigations of Moore and Pressman into the effects of naturally occurring neutral antibiotics on biological membrane systems in 1964–1965 [56]. Valinomycin (Figure 1-3) was reported to change the permeability of cells for potassium by a factor of 4×10^4. Two years later, the highly selective and reversible complexing properties for alkali metal ions were described by Stefanac and Simon [57]. In the meantime, Ross in the United States and Simon in Switzerland had both applied for a patent covering the K^+-selective electrode; the patent application of Simon was accorded priority [58].

Simon was certainly the first to introduce the class of chemical sensors based on *neutral carriers*. Subsequently, in 1970, Frant and Ross described how the valinomycin K^+-selective electrode was first employed in serum measurements [59]. Orion received a licence under the Simon patent and developed a prototype electrolyte serum analyzer for NASA's Space Shuttle and, subsequently, the first commercialized sodium/potassium analyzer SS-30 for whole blood. Ironically, spin-offs from the Orion project led to the business becoming commercially successful. In 1972, another clinical analyzer using a valinomycin–based sensor, namely the STAT-ION (Technicon/ Photovolt Corp., USA), was commercialized [60].

In 1967, the term "ionophore" was coined by Pressman et al. [61]. In the same year the structure of the first macrotetrolide–ion complex was elucidated (see Figures 1-3, and Appendix 1) [62]. The ionophores were, typically, lipid-soluble peptides with a relative molecular mass < 2000. Some of them, such as those in the valinomycin class, had in common molecular masses of 500–1500 and a curious alternation of D- and L- configurations of the participating aminoacids as well as a lack of ionizable groups. The structure of the K^+-valinomycin complex was elucidated in 1969 by Pinkerton [63]. In contrast, the neutral ionophores with lower molecular mass were classified as "carriers". Today, the selectivity of valinomycin for potassium ions still seems striking, and compares favorably with the properties of other ligands developed later.

After testing other naturally occurring antibiotics (macrotetrolides) with remarkable selectivities, Pedersen [66, 67] , Lehn [68, 69], and Cram [70] began to study synthetic ligands (crown compounds, synthetic macrocyclic polyethers, macrohetero–bicyclic ligands, cyclophanes and others). Cram [71] uses the term *host* for the synthetic compounds that are the counterparts of acceptor sites in biological chemistry, and the term *guest* for compounds that are the counterparts of substrates or inhibitors in the acceptor sites, according to Kyba [72]. Pedersen, Cram, and Lehn were awarded the Nobel prize for these achievements in 1987.

In the early development of the industrial electrode, organic ion exchangers were used in a solid configuration. Moody, Oke, and Thomas showed that incorporating the ligands into a plasticized PVC membrane prevents the membrane components from becoming fully hydrated and allows the active components to be sufficiently mobile [73]. This technique enabled ion-selective electrodes to become not just practically, but also commercially feasible. The influence of ion-selective complexing agents on the ion selectivity of liquid membranes was discussed theoretically by Eisenman's school [74, 75], by Covington [76], by Pungors' group [77] and by Sandblom [78] and Orme [79] and later by Buck [80] and by Wuhrmann, Morf and Simon [81,
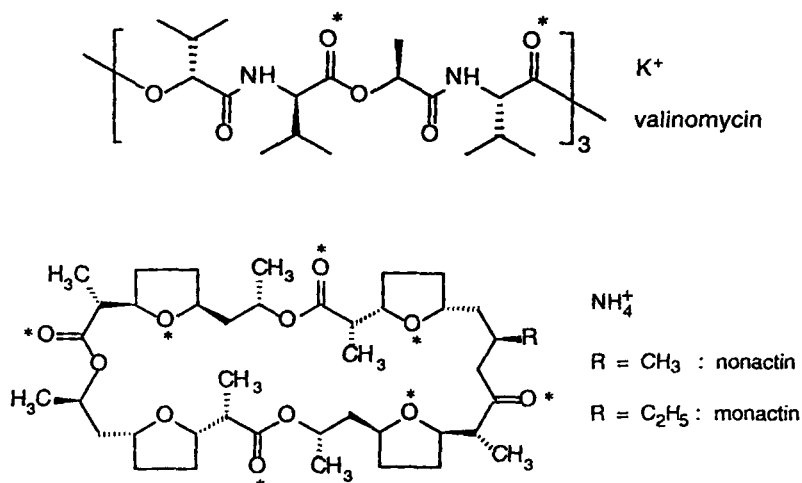
Figure 1-3. Constitution of valinomycin, as presented by [64], and the macrotetrolide antibiotics monactin and nonactin [65]. The ligand-cation complexes are positively charged. The alkali- or ammonium ions are complexed by 5–8 polar oxygen atoms. The conformation of the complex is characterized by an outer nonpolar shell and the polar groups oriented towards the center. Thus the molecule is mobile within a nonpolar membrane phase. O* = coordinating oxygen atoms

82]. Electrically neutral and electrically charged ligands were strongly debated. Eventually, charged ligands were shown not to work in nonpolar phases. The working priniciple and the functional distinction between charged and neutral ligands was accepted empirically rather than being rigurously defined and investigated. The complex formation was described for the interface between an aqueous phase and a relatively nonpolar membrane phase, where the selective transport of cations was to be expected.

More than 12 years later, the first optical potassium test, based on dry reagent chemistry was evaluated by the author, and commercialized subsequently by the Ames Division. The evaluation of silicone rubber membranes for the valinomycin electrode (see Figure 1-3) has led to extensive collaboration between the groups of E. Pungor and W. Simon, within the context of a friendship which has not prevented occasional decisive discussions of fundamental issues [83].

At the same time, the concept of the biosensor was proposed by L.C. Clark Jr. et al. in 1962 [84]. They measured pH, pCO_2, and pO_2 for intravascular continuous monitoring (see section 1.4.3). Also in 1962, Enson, Briscoe, Polanyi, and Cournand [85] introduced intravascular reflection oximetry. Bergman [86] described the first oxygen fluorosensor in 1968, which was introduced into medicine by Lübbers and Opitz in 1975 [87, 88]. At first it was thought that, unlike electrochemical sensors, optical sensors would not require a reference element. Satisfactory results were obtained by normalizing the optical signal of the analyte to a second reference wavelength, which involved evaluating relative intensity changes. Enson et al. [86] proposed the use of the isosbestic point as a reference.

Considerable progress in developing medical sensors has been made during the past few years [89–91]. In nearly all cases of optode design sensitive to the chemical properties of the analyte, the optical detection principle is based on either quenching of luminescence by oxygen or on a change in luminescence intensity due to pH alteration. Pulse oximetry, as well as some other new approaches, are exceptions [12, 92]. Different from other ions, pH is the unique parameter where the ion *activity* is measured and reported. Providing there is reliable calibration, pH assays are relatively unproblematic since no assumptions are necessary for calculating the concentration.

For pO_2 measurements, the sensors are considerably more rugged than those used in the evaluation of enzymatically generated oxygen or quenching compounds such as SO_2, halides, etc. [91, 92]. Thus, assays of most low-concentration analytes based on fluorescence quenching as well as pH evaluations demonstrate that these systems are too unreliable for direct monitoring in blood or in any biological sample. An element for selective recognition of the analyte, acting as a selective filter to take account of interfering entities, would ensure greater reliability.

An important landmark was the introduction by D.W. Lübbers et al. [92] of immobilized indicators into the development of optical sensors for continuous monitoring in biological fluids. They introduced the term "optrode" by analogy with "electrode". The term "optrode", however, is etymologically incorrect; *optode* [93] would be more appropriate.

Enumeration of all the scientists involved in developing combinations of sensing elements and transducers is beyond the scope of this study. Appendix 1 indicates some of the historic landmarks, whereas Table 1-3 shows some of the components and combinations that are possible in constructing chemical sensors and biosensors. Many of these have not yet been tested in real sensor configurations. Apart from the development of the recognition process, the transducers, actuators, or amplifiers have been improved considerably. In summary, the development of physical transducers is much more advanced than is that of the chemical recognition components as used in sensors [94]. At the Optical Meeting in The Hague 1991, the lack of innovation and development in this area was deplored.

1.4 Goals for Future Developments and Trends

1.4.1 Trends

One of the catchier titles in the program of the 1991 Pittsburgh Conference was that of I.J. Higgins et al.: *Biosensors: Philosopher's Stone or Fool's Gold?* The authors presented statistical data and forecasts regarding the sensor market.

Table 1-2 shows an extract from various reports on sales in the sensor market and their forecasts for the future [17, 95–98]. The first row shows figures taken from a report by Prognos AG (Basel, Switzerland) [17]. In 1988, the world market for sensors, physical and chemical, amounted to US$ 24.1 billion. Regional shares in the market reflect the strength of the sensor industries there: US$ 5.2 billion in both the United States and Western Europe, and US$ 3.0 billion in Japan [17]. A wide variety of application–specific demands means that there is a fairly

Table 1-2. Overview of current sales in US$ × 10^6 and forecasts of the sensor and biosensor market respecting different fields of applications (different references)

Year [reference]	Sensor class applications	Market	Sales figures US$ × 10^6	Predicted growth rate
1988 [17]	physical and chemical	worldwide	24100	by 1998
	chemical sensors	worldwide	1200	5–10%
				by 1993
1989 [95]	biosensors	US	13.6	69
	biosensors, processing	US	3.1	24.7
	environmental and security applications	US	1.9	11.3
				by 2004 *
1994 [96]	chemical sensors, (biosensors excluded)	worldwide	500	1380 (9–11%)
				by 2004 *
1994 [96]	biosensors	worldwide	400	1480 (10–15%)
	biosensors, medical	worldwide	ca. 200 (50%)	950
	processing	worldwide	75	250
	environmental	worldwide	25	75

* Projected in 1994 dollars (sales, contract research and development)

heterogeneous market for measuring parameters. According to the report cited, chemical parameters constituted only US$ 1.2 million of the total world market for sensors of US$ 24.1 billion in 1988. The report predicts an annual growth rate of 5–10% for the next ten years.

According to its forecasts, the United States will be the leading vendor and, maybe also consumer in the chemical sensor market by the year 2000 (US$ 14.5 billion), ahead of Western Europe (US$ 13.5 billion) and Japan (US$ 10 billion). This means that, relatively, Japan will have the highest growth rate. It is, however, to be expected that the regional market shares will change.

Another report [95] estimated that the growth of the United States biosensor market would be from only US$ 3.1 million in 1989 reaching US$ 69 million by 1993. This report foresaw the application with the fastest growth during this time to be the monitoring and control of

process industries. It suggested that the largest market segment in 1993 would be in health care (US$ 29.4 million), and that it would consist mainly of single–analyte instruments.

Depending on the source, the forecasts (Frost and Sullivan, Desjardin, Battelle, Market Intelligence Research) differ considerably. Whereas the figures for chemical sensors by Prognos AG include biosensors, the report by Taylor [96] clearly separates the two classes, so that the worldwide sales figures for chemical sensors and biosensors in 1994 have to be added together in order to compare them with the Prognos figures for 1988. Rather surprisingly, sales of chemical sensors and biosensors actually went down during the 6 years between 1988 and 1994, which might indicate some "cleaning" process in view of unsuccessful developments in chemical sensor technology. Notwithstanding this decline, a sharp upturn in the market, which may be entering the "take-off stage" [95], is predicted in both reports [95] and [96]. By way of comparison, *in vitro* diagnostics had a market of US$ 9 billion worldwide in 1992 [97]. 5% of this market consisted of consumer testing (US$ 450 million), 20% of which was dedicated to decentralized testing. Medical *in vivo* monitoring has, on the other hand, declined as it has met with ethical and legal barriers, and is now limited to a few applications.

Table 1-3. Possible components of a biosensor / chemical sensor characterized by selective molecular recognition and solubilization of target analytes

Selective elements	Transducers
synthetic ionophores	electrochemical:
synthetic carriers	– potentiometric
supramolecular structures, clusters	– amperometric
solid layers: metals	– conductimetric
– metal oxides, crystals	– voltammetric, polarographic
– polymers, conducting polymers	– impedimetric, capacitive
organisms	– piezoelectric
microorganisms	optical:
plant and animal tissues	– transmission / absorbance / reflection
cells	– dispersion, interferometric
organelles	– polarimetric
membranes, bilayers and monolayers	– circular dichroism, ellipsometry
enzymes	– scattering
receptors	– emission intensity, photon counting
antibodies	(luminescence) decay time
nucleic acids	calorimetric
natural organic and inorganic molecules	acoustic / gravimetric:
micelles, reversed micelles	– surface photo-acoustic wave
	– quartz microbalance