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Required Technology Breakthroughs to Assume Widely Accepted Biosensors

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ABSTRACT

Silicon microsensors have been very successful over the last decade in a wide variety of applications. Although commercialization of silicon-based biosensors has been slow, careful applications of microfabrication technologies to the development of biosensors will drive the formation of many new markets. The most promising high-volume, emerging markets include clinical analysis, health care, and environmental. For example, the worldwide sales of clinical sensors are expected to reach several hundreds of millions by 2000, whereas the total worldwide market for biosensors is forecast to reach $1 billion by the year 2000.

In this article, an overview of current and potential markets is presented with an emphasis on technological barriers to overcome before biosensors will become more widely accepted. We start by explaining the relative success of physical sensors compared to biosensors. Subsequently, we review several biosensor approaches and techniques and their associated problems. Finally, the markets that these sensors are meant to serve are analyzed.

Index Entries: Biosensors; microsensors.

INTRODUCTION

Physical-type silicon microsensors, such as pressure sensors and accelerometers, have been very successful over the last decade in a wide variety of applications. These micromachined devices are manufactured

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utilizing many of the same fabrication techniques employed in the semiconductor industry. Silicon microsensor technologies, which embrace batch processing techniques, exhibit some inherent features, including:

- High-volume production capabilities;
- Low manufacturing cost;
- High reliability;
- High sensor-to-sensor reproducibility;
- Small size.

Not only are silicon microsensor technologies being incorporated into new products, but they are also replacing the large, costly, and less reliable existing "old-fashioned" sensors in many applications. A clear example of the commercial success of these physical sensors is the micromachined pressure sensor, which can have a retail cost of approx $10 or less in large quantities of approximately 1 million/yr. General Motors is probably the world's largest manufacturer of silicon microsensors, producing about 750,000 sensors a month for detection of air pressure in engine intake manifolds. More recently, micromachined accelerometers have become available for approx $100, and one can expect the price to drop as the market matures.

To this day, however, there are only a handful of silicon-based biosensors on the market that take advantage of the inherent features of batch-processed silicon microsensors mentioned above. Commercial developments of silicon-based chemical microsensors have been slow because of the difficult environments in which sensors must operate, and because such issues as packaging, encapsulation, and biocompatibility must be appropriately addressed. However, the industry is starting to recognize that careful application of microfabrication technologies to the development of biosensors will drive the formation of many new markets and is beginning to feel the impact of these revolutionary technologies.

The fact that there always is a chemical/biological reaction taking place in a biosensor (as opposed to a mechanical sensor, where only a reversible mechanical phenomenon [force, flow, and so forth] is present) constitutes probably the most direct reason for the faster acceptance of physical sensors. Indeed, there is no requirement for the active sensing element in a mechanical sensor to be directly in contact with the outside environment, and the active element can be protected from the environment by proper packaging through which the physical property can be measured. Conversely, a chemical or biosensor must be in contact with the sample on a molecular level, requiring much more complex packaging designs and sensing elements that are not degraded or poisoned by the environment. Fortunately, since biosensors often can be disposable, irreversibilities stemming from chemical reactions need not be a barrier for commercialization.

Increasing demand for efficient and accurate chemical sensors or biosensing devices for use in bioprocessing, medical applications, and food processing will lead to explosive growth in biosensing. The world market for biosensors—small analytical devices that combine a transducer with a biologically sensitive material (such as enzymes, antibodies, or receptors) for the detection of biological substances—is forecast to reach $200 million by 1995 and $1 billion by 2000 (*1*). The three most important markets will be the United States, Europe, and Japan, in that order.

Table 1 describes some of the desired functional characteristics of biosensors. Table 2 is a comparison table of biosensor designs.

DIFFERENT TRANSDUCTION TECHNOLOGIES IN BIOSENSORS

The operating principle of the most common sensor technologies will be reviewed here.

Electrochemical Sensors

A majority of the chemical sensors studied so far are based on electrochemical techniques. These can be divided into four types based on their mode of measurement: potentiometric, amperometric, capacitive, and conductimetric. There are several features that make electrochemical techniques especially attractive. Since accuracy is achieved independently of color and turbidity of the solution, direct and rapid measurements are obtained without tedious sample preparation. Electrochemical sensors are easy-to-use analytical tools, and measure ions and neutral molecules in a range from 1 M to 1 μM. Coupled with chemical amplification, detection limits of pM have been attained. In addition, electrochemical sensors are inexpensive and do not require complex measurement equipment.

Potentiometric Sensors

Ion-Selective Electrodes (ISEs)

The broadest area of potentiometric sensor applications is in the utilization of ion-selective electrodes (ISEs). Ions in solution are quantified by measuring the change in voltage resulting from the distribution of ions between a sensing membrane and the solution. This potential is measured at zero current, with respect to a reference electrode whose potential should be independent of the sample composition. The potential measured is proportional to the logarithm of the analyte concentration.

Potentiometric sensors are used for the determination of ionic species. The oldest and the most well-known ISE is the pH sensor, which is based on a glass membrane. More recently, sensors for other ions of

Table 1
Comparison of Biosensor Functional Characteristics[a]

Feature	Requirement	Example/Comments
Selectivity	Ideally specific, no interferences	Ion-selective electrodes: range 10^{-1}–10^{-5} or better for competing ions Enzymes are selective for classes of chemical compounds Antibodies are generally highly selective Nonselective adsorption of organic materials onto surfaces coated with protein is a common problem
Sensitivity	Linear concentration response curve, detection of < 1% concentration change	Potentiometric systems limited in linearity in response governed by Nemstian equation and ionic strength Piezoelectric systems limited by equations of motion and energy transfer to the local environment Optical system limited by thickness and concentration rules of Beer's Law
Detection limit	Better than nanomolar for most biochemical analytes of interest	Potentiometric generally μM Piezoelectric, amperometric, and fluorescence systems may work reproducibly at this level
Reversibility	Recovery of full analytical response within seconds of cleanup cycle	Generally determined by chemistry, lower concentration of analyte usually leads to longer reverse times General reversibility: enzyme > molecular receptor > antibodies
Response time	99% maximum signal development within a few seconds	Related to analyte concentration and diffusion rates; generally 1–30 min for low analyte concentration
Size	Miniaturized laminar flow systems generally provide improvement of response times and reversibility	Piezoelectric devices approx cm^2 surface area Optical fibers approx 0.5 mm diameter and cm in length Field effect transistors few mm^2
Ruggedness	Insensitive to minor physical or electrical shock	Solid-state systems and covalently bound chemistry preferred
Reliability	Calibrated system with minimal to no drift, lifetime of months	Possible advantages for fiber-optic systems, which can be self-calibrating; all systems can be partially controlled by use of dual channel difference signals
Cost	Low cost for disposable or continuous widespread use	Possible advantages for lithographic technologies using semiconductor or piezoelectric devices
Signal recovery	Signal is reliable, easily transmitted, and free from interference	Possible advantages for fiber-optic devices, which are also useful for remote distributed sensor networks

[a] Adapted from U. J. Krull, (1990), *CHEMTECH*, June, 372.

Table 2
Comparison of Biosensor Designs[a]

Type	Measurand	Sensitivity[b]	Response, min	Stability	Comments
Enzyme	Electrical/ optical	ppt-ppn	1–10	Days to months	Difficult to fabricate consistently and economically, tend to become contaminated
Microbial	Electrical/ optical	ppt-ppn	5–60	Days to months	Same as enzyme
Immunosensor	Electrical/ optical	ppm-ppb	1–20	Days to months	High precision, but requires expensive transducers, multistep procedures, and has low reversibility
CHEMFET and ENFET	Electrical	ppm	1–10	Days	Developmental; difficult to fabricate consistently
Interdigitated chemiresistor	Electrical	ppm-ppb	0.1–5	Months	Easy to fabricate; not specific
Surface acoustic wave	Acoustical	ppm-ppb	0.5–5	Days to months	Developmental; potential high sensitivity and stability
Piezoelectric	Mass balance	ppt-ppn	5–20	Hours to days	Experimental
Enzyme thermistor	Thermal	ppt-ppn	5–20	Days	Temperature insulation problems
Spectroscopic	Includes absorption, scattering, refractive index, polarity, and interference	ppm-ppb	1–20	Days to months	Rugged and stable, needs better immobilization procedures

[a] Adapted from Author D. Little, Inc., Research Report on Biosensors, 1989.
[b] ppt, parts per thousand; ppm, parts per million; ppb, parts per billion.

biological interest have been developed using membranes formed by incorporating different ionophores into polymeric membranes. The potentiometric sensing principle also has been employed for direct detection of antigens or antibodies, and of several drugs.

Potentiometric sensors possess a wide measurement range for several ionic species. It is possible to miniaturize the sensors for in vivo use. Disadvantages include poor detection limits for some applications, the need for a stable reference electrode, and in some cases, poor selectivity.

Chemically Sensing Field-Effect Transistors (CHEMFETs)

CHEMFETs are a logical extension of ISEs. They can be conceptualized by imagining that the lead from an ion-selective membrane, which is attached to a field-effect transistor in the potentiomenter, is simply made shorter and shorter until the lead no longer exists and the membrane is attached directly to the FET, resulting, as it were, in a situation where one places the "instrument" in solution.

Since the sensing mechanism in CHEMFETs is the same as in ISEs, the type of analytes that can be determined by both techniques is the same. The advantages of CHEMFETs are in the dimensional precision obtainable by fine-line photolithography, on-chip signal processing, the ability to place multiple sensors on the same chip, and possible cost savings realized by batch fabrication and calibration.

The development of FET-based chemical sensors, which seemed very promising in the early 1970s, has been plagued by a tremendous number of technological and fundamental problems, such as low reproducibility of performance, signal drift, and degradation of the sensor with time (3). The main technical obstacle to commercialization of ISFETs-based chemical sensors is the problem of *insulation* (encapsulation). The sensing part of the chip should come in contact with the analyte solution, and at the same time, the rest of the electronics should be well protected from the same. This difficulty has held back the promise for this specific form of chemical sensors.

One of the few examples of commercial products based on ISFETs is a hand-held pH meter (pH BOY-C1) developed and introduced in the Japanese market in 1990 by Shindengen Electric Mfg. Co., Ltd. The pH BOY-C1 has a pen-shaped pH electrode part that has an ISFET-based pH sensor that requires only a few microliters of sample and that is claimed to be almost "drift-free" through the use of a specially developed gate material based on tantalum oxide and a careful encapsulation design (4). In general, however, FET-based chemical sensors are presently not well suited for long-term, in-line chemical analysis, and their development is mainly geared toward disposable-type biomedical sensors. In the case of in vivo use, biocompatibility is still a major difficulty.

POTENTIOMETRIC GAS SENSORS

These sensors consist of an ISE covered with a hydrophobic membrane with a thin film of an electrolyte sandwiched between them. When the gas diffuses through the membrane and changes the activity of the ion to which the ISE is selective, the sensor is able to measure the gas concentration indirectly.

Sensors for CO_2, NH_3, SO_2, HCN, and so on, can be designed based on this principle. However, many of these species are mutually interfering. Some selectivity can be obtained by varying the ISE used in the sensor, the internal electrolyte, and the hydrophobic membrane.

Amperometric Sensors

In the amperometric technique, a fixed voltage is applied (by means of a potentiostat) to a working electrode that induces an electrochemical reaction (oxidation or reduction) of the desired analyte. This reaction produces an electrical current that is proportional in magnitude to the concentration of the analyte. Analytical information is obtained from this current-concentration relationship.

Amperometric sensors have inherently better detection limits than potentiometric methods because of the zero background found in amperometric measurements. Also, amperometric measurement can be used for a wide spectrum of analytes, in contrast to potentiometric methods, which are suitable only for ionic species. By far, the most well-known amperometric sensor is the widely used oxygen sensor called "the Clark electrode."

For many years, the emphasis in silicon microsensor research and development has been on potentiometric-based devices, such as ISFET-based chemical sensors. However, the emphasis has recently been shifting toward amperometric-based devices, in which the response curve is ultimately one of current vs concentration. In general, amperometric processes involve a heterogeneous electron transfer as a result of the oxidation/reduction of an electroactive species at the electrode surface. The advantage of amperometric techniques over potentiometric methods include:

- Amperometric measurements offer inherent improvement in the limits of detection (1–100 nmol/L) for direct determinations
- Stability of the reference potential is not critical in the case of amperometric measurements
- Planar microelectrode arrays suitable for multispecies sensing can be made inexpensively using microelectronic fabrication technology
- Instrumentation is simple. With downscaling amperometric sensors comprising microsized electrodes by applying microelectronic manufacturing techniques, amperometric devices

offer several distinct advantages over potentiometric devices, including;

- Shorter response time;
- Arrays without crosstalk;
- Improved signal-to-noise ratio;
- Permission of measurements in highly resistive media;
- Expanded stability window;
- Permission of study of faster kinetics;
- High collection efficiencies.

As an extreme case of downscaling, it is possible to detect amperometrically airborne chemicals directly in air without needing an electrolytic medium in a device made up of closely spaced metal electrodes (*5*). In this setup, the electric field is present over such a small gap—< 1 μm—that the mean free path for ionized gas molecules is larger than the electrode spacing; hence, electric breakdown resulting from ionization does not occur.

Because the magnitude of the measured currents depends on the physical dimensions of the electrodes (unlike potentiometric devices, which are size independent), amperometric devices require high-quality manufacturing control sufficient to ensure the required reproducibility from sensor to sensor, making fabrication difficult in general. Miniaturization of amperometric sensors can lead to an important additional benefit not found with miniaturized potentiometric devices, e.g., increased sensitivity over macrosized sensors because of the analytical advantages of microelectrodes.

Conductimetric Sensors

In conductimetric sensors, analytic information is obtained from the relationship between change of resistance and concentration. The selectivity of conductimetric sensors is not good, because the change in concentration of any ions will change the conductivity. Conductimetric sensors will not be discussed any further.

Capacitance Sensors

When an electrode is placed in a solution, there is always a potential difference between the electrode and the solution of ion distribution at the interface. There is usually a large capacitance associated with the electrode-solution interface. The change in composition of the solution or adsorption of some material on the electrode can change the capacitance. By measuring this capacitance, the composition of the sample can be determined. Like conductimetric sensors, the selectivity of this type of sensor is poor.

Optical Sensors

Fiber-optic sensors combine the recent advances in optics and optoelectronics with well-established analytical techniques, such as absorptimetry, fluorescence, luminescence, and so on. The main disadvantage of the above mentioned techniques has been that they are all based on heavy and expensive instrumentation, and that these instruments could not be exposed to conditions outside the benign environment of an analytical laboratory. The solution to this problem is optical sensing, where chemicals reactive to the analyte of interest are immobilized at the tip of a fiberoptic waveguide, and the guide is used to transport optical signal from the sample to the meter where signals are monitored.

Optical techniques have some advantages and disadvantages when compared to electrochemical methods. The advantages are:

Because the signal is optical, there is no electrical interference.
Optical sensors can be developed for analytes for which electrochemical techniques are not available.
Sensors can be developed that respond to two or more analytes by multiwavelength measurements.
In some cases, the sensor can be self-calibrated, which is a great advantage for continuous monitoring.

The disadvantages are:

Optical sensors have typically limited dynamic range compared to electrochemical counterparts.
Sensors with immobilized dyes have limited shelf life because of poor stability of these dyes, and limited long-term stability because of photobleaching or wash-out.
Commercial accessories of the optical system are optimized for telecommunications, not for sensor applications, and are expensive.

What follows describes two more recent popular optical sensing principles.

Evanescent Wave Spectroscopy

When a light beam strikes the interface between two transparent media coming from the optically denser medium $n_1 > n_2$, total internal reflection occurs when the angle of reflection is larger than the critical angle Θ_c.

$$\Theta_c = sin^{-1}(n_2/n_1) \quad (1)$$

In this case, an electromagnetic component of the light penetrates a characteristic distance of the order of a wavelength beyond the reflecting surface into the less optically dense medium. The electric field intensity in the lesser medium is called an evanescent wave, and the volume in which the exponential decay occurs is often called the evanescent volume.

Because of the short penetration depth and the exponential decay of the intensity, the incident evanescent wave is absorbed mainly by compounds close to (or affixed to) the interface and minimally by bulk solution. Since the effect from constituents further away from the surface is only minimal, reactions at a surface can be measured in the presence of a bulk solution. Evanescent wave techniques can be used in sensors, especially if the medium of high refractive index is the surface of an optic fiber, and the medium of low refractive index is the sample to be characterized. One of the main applications of evanescent wave is in immunoassays, where surface-bound chemicals have to be distinguished from those in the bulk.

Surface Plasmon Resonance (SPR)

Instead of a dielectric/dielectric interface used in the case of evanescent wave sensors, it is possible to arrange a dielectric/metal/dielectric sandwich such that when light strikes the metal surface, a wave is excited within the plasma formed by the conduction electrons of the metal. A resonance between the incident light and the plasma wave is created, depending on the angle, wavelength, and polarization state of the incident light, the refractive index of the metal film and the refractive indices of the materials on either side of the metal film. This phenomenon is called surface plasmon resonance and can be used for sensitive measurement of variations in refractive index of the medium immediately surrounding the metal film if all other parameters are kept constant. The technique has been used for the analysis of immunochemicals and for the detection of gases. The main limitation of SPR is that the sensitivity depends on the optical thickness of the adsorbed layer, and hence, small molecules cannot be measured in low concentrations.

Mass Sensitive Devices

Piezoelectric devices are based on specially cut crystals that mechanically oscillate when subjected to an alternating electrical potential. Deposition of a thin film (of any species of interest) on the crystal will decrease the frequency of this vibration in proportion to the mass of the film. This phenomenon can be utilized for chemical analysis. In short, this is a mass sensor. Most of these sensors are based on quartz crystals and are called quartz microbalances.

The main advantages with quartz microbalances are the high sensitivity, simplicity, reliability, and the frequency output. Selectivity is obtained from coating used.

Another type of sensor that resembles a quartz microbalance is the surface acoustic wave sensor or SAW sensor. A SAW device is a transmission line where an acoustic wave is generated piezoelectrically in one oscillator, propagated along the surface of the SAW substrate, and transformed back to electrical energy in the receiving oscillator. Analytical

information is obtained from the interaction of the sample with the traveling wave. The advantage of SAW over a quartz microbalance is that in the former only the surface of the acoustic waveguide is affected by the sensing process. Also, it can be made by depositing a piezoelectric film on a substrate, such as silicon, and hence, small sensors with on-chip electronics could be fabricated.

Sensors for gases have been made based on the SAW principle. However, they cannot be used for accurate measurements in liquid phase because of problems associated with the leaky propagation of waves in liquid media. Alternative technologies for sensing in liquid media have been proposed based on other types of elastic waves.

Chemical Amplification and Selectivity

For both chemical amplification and selectivity, only one representative example is given here. Enzymes are an example for amplification and selectivity at the same time. Antigen/antibody-based sensors are the best example of how to induce selectivity.

Enzyme Sensors

Nature provides us with one of the best examples of chemical amplification. It is the usage of the catalytic effect of enzymes. An enzyme sensor is fabricated by combining a thin layer of enzyme immobilized in close proximity to the active surface of any of the conventional sensors described above. The enzyme catalyzes the breakdown of the analyte of interest, and the sensor underneath monitors the progress of the reaction by measuring a reactant or product of the enzyme reaction. The output of the sensor can then be correlated to the concentration of the analyte.

The use of enzymes is based on their unsurpassed catalytic activity, exceeding nonbiological catalysts 10^8–10^{13}-fold, and their clear selectivity. Enzymes are capable of producing hundreds of thousands of molecules per second. Thus, during the course of analyte recognition, rapid product multiplication occurs, and detection of even very small quantities is possible.

Immobilized enzyme sensors facilitate analyte determination using small volumes and enable the recovery of enzyme for repeated use. The usefulness of immobilized enzyme electrodes is affected by several factors, such as the chemical and physical conditions of use, the stability of the enzyme layer, the stability of the base sensor, the storage conditions, and so forth.

At this time, about 3000 enzymes have been identified, and about 200 of them are commercially available. However, enzyme electrodes are made only for about 80 analytes. Among these, amperometric enzyme electrodes are the only ones that have achieved a measure of commercial success. A lactate analyzer was launched by Roche in 1976. The most celebrated among enzyme sensors is the glucose sensor, developed by

Clark and marketed by Yellow Springs Instruments. In 1987, a new concept in biosensor design was introduced by MediSense. This device, a pen-sized glucose sensor, may be considered as a second-generation amperometric enzyme sensor.

Immunosensors

Immunosensors are another class of sensors used for the analysis of biochemicals. These are based on antigen-antibody reactions. Antibodies are part of the immune system of living things and, in contrast to the enzymes, show no catalytic activity, but exhibit even greater specificity in binding. Antibodies are very diverse, and an antibody with unique specificity could be produced for almost any biochemical species of interest. The antigen-antibody binding constant is usually of the order of 10^9–10^{12}. This high specificity together with the high-binding constant allows selective determination of very small quantities of materials in the presence of a high concentration of interfering substances.

There are two types of immunosensors: direct and indirect sensors. Direct sensors convert the binding of antigen to antibody directly into a measurable signal. They consist of an antigen or antibody immobilized on a surface. The antibody-antigen binding will result in the change of some measurable property, such as potential, capacitance, or mass. These sensors include potentiometric, capacitive, piezoelectric, and surface plasmon resonance sensors. These sensors have in common their fundamental nonspecificity. Any nonspecific adsorption also will give rise to a signal, thus interfering with the measurement.

Indirect sensors are not sensors in the strict sense, because they involve a number of manual manipulations, including several washing steps and addition of reagents. For example, in one configuration, a limited amount of antibody immobilized on the transducing surface is used. The measurement is started by mixing the sample (which has the analyte of interest or what is called the antigen) with a known concentration of antigen that has previously been labeled. The labeled antigen competes with the sample antigen for the limited amount of antibody sites. Once the equilibrium is reached, the amount of labeled antigen bound to the antibody is inversely proportional to the amount of antigen in the sample. Before determining the amount of labeled antigen, any unbound labels have to be washed off completely, since they can lead to false readings. Consequently, most of the immunosensors developed to date do not eliminate operator dependence. However, they offer selectivity and sensitivity.

A wide variety of chemicals have been used as labels, although the most commonly used ones are enzymes. Enzyme labeling provides good sensitivity because of the amplification resulting from the enzyme reaction (*see* previous section).

TECHNOLOGICAL ISSUES

Biosensors can revolutionize the way in which analytical information is retrieved. However, in spite of two decades of research and commercial interest, only limited success has been obtained so far. One reason for this lack of commercial success is that sensors functioning well in the laboratory may not meet the needs of the marketplace. Even in the case of those that met the requirements, transfer of technology from laboratory to production was often much slower than expected. Also as indicated in the introduction, the use of a biosensor by definition involves a chemical/biological reaction often inducing an irreversible change in the sensor. For an on-line sensor, this can pose a serious problem. But for a disposable, one-time use sensor, this is often not an impediment to successful operation.

The requirements of a biosensor (*see* Table 1) are obvious, even though the order in which they are ranked can vary from application to application. They should be sensitive, accurate, reproducible, stable, selective, inexpensive, and user friendly. In addition, size, sterilizability, and biocompatibility may be important for certain applications. The important analytical characteristics of biosensors are described below.

Dynamic Range

The measuring range of the sensor has to be adapted to the respective analyte concentration. For example, in blood or fermentation systems, the concentration of metabolites like glucose, lactate, and so on, exceeds the upper limit of the calibration curve of the enzyme electrodes. Fortunately, this problem can be reduced by limiting the amount of substrate that diffuses to the electrode. On the other hand, the concentration of hormones, drugs, and toxins is sometimes below the detection limit of enzyme electrodes, and there is no simple method to amplify the signal further. Immunosensors should be able to tackle this problem, since they have the capability to measure down to picomolar concentration. However, no simple, user-friendly immunosensor has yet been developed.

Selectivity

Selectivity is another major problem that has hindered the commercialization of sensors. In the case of biosensors, selectivity is greatly enhanced by the use of biochemicals, such as enzymes. However, interference can be caused by alternative substrates or inhibitors, that may cause problems in practical applications. Selectivity can also be lost at the

level of the transduction reaction following the enzyme reaction. For example, reducing substances can be cooxidized along with enzyme reaction products and can cause interference. This has been the major problem in commercializing a disposable, electrochemical glucose sensor. The problem was solved by using mediators, that are oxidized at a lower potential than the interferences.

Reliability

Reliability is another major issue. Once the sensor is installed, periodical calibration is very difficult or cumbersome, and virtually drift-free operation is especially necessary for continuous measurements. However, drift can be caused by different factors. For example, chemical changes at the transducer surface can cause drift. Also, degradation or leaching of the reagents can lead to drift. Improvements have been made in this area by understanding more about the reasons for drift and modifying the transducer surface, using more stable reagents, and using better immobilization schemes. More improvements are necessary in this area, however.

Versatility

For a sensor to be commercially successful, it should be versatile. In order for the sensor to be widely used, it should measure any species necessary. The marketplace will not be interested in replacing presently installed instruments, if only a few analytes can be measured with sensors, and conventional instrumental methods have to be adopted for the remainder. An example is ion-selective electrodes for blood electrolytes, like H^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Li^+, Cl^-, and HCO_3^-. In the case of H^+, K^+, Na^+, Ca^{2+}, and Cl^-, ion-selective electrodes have completely displaced flame photometry, atomic absorption spectroscopy, and coulometry. However, for ions, such as Li^+, Mg^{2+}, HCO_3^-, and so on, alternative techniques still have to be used. Considerable progress has been made in making newer and improved sensors, but more work is needed. Even though everybody sees advantage in using sensors, their development has been a chicken-or-egg type of problem. Until there are sensors ready for many more analytes than are currently available, existing analytical techniques cannot be replaced, and until there are more signs of sensors completely replacing large analytical instruments, manufacturers will not be willing to fund sensor development on a large scale.

Size

Size is a problem at least in medical applications, especially for in vivo applications. The sensor must be small enough to fit in a certain location or to make measurements using small sample volumes. Size is normally

not an issue for industrial applications. These sensors can be bulky structures with a built-in protective housing.

Biocompatibility

Biocompatibility is a major issue for several potential applications of sensors. The sensor must come into contact with the sample, which leads to fouling of the surface. This fouling is common to any artificial material coming into contact with a biological matrix. Fouling can lead to drift and final death of the sensor. Sensors in contact with fermentation media or food are also subject to biofouling. Substantial progress has been made in dealing with this problem, and sensors have been implanted in living animals for several months without much deterioration in sensor performance.

Sterilization

Sterilization issues must also be addressed before sensors can be used for many applications. Typical sterilization procedures involve autoclaving, γ radiation, or ethylene oxide treatment. The effects of ethylene oxide residues on the chemical selectivity and sensitivity of the sensor should be determined. γ radiation could alter the materials used in the fabrication of sensors, changing their characteristics. Several components of the sensor, such as heat-sensitive enzymes, may not withstand conventional steam-sterilization.

Packaging

Sealing and packaging of sensors is an area where insufficient work has been done. The packaging keeps all unwanted elements, such as water, dust particles, or fouling materials, from coming into contact with the sensor, or adjacent metallic or electronic components. Packaging is especially important for sensors to be used in liquid environments. In this case, the sensing component should come into contact with the liquid, and at the same time, the electronics and leads should be protected from water and even humidity. This has been the main failure mode of the much-heralded CHEMFETs. So, it is clear that the success or failure of a sensor can depend on good sealing and packaging.

Cost

Cost could be another issue that will delay the widespread introduction of sensors. Most of the sensors, with the exception of some recently introduced ones, are individually made and calibrated. So, the cost per sensor has been relatively high. It is necessary to use batch fabrication processes to bring down the cost and to improve the quality. However, most of the processes used in semiconductor fabrication are not directly

transferable to sensor fabrication; new materials are used, and the performance requirements of different components are different. Sensor packaging is the best example of this difference. Nobody would think of exposing a transistor to humidity, let alone putting it into a solution. However, sensors must be placed directly in the measurement environment. So, each packaging technology has to be reworked for sensors. The same differences are found in the fabrication of the sensors themselves. Many materials that are nonstandard and foreign to the IC industry are used in sensor fabrication. Although the same batch fabrication techniques can be used, alteration and modification to these techniques are necessary in order to manufacture sensors with the same high degree of reproducibility. Several researchers are working in this area, and considerable progress has already been made.

COMMERCIAL PERSPECTIVES

Health Care

In terms of market size, health care is the largest area of application for sensors and has been the largest area for advanced sensor research. The size of the market, and the industry's willingness to use new technologies and products may be the reason for such a large effort in this area.

The largest applications of chemical sensors in health care are in clinical chemistry. The largest sensor market will be for clinical analysis of samples for blood gases (oxygen and carbon dioxide) and pH, glucose, potassium, sodium, calcium, chloride, bicarbonates, urea, creatinine, bilirubin, and cholesterol. Currently, the blood monitor market is $400 million in the United States alone. Systems for this market today are generally bench-top analytical devices. A blood sample is drawn from the patient and transported from the bedside to a laboratory for analysis. The use of biosensors in clinical testing at a patient's bedside or in a physician's office instead of at centralized laboratories allows therapeutic decisions to be made rapidly and thus improve patient care. Many of the major medical laboratory suppliers are developing smaller bench-top general chemistry analyzers designed for use in the physician's office. Eastman Kodak (Rochester, NY) is developing a compact, but complex health maintenance facility for NASA to be used on the space station, and this device would also fill this niche. The fully automated Kodak Ektachem 400 system has been on the market for some time and performs 10 different assays in seconds. The SERALYZER from Ames Division of Miles Laboratories, the REFLOTRON from Boehringer Mannheim, the ANALYST from DuPont, and other similar instruments cover a wide range of blood chemistries for the physician's office.

Two technologies have driven the development of these smaller instruments. The first is the development of solid-state stabilized reagents. Although quantitative test strips used in clinical chemistry have been available since the 19th century in the form of litmus paper, recent advances in thin-film technology and dry reagent chemistry have allowed automation to increase the precision and capacity to perform semiquantitative analysis on many clinically important compounds and ions. Dry reagent chemistries are presently available for over 30 biological compounds. Biosensors for vital functions, metabolites, therapeutic drugs, and anesthetic levels are being developed for continuous monitoring during critical care. The second major development is that of microsensors and microelectronics. The advanced fabrication techniques used in the electronics industry has enabled the development of a new generation of small instruments. These microsensors, with accompanying electronics, can be made to the dimensions of a human hair and at a fraction of the cost of conventional instruments. This allows them to be massively deployed for redundant sensing and even disposed of after one-time use.

The large analytical instruments currently used in clinical laboratories require transport of the sample to the laboratory, calibration of the instrument, and sample preparation, which often result in critical time delays before results can be delivered to the physician. In vitro diagnostics is undergoing a transition from these centralized laboratories to an alternate site testing (AST), for example, bedside, physician office, and home testing. As reliable, disposable sensors appear, clinical laboratory analysis will be moved to these alternative testing sites. The requirements for the latter are different from that of centralized testing. AST demands simple, reliable, and inexpensive instrumentation. The instrument should be small and capable of analyzing a set of parameters with a drop of sample without any processing of sample or addition or reagents.

In addition to decentralizing clinical analysis as a whole, sensors will also enable continuous monitoring of blood gases, electrolytes, hormones, drugs, and so on, which is not possible by instrumental methods of analysis. The market for sensor devices that monitor patients continuously is likely to grow as sensor technology breakthroughs occur and as potential users become aware of the benefits the new technology can offer.

Another area where biosensors could make a major impact is in immunoassay. Immunoassays are used as diagnostic tools to determine the presence of bacteria, hormones, cancer agents, poisons, and so forth. The fertility and pregnancy test kits that can be found in drugstores and supermarkets are examples of this technology. Again, the sensors should meet the requirements for the AST market. For the sensors that have been developed so far, ease of use is inadequate. The need for a wash step to separate bound labeled antigen from free ones introduces a source of error and operator dependence. New technologies, combined with microfabrication techniques, are trying to address these problems.

Several companies are developing the next generation, hand-held size, portable instrument for bedside analysis to produce a printed record of test results from a drop of blood depending on the panel of tests desired. Each panel will contain the most common tests for a specific organ or disease state. Examples would be a panel for general chemistry, hepatic condition, hematology, renal condition, protein, lipid, or immunoassay. Each panel would include a range of tests, such as uric acid, calcium, phenolsulfonphthalein, creatinine, blood urea nitrogen, and glucose for the renal panel. The GEM-PREMIER blood gas/electrolyte system is available from Mallinckrodt Sensor Systems. Each panel is incorporated in a GEM Premier Pak that incorporates established electrochemical sensor technology that has been miniaturized and packaged into a disposable cartridge. Panels can be purchased to measure pO2, pCO2, pH, sodium, potassium, calcium, and hematacrit. PPG's STATPal is a similar instrument. The I-STAT Portable Clinical Analyzer from I-STAT Corp. is a hand-held system that can perform simultaneous assays on whole blood. It provides results on sodium, potassium, chloride, urea nitrogen, glucose, and hematocrit in <2 min. The ACCUMETER cholesterol test from ChemTrak is designed for a lipid screen to evaluate cardiovascular disease risk. ABAXIS's MiniLab MCA will be a general chemistry analyzer that uses electrochemical sensors with a novel fluid handling scheme using a centrifugal analyzer, but this system is not currently on the market. Although all of the above instruments are based on electrochemical detection, new optical immunoassay techniques are being tested by BioStar Medical Products in a silica test strip format. A change in color of the silica strip occurs when the antibody of interest binds in a layer resulting in interference of the reflected light. Semiquantitative tests for determining the presence of bacteria, poisons, hormones, and other biochemicals can be determined by this immunoassay approach.

Home health care technology has been available since the 1960s with the development of semiquantitative glucose, urine, and pregnancy test strips for consumer use. Patient self-testing was greatly accelerated in the 1980s with the introduction of electronic glucose monitors for diabetics. As a current example, the ExacTech Blood Glucose Monitoring System by MediSense is the size of a pen with disposable printed carbon electrodes on polyvinyl strips. It is calibrated for use with capillary blood and provides results on a liquid crystal display in 20 s with an assay range of 40–450 mg/dL. Other glucose instruments, such as LifeScan from Johnson & Johnson and Answer from Wampole Laboratories, use optical reflectance meters and dry chemical test strips. Both types have vastly improved the management of diabetes, and allow accurate test results with little training and no laboratory skills. Other biosensors that are being investigated for measurement in this format are lactate, pyruvate, urea, lactose, galactose, alcohols, and L-amino acids.

Miniature, implantable drug delivery systems will not reach their full promise until biosensors with long lifetimes are available for closed-loop feedback to help regulate drug delivery rates. As has been the case in biosensor development to date, glucose has received the most attention. Advances in closed-loop insulin infusion or artificial pancreas will require a miniature implantable glucose sneosr. A number of different catheter types have been tested that constantly monitor blood and tissue glucose concentrations, so as to help regulate insulin flow rates for maintaining sugar levels after implantation. Unfortunately, long lifetimes have not been estabilished because of biocompatible materials that have not yet lived up to their considerable potential. The complexities of developing a sensor to work in the environment of the human body are enormous, and there are no simple criteria that define a material as biocompatible, since biocompatibility will vary depending on the function and location in the body.

Biosensors in Food Industry

Applications of sensors within the food industry have been traditionally limited to physical variables, such as temperature, pressure, flow rate, viscosity, color, and moisture. It is clear that new types of sensors, especially those that measure chemical and biological substances, are desired. Opportunities of chemical sensors in the food and beverage industry will be extremely diverse, ranging from detection of freshness in fish to detection of sulfur dioxide, which is used as a preservative in many foods and in beverages, such as wine. For example, a microsensor that can detect biological contaminants in food would be highly desirable as an alternative to the tedious laboratory analyses currently employed. Salmonella testing, which still takes days conventionally, is one of the most frequently performed assays with an estimated 5 million tests anually ($40 million to $50 million per year). The US market for biosensors for bioprocess control in the food industry is expected to grow from about $62 million today to over $180 million by 2000 (2).

New Trends and Opportunities

Combining solid-state chemical sensors with advanced microelectronics and computer technology results in advanced microchemical systems that have the intelligence and the ability to interact with their surroundings. The benefits will be seen in considerably improved accuracy and reliability at reduced cost for existing applications, and in the ability to instrument entirely new areas in emerging industries.

The advent of the new generation of chemical sensors will be exceedingly helpful in any process that requires rapid, *in situ* identification and monitoring of chemical or biological species. The most promising high-volume emerging markets for advanced microchemical systems in

the coming decade include environmental monitoring and control (HVAC), health care, industrial process control, and advanced automotive combustion control systems.

A report published by Frost & Sullivan titled "The U.S. Market for Smart Sensors," states that although the biosensor industry is small at the moment, commercially speaking, it is expected to grow 45%/y over the 1990–1995 period. The promise is the conversion of much of the $54 billion a year medical laboratory service business into point-of-care analysis, with the addition of several new markets not yet available. The enthusiasm for the technology is rampant; unfortunately, the consistency and accuracy required by the clinician are not yet available in most sensor types. This leaves a large market open to those innovators and venture capitalists who can accept the risk in view of the growth potential.

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