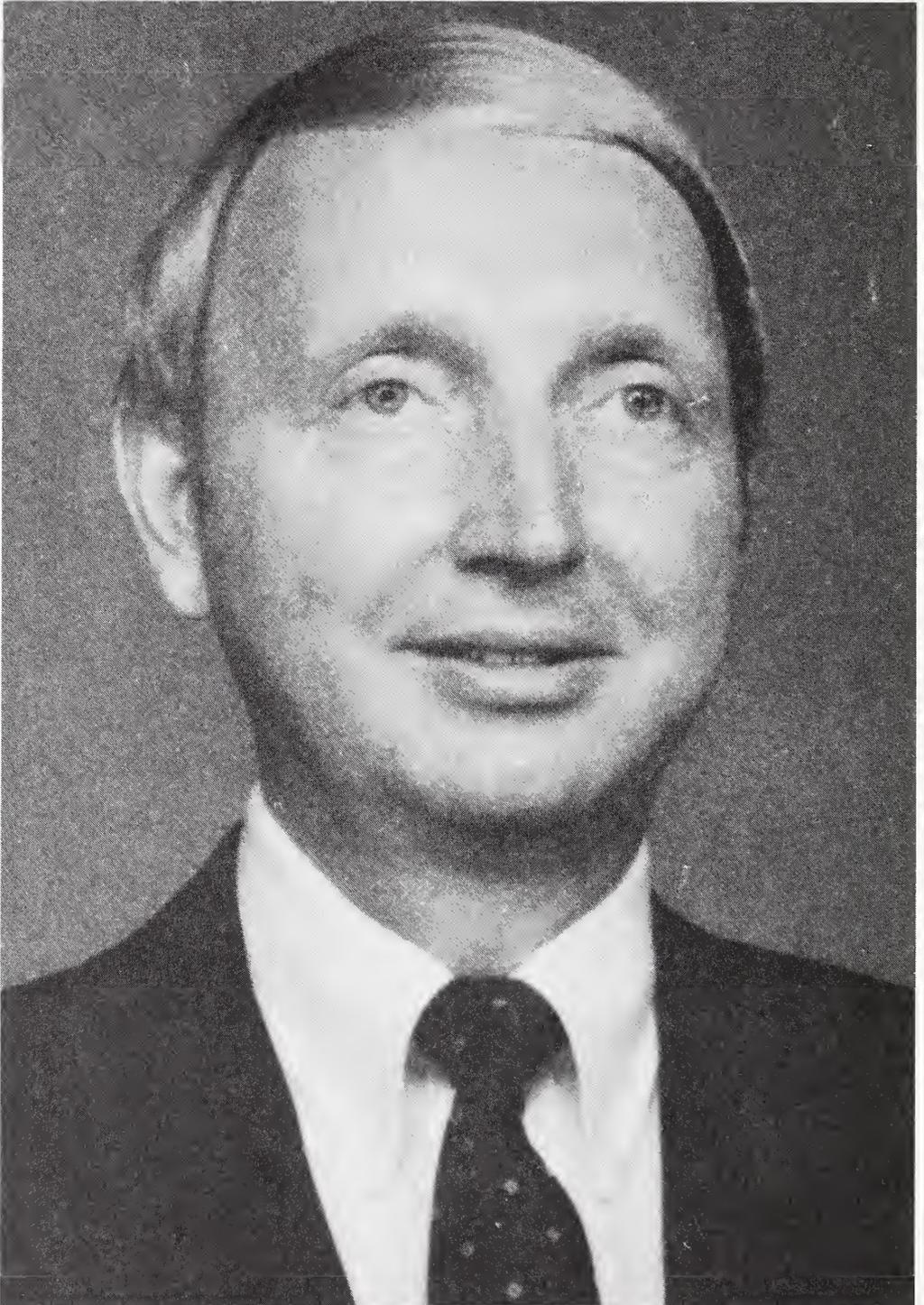


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PRESIDENTIAL ADDRESS

Biosensors: Recent Developments and Concerns

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Recent developments in biosensor technology have been hailed as the “marriage of the century—the marriage of biotechnology and microelectronics” (5). These developments, along with other advances in the field, have stimulated expectations that significant capabilities for monitoring and controlling an increasing number of biological and chemical species will be commercially available in the near future. My purpose in this talk will be to very briefly mention some of the important developments of biosensor technology which have occurred in the last twenty years and then focus on some of the very recent developments and possible future directions for this important emerging technology.

In order to appreciate the capabilities offered by some of the most recent advances, we need first to discuss some of the general classes and operation of biosensors. Biosensors can be classified in two general categories, devices which sense biological molecules and devices which use biological molecules as part of the sensing mechanism.

Biosensor technology is an outgrowth of ion selective electrode technology. One of the first and most commonly used ion selective electrodes is the glass electrode used for pH measurements. The first ion selective electrodes were very similar in size and use to the glass electrode. Similarly, the first biosensors were similar in size and use to ion selective electrodes—except that they either used or sensed biological or organic molecules rather than inorganic ionic species. For purposes of our discussion, I will categorize these biosensors as representing a “conventional” approach in contrast to some of the more recent devices which employ solid state microcircuits and/or fiber optics and which I will classify as the “microtechnology” approach.

Biosensors can be grouped into six major kinds:

1. Enzyme sensors
2. Microorganism sensors
3. Hybrid sensors
4. CHEMFETS/ISFETS
5. Multisensors
6. Optrodes

The first three of these are in the conventional category and the last three are in the microtechnology category.

Figure 1 shows a conventional biosensor in generalized form. The supporting membranes are normally porous or selectively permeable. The bioactive substance sandwiched between the supporting membranes can take any one of a number of different forms. Typical forms include an enzyme immobilized on a polymer substrate, a specific chemical compound, a microorganism immobilized on an appropriate substrate or even a thin slice of plant or animal tissue which contains the desired enzyme or microorganism. The specific bioactive substance determines the type of conventional biosensor. Frequently, the biosensor contains a conventional gas sensing electrode above the upper supporting membrane.

GENERALIZED BIOSENSOR

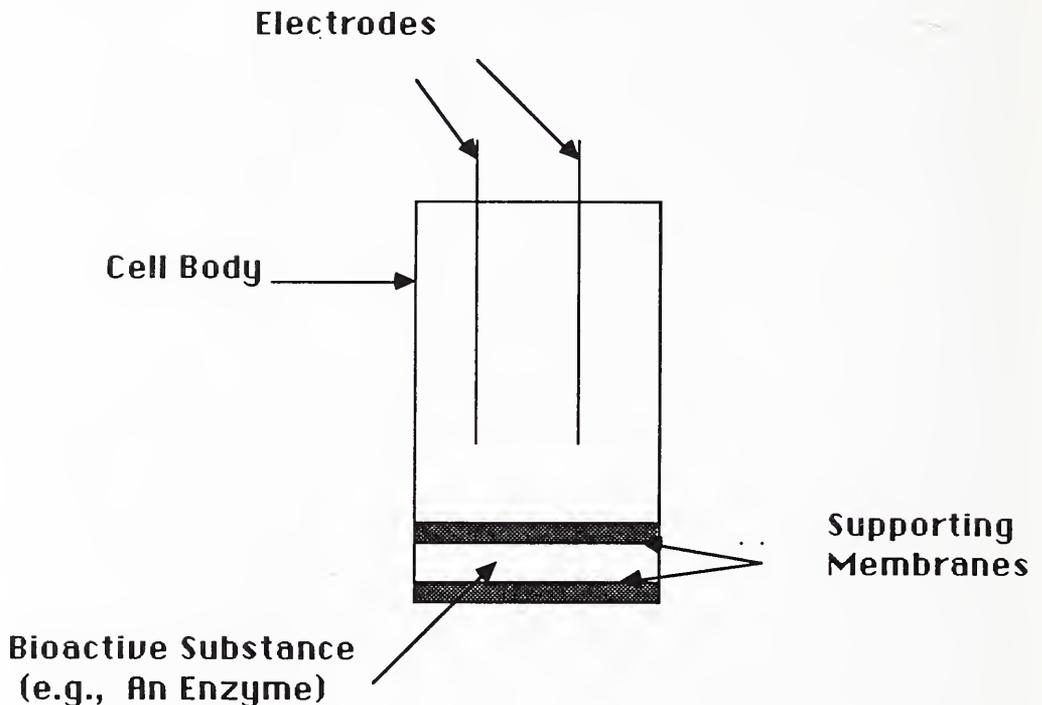


FIGURE 1. Simplified diagram of a generalized biosensor.

The general operation of a sensor consists of a chemical reaction catalyzed by or promoted by the bioactive substance. Typically, this reaction would either produce or consume a gas. The molecules of gas diffuse across the upper supporting membrane, which is selectively permeable to them, and cause changes in current or voltage at the electrodes. Monitoring these changes provides an indication of the amount of analyte present in the solution. Response times vary greatly with the specific electrode but generally are in the range of from 30 seconds to several minutes.

Since the enzymes or microorganisms are highly specific for a given type of reaction, the electrode is highly selective for a given type of analyte. The analyte molecules can be inorganic, organic or biochemical. Continuous or remote monitoring of concentration changes is possible. Many of the analytes for which biosensors are now available are species for which more traditional methods of analysis are very tedious, time consuming and/or not easily adaptable for continuous or remote monitoring.

Applications for biosensors can be found in many diverse areas. Both measurement and control functions in environmental, physiological, agricultural, industrial and geological settings are but a few of the wide variety of areas which can or do already use biosensors. Advances in the microtechnology approaches permit extremely small sensors to be constructed so that remote, continuous, *in vivo* monitoring for several species simultaneously becomes possible in plants, animals and humans. Such techniques promise

to open the door to information about these systems which has previously been impossible to obtain and they offer the potential for greatly increasing our understanding of these systems.

BIOSENSOR MARKETS

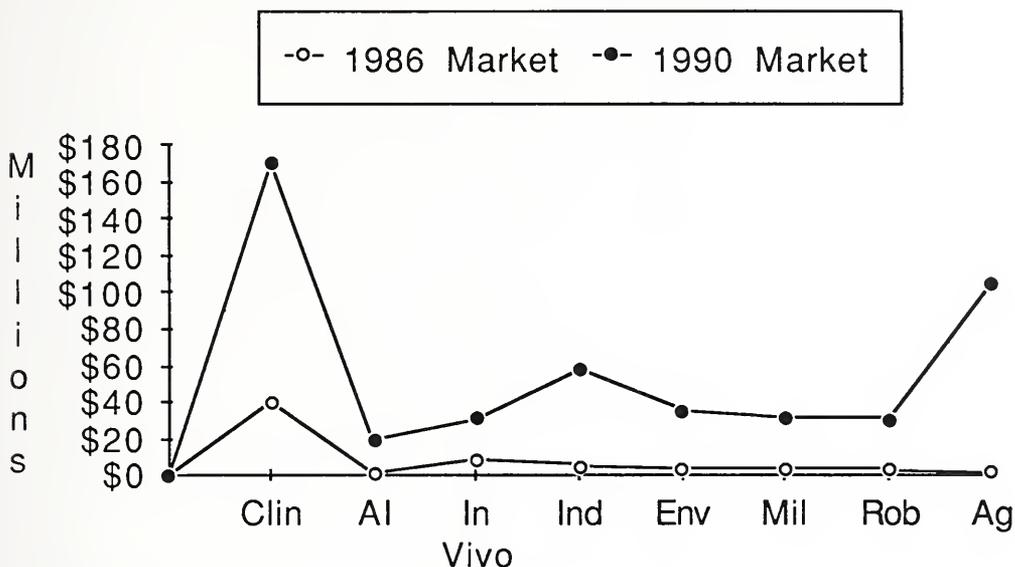


FIGURE 2. Projected markets for biosensors in various application areas. Clin = Clinical, AI = Artificial Intelligence, In Vivo = In vivo monitoring, Ind = Industrial application, Env = Environmental monitoring, Mil = Military, Rob = Robotics, Ag = Agricultural.

Figure 2 shows a plot of market research data for selected applications of biosensors based on data reported in 1985 (2). The lower of the two lines represents the market for biosensors in various areas in 1986 and the upper line the projected markets in 1990. Each of the application areas show is discussed briefly below.

Clinical

This area refers particularly to the development of multiparameter satellite medical testing facilities which can be installed in physicians' offices or taken to the bedside of hospitalized or homebound patients. This area shows the greatest market potential for the immediate future.

Artificial Intelligence

Biosensors can be used for feedback input into AI software for decisions regarding control and human intervention in chemical, physiological, environmental or other types of processes.

In Vivo

Continuous and remote measurements in human, plant and animal systems are becoming possible using microtechnology and invasive techniques.

Industrial

Monitoring and control of bioreactors, fermentation broths, food production, etc. are areas offering great potential use.

Environmental

Hazardous substances in wastewater, air, etc., can be monitored continuously and remotely.

Military

Monitoring for toxic chemicals and biological weapons is a primary concern of military applications.

Robotics

Sensors to provide robots with touch, taste and tactile sensing will involve biosensors in many applications.

Agriculture

Quality control in food factories or monitoring soils for certain bacteria types are significant application areas. For example, a nitrogen fixing bacteria has been found to be the limiting factor for growing tomatoes. A bacteria has been developed which, when injected into the soil, increases tomato production. Monitoring these and other bacteria is necessary for continued use, research and application to other plants. This area is expected to offer a very substantial commercial market for biosensors.

CHEMFETS/ISFETS

Some of the most exciting recent developments in biosensor technology have been made possible by innovative applications of solid state microcircuitry and fiber optics. We will first consider the solid state sensors.

“CHEMFET” is an acronym for “chemically sensitive field effect transistor.” “ISFET” (“ion sensitive field effect transistor”) is another acronym for the same type of device. To appreciate this technology, it is helpful to review some characteristics of an insulated gate field effect transistor (IGFET).

IGFETs are tiny electronic devices which have for a number of years been used for amplifying or switching electronic signals. They are suitable for use in “keyless” control panels on microwave ovens, in elevators and cash registers. They can be manufactured to occupy a very small space, lend themselves well to integrated circuit manufacturing techniques and are frequently a basic component of solid state memories for computers. Literally thousands of these devices can be packed into a chip which is only one or two millimeters on a side.

Each IGFET typically has three leads designated as the source, the drain and the gate lead. If a current source, such as a battery, is connected between two of the leads (the source and the drain), a very small charge applied to the gate lead causes a significant change in the current flowing between the source and the drain. In the “keyless” control panels referred to above, the electrostatic charge on the operator’s finger would be sufficient to change the current between the source and the gate leads from completely “off” to completely “on.” The general principle is that a very small change in charge applied to the gate causes a relatively large change in the source to drain current. This is amplification.

In recent years, innovative chemists and physicists realized that there is no reason that the charge applied to the gate must come from a person’s finger or from a voltage source which is part of an electronic circuit. Ions in solution would have the same effect. Therefore, if an IGFET were mounted on the end of a probe and modified so that when the probe was immersed in a solution, ions from the solution could come in contact with the gate, the current flowing between the source and the drain leads would be changed according to the number of ions contacting the gate. This concept gave rise to the chemically sensitive field effect transistor (CHEMFET) or ion sensitive field effect transistor (ISFET).

To make the ISFET selective for specific ions, a membrane which is permeable to only a given type of ion can be used to cover the gate lead. Only those ions which can permeate the membrane reach the gate and therefore effect the current through the ISFET.

Since ISFETs are easily fabricated with integrated circuit technology and can be made to occupy a very small space, they have been mounted on a tiny chip at the end of a standard catheter and used for *in vivo* monitoring of physiological fluids in humans and animals. The signal leads from the chip can be run through the catheter to the electronic monitors at a patient's bedside. This configuration provides continuous readout of the physiological variables being sensed. Since several sensors can be fabricated on a single IC chip, several species can be monitored simultaneously. Typical sensors available for monitoring physiologic fluids include potassium, calcium, ammonium and sodium. An *in vivo* potassium monitoring system has been reported in which the performance was sustained for several hours. After the sensor was removed from the patient, it continued to give accurate measurements on standards for several hours. (3).

In vivo monitoring of physiological fluids is particularly attractive for use with critical care patients. Physicians now know that frequent interval measurements provide the first clues to potential dysfunctions otherwise unsuspected at that time. Critical care patients are commonly intubated with several catheters which could be used to also contain sensors for continuous monitoring without additional invasive procedures on the patient.

Possible future uses of continuous *in vivo* monitoring include the direct interface of the sensors with a computer. Using appropriate artificial intelligence software systems, current data could be compared with data banks and provide interpretations of trends in terms of differential diagnoses—including the reporting of relative probabilities for different courses of action. Based on these interpretations, alarms could be sounded when critical values were encountered and suggestions made to the attending physicians regarding possible therapeutic interventions.

While biosensors interfaced to a data base with an expert system to interpret trends sounds somewhat esoteric, the feasibility of the system depends most strongly on the biosensor. The ultimate use of such systems will likely come to rest on the availability of rugged, accurate, reliable and safe sensors.

Ex vivo use of biosensors for monitoring physiological fluids is also possible and avoids some of the problems associated with the *in vivo* sensors. Biosensor systems for *ex vivo* testing have the potential to greatly reduce costs for blood and urine analyses. Since hospitals and the medical profession in general are being forced to become increasingly sensitive to costs for medical care, a large market appears to exist for *ex vivo* systems.

Flow injection analysis (FIA) ISFET systems will be able to provide the capability for *ex vivo* bedside multivariable testing. With these systems, a nurse would withdraw a fraction of a milliliter of blood from a patient through a catheter and inject the sample into a near bedside FIA ISFET unit. This unit would automatically test for a variety of blood or urine components. Such testing would decentralize frequent and routine testing and thereby reduce costs as well as reduce delay time between sampling and sample reporting.

While a qualified technician would likely be needed to calibrate such instruments in a hospital setting, the bulk of the testing would be done by nurses. Thus, substantial savings would be realized compared to staffing a centralized laboratory.

Another market for *ex vivo* biosensor systems is projected to be in individual physician's offices. Again, both cost and time considerations are likely to encourage the development and use of such systems.

MULTISENSORS

The ability to measure a number of different analytes simultaneously is a characteristic of a device now under construction by Molecular Devices. A four by four inch silicon wafer has room for over 1,500 different test sites. LEDs at the back of the chip can be

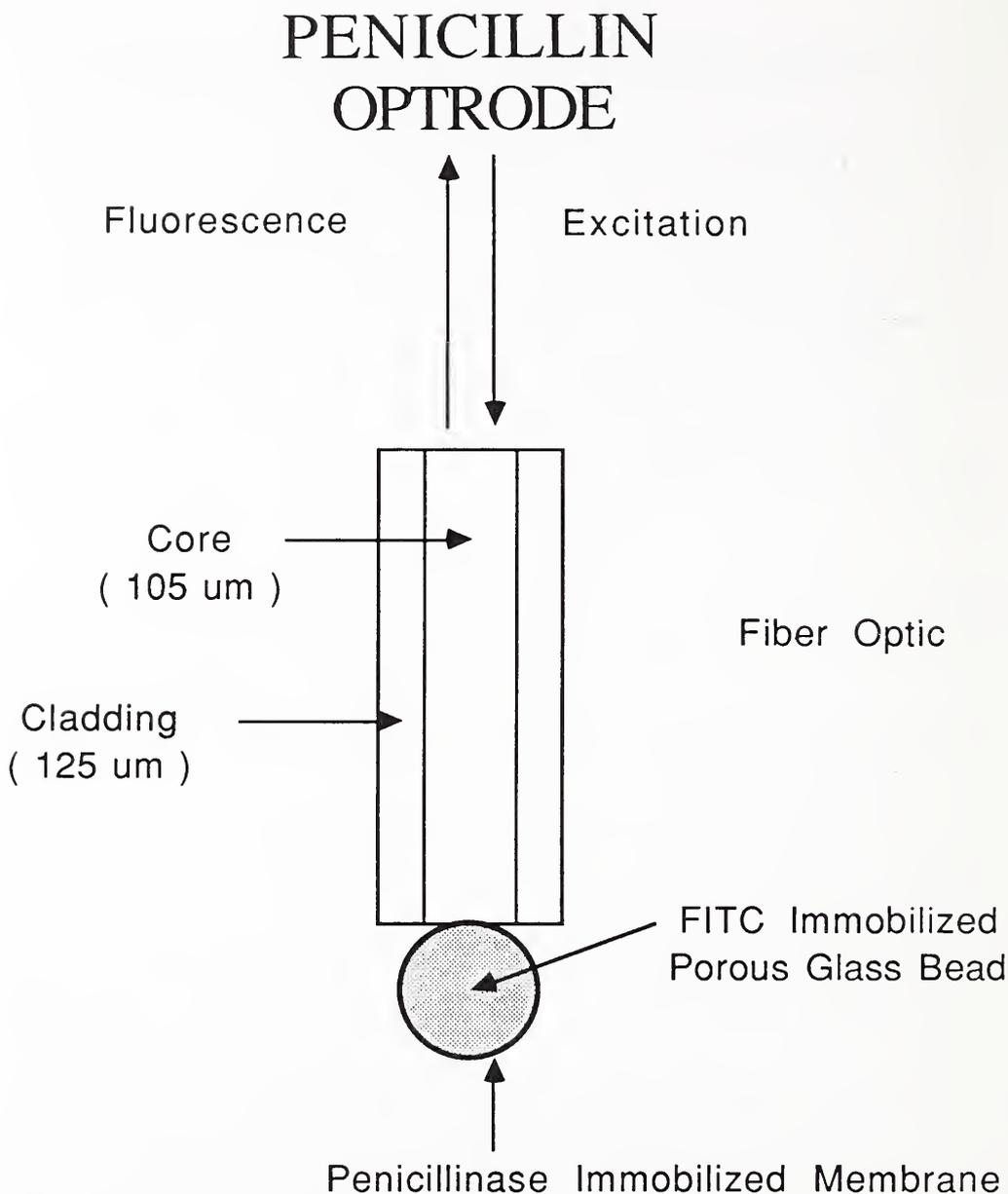


FIGURE 3. Penicillin optrode. Used with permission from Fuh, M.S., Burgess, L.W., and G.D. Christian. "Single Fiber Optic Fluorescence Enzyme Sensor." Preliminary manuscript for paper presented at FACSS meeting, November 1987.

turned on one at a time. The light emitted from an LED causes a photoresponse at a given area of the chip at which a specific test site is located. When there is a chemical reaction at that site, such as an antigen-antibody binding reaction in an immunoassay, the current flowing in that portion of the chip is changed and is detected. This device is scheduled to be marketed as early as sometime in 1988 (5).

OPTRODES

A characteristic of all of the solid state sensors listed previously is that they must transmit electrical signals from the sensor to the detector through electrical connections. This means that if they are used in environments in which magnetic fields exist or in situations in which stray currents can be hazardous (such as in *in vivo* situations) either

electrical interference or potential danger to the patient results. A relatively new approach which eliminates many of these problems involves the use of fiber optics to transmit the information from the sensor area to the detector. These devices are known as "optrodes."

Figure 3 shows a diagram of a penicillin sensor using this approach which was recently reported by Fuh, Burgass and Christian (1). This sensor consists of a small, porous, glass bead attached to the end of a single optical fiber. The bead contains fluorescein isothiocyanate (FITC) immobilized on its surface. When radiation of an appropriate wavelength is transmitted through the fiber to the bead, the FITC fluoresces. The fluorescing radiation is transmitted back to the detector through the same fiber. The intensity of fluorescence has been shown to be proportional to the pH environment of the bead. Therefore, the fluorescence is an indication of pH.

The enzyme penicillinase can also be immobilized on the same bead. If the resulting bead is immersed in a solution containing penicillin, the penicillinase catalyzes the hydrolysis of penicillin to penicilloic acid and hydrogen ions. The increase of hydrogen ions at the bead surface causes changes in the fluorescence of the FITC on the bead. The resulting fluorescence has been shown to be linearly related to the amount of penicillin in the solution. The response time for this electrode is about 30 seconds and the working range is approximately 0 – 10 mM, although it varies somewhat with the buffer system used.

Another recent innovation employing fiber optics is the chemfuse (4). In contrast to the sensors described previously, these devices have only two significant output states rather than a varying output which is proportional to the concentration of some analyte. The operation of chemfuse is based on the fact that a change in some physical characteristics of a chemical (such as a change from the solid to liquid state) causes changes in its ability to reflect light. If a perturbation in the physical environment of this chemical occurs which is sufficient to cause the change in its physical state, the resulting change in reflective properties signals the presence of this perturbation.

Figure 4 shows the configuration of a generalized chemfuse. Suppose such a device were to be constructed so that it would indicate if a particular liquid chemical were spilled from a reactor. A solid substance which was soluble in the chemical of interest would be placed in the bottom of the 13-gauge hyperdermic needle. Light from the LED source would be transmitted through one of the optic fibers to the surface of the solid. A portion of the reflective light from the surface would be conducted to the detector through the second fiber. A steady state intensity of reflected light would result as long as the substance in the hypodermic needle remained solid. If the needle were subjected to an environment containing the spilled chemical of interest, this substance would enter the needle and dissolve the solid. Since the reflective properties of the resulting solution in the needle would be significantly different from the solid, a marked change in intensity of reflected light would be observed. This change could be used to sound alarms or initiate some automatic shutdown procedures. The solid substance originally placed in the needle could be chosen to be either very specific for one substance or specific for a range of substances.

Chemfuses are simple, small, rugged, and reliable. They require no calibration. As is characteristic for all optrodes, the light transmission is not effected by electrical or magnetic interferences which would cause problems for electrical signals.

Chemfuses can be configured to act as temperature fuses by choosing the solid initially placed in the needle to have a melting point at the temperature of interest. A ferrographic sensor has been reported which detects the build-up of magnetic material in oil. This is of interest since small particles of iron are known to accumulate in, for example, motor oil, the longer it is used. The ferrographic optrode can continuously monitor the condition of the oil during operation of the motor and indicate the need for an oil and/or filter change. The ferrographic optrode is constructed by placing a small magnet, covered with aluminum foil, in the needle. Additional holes are made in the sides of the needle

GENERALIZED CHEMFUSE

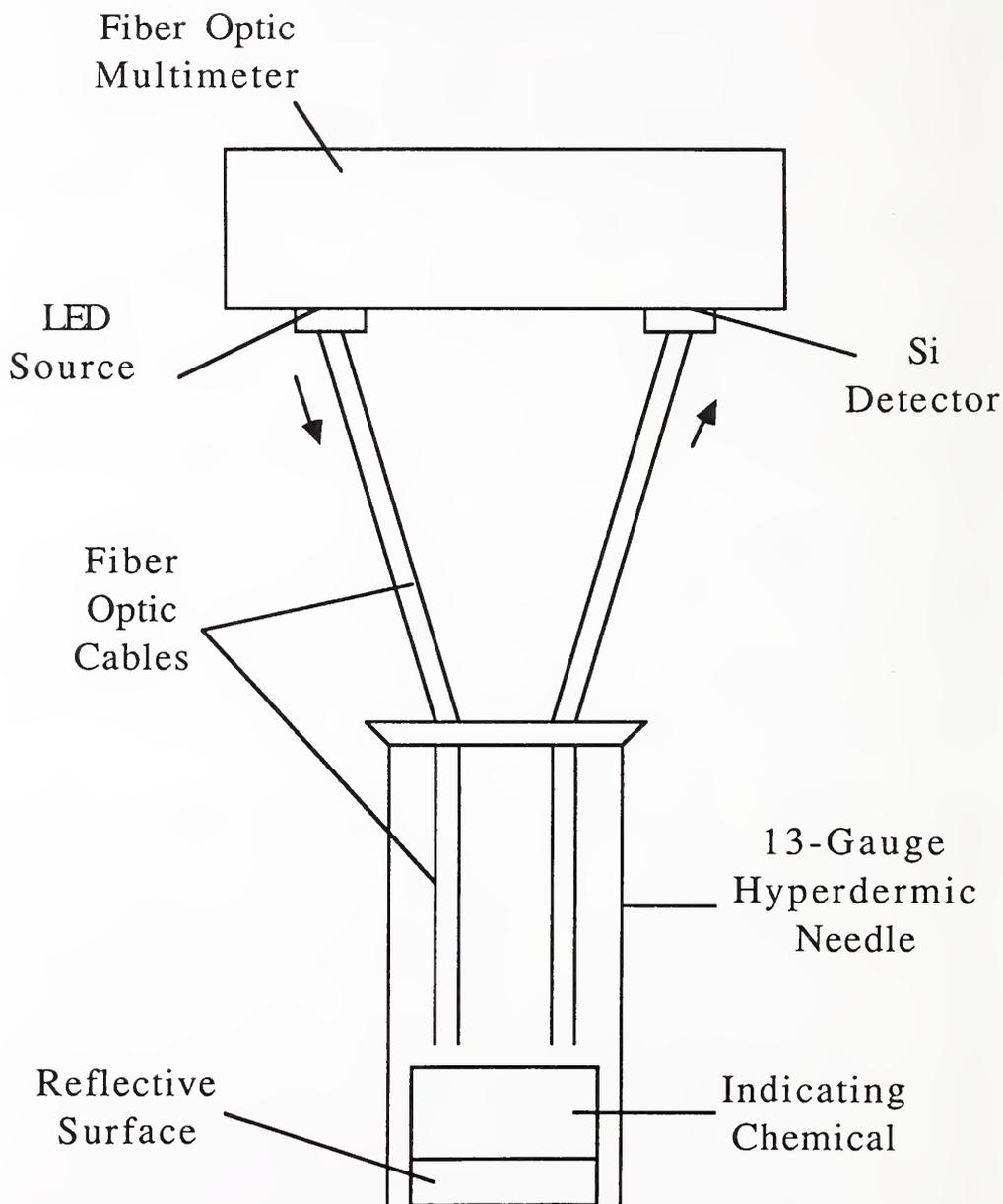


FIGURE 4. Generalized diagram of the chemfuse. Used with permission and adapted from Tenge, B., Grunke, A.Q., and D.E. Honigs. "Chemfuses: Fiber Optic Switches Modulated by Chemical Substances." Preliminary manuscript for paper presented at FACSS meeting, November 1987.

and the needle is immersed in the circulating oil. Iron particles in the oil are attracted to the surface of the aluminum foil as they pass over it and adhere to the surface. The accumulation of these particles changes the reflective properties of the foil.

Since chemfuses are a very recent innovation, only a few possible uses have actually been reported. However, it seems likely that applications of the basic principles will be found for detecting many different substances, including many biological or biochemical substances.

CONCERNS

There are at least two primary areas of concern related to the development and marketing of biosensors. These are technical concerns and liability concerns. We will comment briefly on each of these.

In a Delphi study reported in 1985 (2), experts in the biosensor field were asked to rate critical issues relating to biosensor development. Problem areas were rated according to the degree of importance for the biosensor field to progress as well as their projected degree of difficulty in being solved. Figure 5 shows a summary of some of

PROBLEM IMPORTANCE & DIFFICULTY

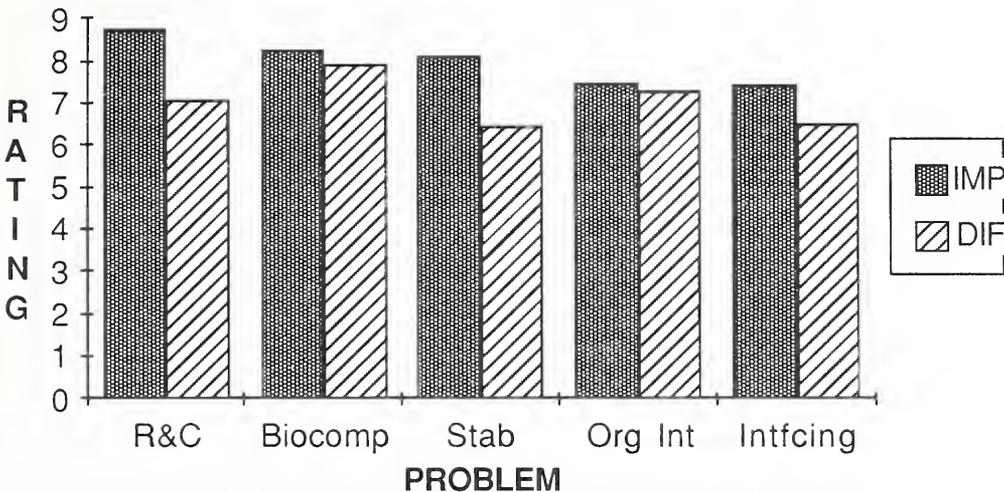


FIGURE 5. Five most important technical problems which need to be overcome for biosensor technology to progress rated by importance and degree of difficulty in solving.

these results. The five most important problems identified are shown and are: reliability and consistency of the sensor (R&C), biocompatibility of the sensor with the environment/body (Biocomp), the stability of the sensor to changes in the environment (Stab), the sensitivity of the sensor to interferences from organic chemicals in the environment (Org Int), and the problems involved in interfacing with the biomaterial (Intfcng).

The left bar in each pair in Figure 5 is the rating of the degree of importance. The higher the number, the more important the problem. The right hand bar in each pair indicates the relative degree of projected difficulty in solving the problem.

Figure 5 shows that reliability and consistency (R&C) of the sensors is the most important problem by a slight margin. Interestingly, the solution to that problem is not seen as being as difficult as for some of the other problems.

The time projected for biosensor technology to penetrate several different market areas is shown in Figure 6. Of the areas listed in this figure, optrodes appear to have the potential to impact the market in the shortest time. Given the magnitude of market potential in dollars described earlier for clinical applications of biosensors, this might seem somewhat surprising. However, liability and ethical concerns are likely to delay the widespread use of biosensors for medical purposes in some areas.

Liability concerns are particularly severe in the medical field for *in vivo* applications of ISFETs. The potential harm to a patient resulting from a device failure or malfunction is likely to be a major impediment to the commercial introduction of these devices (3). Since there is a small electrical current flowing through the ISFET devices, if adequate

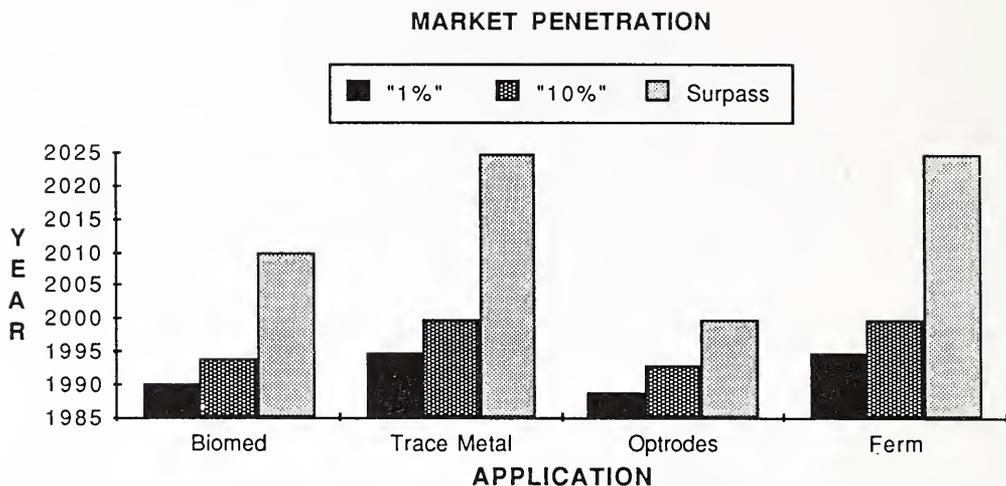


FIGURE 6. Time required for selected biosensor technology or application to penetrate the market place.

electrical insulation around the sensor were lost for some reason, potentially lethal damage to the patient would result. Stray current in in vivo systems could very likely result in exogenous electrical stimulation of the heart and consequent myocardial fibrillation. In addition, as these devices are developed to the stage that they are incorporated into computerized AI systems used for differential diagnosis, automatic control or advice on therapeutic intervention, the risks rise substantially. Arranging for testing of these devices on human patients will likely be cumbersome and expensive at best and fraught with the possibility of ethical considerations relating to the patient's comfort, safety and well-being.

While these considerations alone will likely not stop the ultimate development of in vivo applications of biosensors, they will very likely delay their introduction commercially on a wide scale. The fact that the Japanese have had some commercial versions of simple ISFET probes for in vivo pH and pCO₂ measurements available to the medical community since 1983 indicates that at least some manufacturers, medical personnel, regulatory agencies and patients are willing to accept the risks involved.

If U.S. companies are sufficiently reluctant to accept the liability risks, this reluctance will likely preferentially accelerate the development of ex vivo systems. In any case, biosensors appear to have a niche carved for them in a variety of applications and it is only a matter of time before widespread use of these devices occurs.

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