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Review

Biosensor Development

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This article reviews the recent biosensor developments for medical applications, focusing on the various biological recognition elements used in biosensors and the systems transduction mechanisms. Available instruments utilizing biosensor technology are also examined from a commercial perspective.

KEY WORDS: antibody; biosensor; chemoreceptor; enzyme.

INTRODUCTION

Research, development, and the use of biosensors has led to an expanding diversity of measurement techniques. General reviews on biosensors have appeared recently (1–3), but the literature is rapidly outpaced by the breadth of the research. Biosensors are currently being developed for applications in environmental studies, food, agriculture, biotechnology, medicine, and the military. While there are no clear-cut lines that separate these areas, this Review emphasizes the medical applications.

For the purpose of this article "biosensor" is defined as an analytical device incorporating a biological sensing element which translates the chemical parameters of a system into an optical or electrical signal. Biosensors offer small, selective, and portable diagnostics for key biological substances. Such diagnostics could improve management of patients and allow testing in the home or at convenient outpatient clinics. While most biosensors are still in the developmental stage, current research promises to meet several of the important measurement goals for selected metabolites, drugs, and other important clinical molecules. Enzyme technology was exploited as early as the 1950s with the development of an enzyme test strip (4) for the simple analysis of solutions, and the enzyme electrode is still the classic and most commercially marketed biosensor. The first example of this technique is the combination of glucose oxidase and an oxygen electrode, which measures glucose by detecting the reduction in oxygen when the oxidation of glucose is catalyzed by the enzyme (5). Desired functional characteristics of biosensors are described in Table I.

Selected sensors of the mammal, plant, and insect world are understood well enough to provide design suggestions for sensor development. As an example, the olfactory organs in the antenna of the male gypsy moth have the incredible ability to detect individual molecules of the female moth's pheromone against a background that is 15 to 20 orders of magnitude greater, allowing the male moth to detect her 10 mi away (Fig. 1). This sensitivity is achieved by a combination of diffusive sampling, two-dimensional chromatography, and receptor sites, all in a package that has a volume of 0.01 cm^3 and weighs 10 mg.

Biosensor Development for Cation Detection

Continuous measurement of potassium levels through invasive and noninvasive sensors would provide important clinical indicators of treatment effects and the physiological state of a patient. Potassium concentration in the body varies greatly from cell to cell, but its concentration in human serum remains remarkably constant. The serum K^+ normally remains between 4 and 5.5 mEq/L. A significant drop in this level is an indication of disease, deficiency caused by drugs, or vigorous activity. The most commonly used method for the clinically accurate analysis of potassium is flame emission photometry (6), requiring discrete serum samples. It is not a method for continuous measurement since the patient's comfort and health dictate that as few samples as possible be taken. Further, ion-selective glass electrodes allow direct *in vitro* measurement of potassium concentration (7), but they display a low selectivity of potassium over sodium.

The use of the biomolecule, valinomycin (a natural macrocyclic antibiotic that selectively interacts with potassium) was the first biosensor design for ion-selective electrodes (8). The macrocyclic antibiotic electrodes are up to 1000 times more selective than liquid ion-exchange or potassium-selective glass electrodes. As a group, these electrodes have found widespread application. Potassium-selective electrodes have been used for measurements in serum (9) and blood-simulated aqueous solutions (10). Serum potassium levels using potassium selective electrodes are in good agreement with measurements using flame emission photometry.

Problems that have limited continuous *in vivo* measurements in patients include biocompatibility, errors arising from the perturbation of the liquid–liquid junction by external factors, electrical shock hazard, the requirement of a reference electrode, and cross-talk between other electrical

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Table I. Comparison of Biosensor Functional Characteristics[a]

Feature	Requirement	Example/comments
Selectivity	Ideally specific, no interferences	Ion-selective electrodes: range 10^{-1} to 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 less than 1% concentration change	Potentiometric systems limited in linearity in response governed by Nernstian 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 to 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 in 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 that 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, *CHEMTECH*, June 1990, p. 372.

instruments (11). Some of these have been overcome with the development of ion-sensitive field effect transistors (ISFET) for potassium detection. The ISFETs can be made less than 1 mm in size, work over a wide pH range, and have been used in the *in vivo* continuous monitoring of potassium in animals (12). For mass *in vitro* analysis, Eastman Kodak has developed an Ektachem thin film-coated slide for the potentiometric assay of potassium that reaches a stable potential in 3 min with as little as 10 μl of sample.

Recent biosensor designs focus on the optical detection of potassium. Again, ionophores or channels are the basic recognition elements in these sensors and they provide natural selectivity for potassium over other ions or contaminants. Optical methods for the determination of potassium have been reported using colorimetric (13,14) and fluorimetric analysis (15,16). One method relies on the color reaction of the dye dinitrohydroxyazo with potassium to obtain a colorimetric change (13). All others use a method involving an ionophore that transports K^+ into a nonpolar phase for detection. In one instance a dye molecule comigrates with the potassium to maintain charge neutrality, and in the other a fluorescent or chromogenic crown ether reagent is affected upon complexation of the potassium molecule. A dry reagent strip developed by Ames Division, Miles Laboratories, Inc., can detect potassium by reflectance spectroscopy with only 15 μl of serum sample, and EM Science has a color test strip that can detect potassium ion in solution from 0 to 20 m*M*.

A potassium fiberoptic sensor has been targeted as the next logical step in development along with sodium and chloride for an on-line determination of serum ion concentrations *in vivo* (17). The first reported potassium fiberoptic sensors were developed using potential sensitive dyes that correlate potassium concentration indirectly with a membrane potential (18). A second sensor used a labeled crown ether at the end of the fiber optic and measured the optical absorption change upon complexation with potassium (19). Both the membrane potential- and the optical absorbance-based sensors have a low sodium/potassium selectivity ratio and could not be used in biological samples unless a reference sodium sensor was present. A fiberoptic sensor that solves this prob-

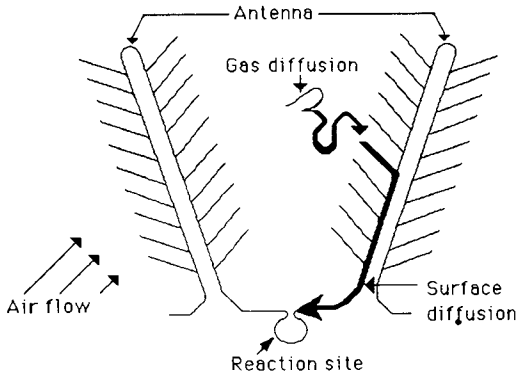


Fig. 1. The male gypsy moth depends on a single molecule sensor to find the female of the species. Nature has been designing portable, low-cost biosensors for 10^9 years, and by now she is quite good. [T. Hirschfield *et al. Science* **226**:312 (1984)].

lem and responds rapidly and reversibly to potassium ion has been described recently (20). Both the ionophore and the color indicator are in a nonpolar membrane, and charge neutrality is maintained by the deprotonation of the indicator in the presence of the charged ionophore–potassium complex.

This overview of methods for detecting a single parameter of interest in biological fluids shows the diversity and complexity of devices available to the clinician. In general, a biosensor is made up of two principal components: a biological recognition element and an instrument that generates the signal for analysis (transducer). This Review focuses on the various biological recognition elements used in biosensors and those biosensors important in clinical applications. Table II lists biosensor designs and comments on their sensitivity, response, and stability.

BIOLOGICAL RECOGNITION ELEMENTS

Antigen/Antibody

Antibodies are ideal candidates for use as recognition elements in biosensors. They can be produced for most biomolecules, drugs, viruses, and cellular material. Also, they have the ability to bind antigens selectively and with a high sensitivity, but interference from proteins and slow reversibility of binding lead to a slow overall response. These two problems have limited their successful use in the laboratory as well as in commercial development.

Piezoelectric Detection. One very sensitive approach to the detection of antibodies is the use of coated piezoelectric crystals. A piezoelectric sensor uses an oscillating quartz element that is coated with an absorbent layer designed to interact selectively with the compound of interest. The crystal is forced to oscillate at its fundamental frequency using an electrical current. Adsorption of the compound of interest onto the crystals causes a change in mass on the detector, which results in a frequency change of the oscillator. Any frequency change qualitatively indicates the presence of the analyte, while the total frequency change is proportional to the amount of analyte present. Guilbault *et al.* use antibodies in making a piezoelectric *Salmonella* detector. The antibody for the bacterium *Salmonella typhimurium* was coated onto

Table II. Comparison of Biosensor Designs[a]

Type	Measurand	Sensitivity[b]	Response (min)	Stability	Comments
Enzyme	Electrical/ optical	ppt–ppm	1–10	Days to months	Difficult to fabricate consistently and economically; tend to become contaminated
Microbial	Electrical/ optical	ppt–ppm	5–60	Days to months	Same as enzyme
Immunosensor	Electrical/ optical	ppm–ppb	1–20	Days to months	High precision but require expensive transducers and 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–ppm	5–20	Hours to days	Experimental
Enzyme thermistor	Thermal	ppt–ppm	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.

a quartz piezoelectric crystal by first precoating the crystal with polyethylenamine and then immobilizing the antibody (21). When the antigen is present, the driven crystal undergoes a detectable frequency change, which has a reported sensitivity of 10^5 to 10^9 *Salmonella* cells/ml, with a response time of 2 to 3 min and a sensor lifetime of seven assays over a 4-day period. Because such frequency monitoring techniques really involve changes in mass or pressure on the piezoelectric surface, no special labeling of the compound of interest is needed, but work on minimization of other adsorption interferences to improve selectivity will be required.

Prusak-Sochaczewski and Luong used a piezoelectric crystal coated with protein A and reacted with anti-human transferrin antibody to determine human transferrin (22). The protein A had a strong complexation with the gold electrode that allowed high binding stability of the sensor, but attempts to reuse the sensor were not successful because of dramatic sensitivity decreases from measurement to measurement. The detection range for human transferrin was linear between 10^{-4} and 10^{-1} mg/ml, but reproducibility between sensors was not reported. This group has created a similar sensor for the detection of human albumin.

Potentiometric Detection. Potentiometric electrodes can be coupled with antibody–antigen reactions. In this case the antigen is chemically bound to a carrier that can change the potential in a selective fashion (Fig. 2). Enzymes or ionophores labeled with an antigen have most often been used to mediate the reaction. A review of this transducer technique for immunoassay is given in Ref. 23. The reaction of the antigen with the antibody modulates the carrier properties and, so, changes the potential. This type of sensor is attractive because it uses proven ionophore or enzyme sensors; moreover, it can be made reversible and reusable. It relies on the fact that the low molecular weight antigen can pass through chosen porous membranes but the bulky antibody is trapped in the sensor arrangement. Such sensors are limited to antigens of low molecular weight, such as those for dinitrophenol or digoxin, and to antibody–antigen systems having binding constants that are rapidly reversible. These systems have problems with nonspecific interferences in the biological fluids.

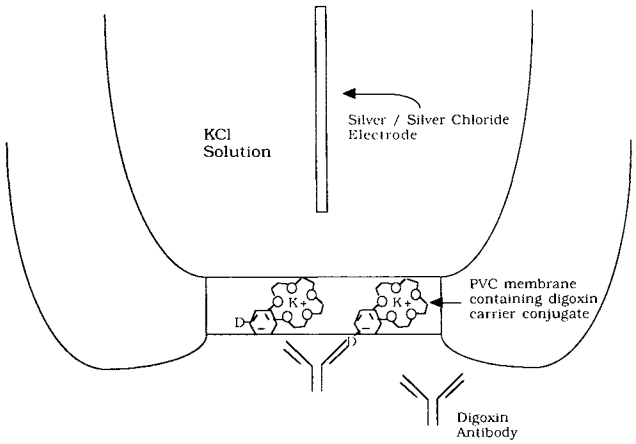


Fig. 2. Potentiometric electrode for antibody measurement using an antigen–ionophore complex. Antibody interaction prevents potassium transport across the membrane.

Optical Detection. A technology that offers several distinct advantages is fiber-optic sensors. Fiber-optic sensors for invasive measurement satisfy flexibility and miniaturization requirements and can be easily inserted into endoscope channels, catheters, or hypodermic needles. Chemical fiberoptic sensors often consist of a thin material that is impregnated with dye and attached to the fiber. When the optrode is placed inside the body, blood constituents diffuse into the probe and cause a change in absorbance or fluorescence of the dye. This intensity change is designed to correlate with the concentration of the analyte. There have been several reviews on fiber optic-based medical sensors and biosensors (24,25). Schultz and co-workers were one of the first groups to develop a reversible-affinity fiber-optic glucose sensor that operates on the basis of competition between glucose and dextran for the binding sites on the lectin, concanavalin A (26). This is achieved by using fluorescein-labeled dextran that is entrapped at the tip of an optical fiber by a membrane. The membrane takes advantage of the high molecular weight of dextran to secure it inside its volume, but glucose molecules are free to diffuse. When the fluorescently labeled dextran is displaced by glucose, an increase in fluorescent intensity is seen through the fiber. This fluorescent increase can be linked to the glucose concentration.

Vo-Dinh *et al.* have developed a fiber-optic fluoroimmunosensor for a DNA/carcinogen adduct product, benzo(*a*)pyrene tetrol (BPT) (27). Their technique uses a phase-resolution detection of the fluorescence lifetime of BPT when it is combined with the anti-BPT antibody. They report that the sensor can detect BPT at femtomole levels in the presence of an amount of interfering benzo(*a*)pyrene that is 50-fold more concentrated.

Ives and co-workers have described modifications of internal reflection spectroscopy techniques relevant to the design of evanescent fiber-optic immunosensors (28). Fluorescently tagged antigen bound in the sensing membrane competes for the trapped antibody with free antigen in the sample (competitive inhibition). This biosensor has been used to measure concentrations of anti-human immunoglobulin G, while other sensors similar in design have been reviewed that measure dioxin and human ferritin antigen.

This class of sensor may be limited by the nature of the antibody. Antibodies need to be found with sufficiently fast dissociation rates to allow reversible measurements in real time. Work on the kinetic properties of the antibody–antigen reaction and optimization of the transducer–detector interface will help expand the use of antibody reactions in all biosensor designs. There is also a need for systems with simple or no pretreatment of the test sample.

Enzymes

Enzyme electrodes under development for the last 20 years have become the most commercially available biosensor type. These sensors are based on immobilized enzymes placed into a layer that can react with the analyte of interest and convert its concentration into a generated signal (Fig. 3). In most cases, the enzyme does not directly interact with the electrode, but close proximity of the enzyme allows the electrode to detect a by-product of the enzyme reaction. Biosensors have been prepared using natural enzymes, artificial

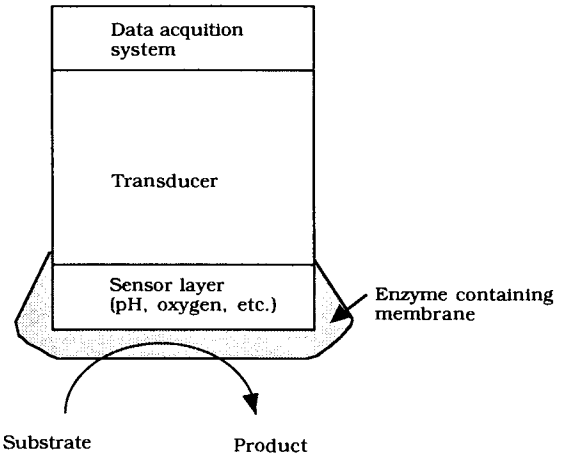


Fig. 3. Enzyme biosensors usually measure a by-product of the enzyme reaction. Substrate concentration is measured when the enzyme reaction alters the microenvironment of a sensor/transducer combination. The sensor is designed so the predetermined variable altered is proportional to the substrate concentration.

enzymes, and enzyme systems isolated from bacterial cells and plant or animal tissue.

In any of the enzyme sensor variations, the analyte's interaction with the biocatalytic layer produces a product that can be read by the transducer and turned into an electrical signal. These products can be monitored to detect and quantify directly a single compound in the biological medium using a signal transducer. Enzyme-catalyzed processes have been coupled to many types of signal transducers in the construction of biosensors. Each transducer usually has its inherent advantage over the other types, so in practice it is the application that dictates one type of sensor over another. Operating parameters of the measurement will most often determine the transducer chosen, and no system has proven ideal for all cases. Operating factors such as high magnetic or electric fields in the vicinity of the measurement could dictate optical over electrical transduction, whereas sensor response time restrictions could indicate the use of electrochemical over optochemical detection.

Encapsulation or immobilization of the enzyme can be one of the most difficult problems in the design of a sensor, and it is one of the most important because it is usually at this phase that the sensor is designed for response time, increased enzyme stability, and sensor lifetime characteristics. Kampfrath and Hintsche have described a deposition technique that will lay down polymer films precisely at the active area of the sensor element (29). In integrated circuit technology, thin-film noble metals are deposited onto silicon wafers using a mask and vacuum deposition. It would be advantageous if an immobilization film could be deposited using the same masks and vacuum deposition process, so mass production of the entire microsensor could be accomplished. Their technique uses vacuum plasma polymerization to lay a thin (100- to 150-nm) polymer film onto a wide variety of substrates such as noble metal electrodes on silicon biosensors. These uniform films retain their functional groups for immobilization of selected enzymes. To demonstrate this technique, glucose oxidase was coupled to the amino groups of a 2-aminobenzotrifluoride acrylic acid film. The response time was 4 sec, but the sensor had a lifetime of 1 week; this was attributed to the small amount of enzyme on the sensor surface. Conventional glucose enzyme sensors have lifetimes approaching 300 days, but the cost reduction gained in mass production would make a sensor life of 1 week acceptable in many short-duration applications. Solution techniques have been used in the past when working with biological material. However, due to the variation in viscosity of the solutions and surface roughness of the substrate, the uniformity of the sensor is not as good as can be obtained by plasma deposition. This research group also works with polyurethane enzyme membranes that show good adhesion to silicon chips, good retention of the enzyme after cross-linking of the surface by polyfunctional isocyanate groups, and low diffusional resistance to both analytes and products (30). Glucose and urea sensors were designed using this technology on ISFETs (ion-sensitive field effect transistors) and TFMEs (thin-film noble metal electrodes). Other recent work on polyurethane membranes (31) and Langmuir–Blodgett films (32) has also been reported for enzyme membrane electrodes.

Amperometric Detection. Enzyme electrodes based on a chamber design for the measurement of glucose and lactate have been described by Schneider's group (33). They claim that the chamber-type structures are well suited to the fabrication of miniaturized biosensors, because the defined volume provides a space for immobilizing biocatalysts in close proximity to the electrode in a reproducible fashion. Studies using glucose oxidase and lactate oxidase immobilized in agarose gel and placed in a chamber-type oxygen electrode exhibited response times of 10 sec, but studies for reproducibility were not reported.

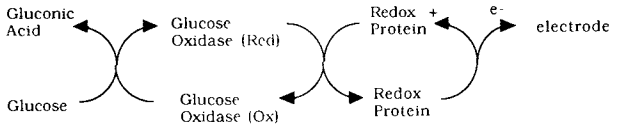
Amperometric enzyme electrodes for glucose measurement have been designed using oxygen, pH, or hydrogen peroxide sensors as the secondary transducer (34).

$$\beta\text{-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \beta\text{-gluconic acid} + H_2O_2$$

Amperometric electrodes have a fixed voltage applied between the working and the reference electrodes to drive electron transfer reactions. The net current flow measured is proportional to analyte concentration. The enzyme, glucose oxidase, is immobilized in the membrane of the detection electrode and catalyzes oxidation of glucose to gluconolactone and hydrogen peroxide with oxygen as the final electron acceptor. Most sensors then measure the glucose concentration by detecting the reduction of oxygen in the microenvironment of the sensor head, but this is not ideal since the production of H_2O_2 may lead to undesirable side reactions, and there must be a constant oxygen supply. The use of oxygen has disadvantages as an electron acceptor and cosubstrate because, under physiological conditions, variations in oxygen tension can cause fluctuations in electrode response, and oxygen concentration can be rate limiting, as it is low compared to glucose levels. In recent sensor designs, oxygen is eliminated as the final electron acceptor and replaced by an organic mediator such as ferrocene (35).

Since the mediator is oxidized by the electrode, the measurement becomes essentially independent of variations in oxygen tension, which can be important for sensor repeatability.

Davidson has reported on a group of redox enzymes that could communicate with the electrode by providing direct electron transfer (36) and so eliminate the need to measure a reaction by-product such as oxygen or H_2O_2. He describes a new class of enzymes called quinoproteins that undergo oxidation and reduction reactions directly and can transfer electrons directly to the electrode. They have the potential to detect important mammalian amines such as dopamine, histamine, spermine, and others. Davidson's paper does not describe stability or sensitivity of biosensors but stresses further understanding of the mechanisms of catalysis and electron transfer so that the logical design of electrodes can proceed.



Rechnitz's group has worked on a biosensor that uses an artificial enzyme with a reported stable lifetime of at least 6 months, using no added cofactors (37). Artificial enzymes (synzymes) are synthetic polymer chains having functional groups that mimic the biocatalytic activity of natural enzymes. The design used partially quarternized poly(ethylenimine) in which 10% of the residues are retained as primary amines. The synzyme catalyzed the decarboxylation of oxalacetate according to

$$HOOCC(=O)CH_2CO^-{}_2 + H^+ \rightarrow HOOCC(=O)CH_3 + CO_2$$

and a CO_2 electrode was used for the transducer.

Another major trend in this area is the use of cellular materials as biocatalytic alternatives for isolated enzymes. The primary advantage of cellular material is that the enzymes exist in their natural environment, with its built-in component stability. No direct evidence has been presented showing that cellular material is actually more stable than laboratory enzyme preparations, but useful lifetimes of 30 to 60 days have been achieved for certain cellular biosensors, whereas their isolated enzyme counterparts last only a few days. These cellular systems have also been shown to be highly selective in their response to a wide range of analytes. Research has been done on trapped bacterial cells, fungi, and tissue from plants and animals. A current example is one from Rechnitz's lab that uses the incorporation of ground beet stem into the graphite paste of an H_2O_2 electrode tip for determining oxalate (46). The H_2O_2 generated by enzymatic degradation of oxalate is monitored with a detection limit of 10^{-4} *M* and a response time of less than 1 min. Since oxalate oxidase is also found in banana peels, moss, and barley seedling, these could also be used. Another example is a banana-based biosensor that can measure the neurotransmitter dopamine when coupled to an oxygen electrode. The browning reaction of the banana converts dopamine to melanin in reaction steps that consume oxygen. The range of biosensors that can be devised staggers the imagination: spinach leaves for catechols, a magnolia flower petal for amino acid measurements, parsley leaves for measuring amino acid and urea, a toad bladder for vasopressin, cabbage for vitamin C, and others (47,48).

Piezoelectric Detection. Lasky and Buttry are developing a piezoelectric glucose biosensor using enzymes (38). The work was based on the enzyme hexokinase entrapped within a cross-linked poly(acrylamide) matrix and coated onto the crystal. Hexokinase phosphorylates glucose to produce glucose-6-phosphate in the presence of Mg^{2+} and ATP. When the glucose binds to the enzyme, there is a frequency change between an active and a reference crystal placed in the solution. The article reports a glucose detection range from 0.1 to 20 m*M*, response times of less than 10 sec, and a continuous operating lifetime of several days. The stated cause of this lifetime limit was primarily delamination of the gel from the sensor surface, so better coating techniques could extend this range. Unfortunately, the average enzyme-based biosensor has a relatively short lifetime (approximately 30 days) due to the stability of the biocatalyst, so more work must be done to incorporate into the sensor those factors that will ensure longer enzyme stability. This will be a difficult task since each enzyme system has a different set of parameters that will determine its lifetime.

Calorimetric Detection. Muehlbauer and co-workers have developed a calorimetric transducer that detects the heat of the glucose reaction (39). The sensor consists of a thermopile next to a glucose oxidase membrane. The temperature rise that occurs because of the enzyme-catalyzed reaction is measured and read as glucose concentration. The major drawback is that external temperature variations also affect the sensor, but the response time (a few seconds) and sensitivity compare favorably with those of other transducer designs.

Optical Detection. The nicotinic acetylcholine receptor can bind drugs of abuse, anesthetics, and antidepressant drugs. Consequently, free drugs in a sample can be determined in a competitive binding assay where the free drug competes with enzyme-labeled drug for a fixed number of receptors. When the enzyme-labeled drug is bound to the acetylcholine receptor, the activity of the enzyme is inhibited, and this loss in activity can be measured colorimetrically. The level of enzyme activity is directly related to the amount of free drug. The enzyme marker system used by Hallowell and Rechnitz was glucose-6-phosphate dehydrogenase (G6PD) labeled with the drug phencyclidine, commonly called PCP (40). Activity was related to absorbance by monitoring the conversion of NAD to NADH at 340 mm by the active G6PD. This method has been shown to respond to nanomolar concentrations of the drugs PCP, trifluoperazine, and desipramine with little or no interference from non-receptor-binding proteins. The sensitivity of this assay is directly correlated with the Beer's law variables of molar extinction coefficient of the absorbing species and optical path length and the ligand's affinity constant since the event being measured is the binding of the analyte. While the en-

zyme immunoassay provides a semirapid (30-min incubation times were given in this paper) screening for analytes with very little sample preparation, it fails in discriminating between structurally related compounds that can show up in other prescription medication.

Enzyme fiberoptic sensors require an indicator system, usually fluorescent, to mediate between the primary enzyme-catalyzed reaction and the optical transducer. These indicators are coimmobilized with an enzyme and respond to the by-products of the enzyme reaction to the substrate of interest. A joint group led by Culp *et al.* used fiber-optic sensors for the measurement of penicillin (41). Penicillin can be detected in combination with a fiber-optic pH sensor due to a pH change in the sensor region because of the enzyme, penicillinase's, reaction product. The enzyme catalyzes the cleavage of the β-lactam ring of penicillin to form penicilloic acid, thus producing a pH change in the sensor microenvironment. A more general coupling scheme has since been described for enzyme optical fiber sensors that has been shown to work with penicillin, organic esters, and urea (42). A second type of optical biosensor was designed to detect ethanol through the fluorescence detection of NADH and the enzyme alcohol dehydrogenase (43). This type of sensor does not require an intermediate chemical reaction, such as pH, because it uses the enzyme reaction directly to generate the optically measurable species NADH.

Trettnak and Wolfbeis have constructed a fiber-optic sensor for cholesterol where cholesterol oxidase is immobilized covalently on a nylon membrane (44). Cholesterol is measured by monitoring the consumption of oxygen with an oxygen optrode as the transducer. The oxygen-sensitive fluorescent dye is incorporated into a thin silicone membrane beneath the enzyme layer. The dynamic range of the sensor is 0.2 to 3.0 m*M* cholesterol at pH 7.45 and reaches full steady state in 7 min, but *in vivo* analysis with this sensor is unlikely since it is affected by changes in pH, O_2, salinity, etc. A fiber-optic lactate sensor designed by this same group also is limited by some of the same problems.

Blum and co-workers have measured NADH with detection limits of 2 and 0.3 n*M* using the bioluminescent enzymatic systems from the marine bacteria, *Vibrio harveyi* and *V. fischeri,* respectively (45). These biosystems were immobilized on preactivated polyamide membranes and observed with an optical fiber. The system had excellent operational stability and reproducibility, a linear range of three orders of magnitude, and 1-min response times. Other work with luminescence-based fiber-optic biosensors have determined ATP, hydrogen peroxide, ethanol, sorbitol, and oxaloacetate.

Enzyme biosensors are strongly affected by pH, ionic strength, heavy metal ions, and organic inhibitors. They also irreversibly lose activity at 50°C and above. There is a need to develop techniques for packaging biosensors that allow use in these hostile environments or development of artificial enzymes that will overcome some of the problems presented by the complex natural molecules. Sensors based on the use of oxidases require a constant oxygen supply, and the elegant method of shuttling electrons directly from the active center of the enzyme onto an electron acceptor still needs to be optimized. Finally, enzyme sensors are not sensitive enough for a variety of applications, and the sensor response needs to be optimized.

Chemoreceptors

Chemoreceptors are biomolecular assemblies of cells that make up the chemical senses, such as olfaction, taste, and other physiological functions. The laboratory of Rechnitz is developing the use of chemoreceptors as biosensors (49), such as intact chemoreceptor structures from crustaceans and fish, to detect chemicals ranging from salts and amino acids to steroids and pheromones. Because aquatic organisms live in fresh or salt water, they have the physiological arrangement necessary for solution sensing and, thus, are excellent candidates as dissolved materials biosensors.

In recent studies (50), portions of the sensing antennules of the blue crab, *Callinectes sapidus,* acted as an amino acid recognition element by joining the chemoreceptor nerve fibers to a micropipette electrode (Fig. 4). They have detected concentrations of the excitatory amino acids, kainic acid and quisqualic acid, at concentrations as low as 10^{-15} *M*. Interaction of the stimulant biomolecule with the chemoreceptor site produces a burst of electrical spikes (action potentials) that travel through the nerve cells and whose frequency is a function of stimulant concentration. The triggering of an ac-

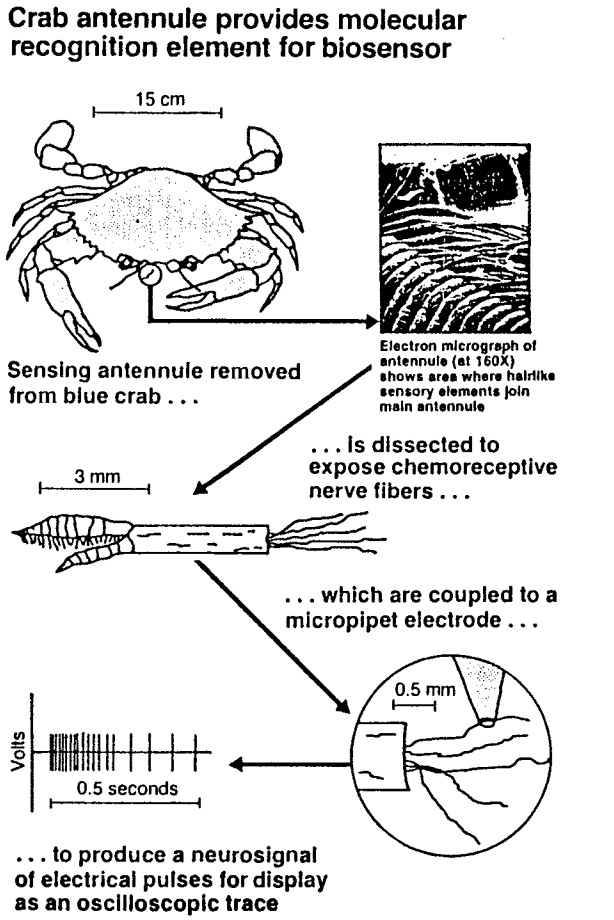


Fig. 4. Chemoreceptor biosensor using the antennule of a crab. Reprinted with permission from Ref. 49.

tion potential and the reestablishment of the resting potential have a response time of only 2 to 3 msec, and the theoretical analytical detection range of the stimulant is almost three orders of magnitude; however, the crab antennae do not respond quantitatively, probably because of other receptors and regulators.

Not all sensors use the intact chemoreceptor structures, but operate with isolated receptors. Karube's group placed a lipid layer containing nicotinic acetylcholine receptor from the electric fish, *Torpedo californica,* on an ion-sensitive field effect transistor (ISFET) and observed limited acetylcholine responses in the micromolar concentration range (51). The sensor is based on the fact that a channel in the receptor is opened in the presence of acetylcholine, which will allow a flux of sodium ions. Since the transistor gate potential is sensitive to ions, a reversible signal can be established that correlates to acetylcholine concentration. More recent work on the determination of acetylcholine and choline (52), as well as L-alanine (53), has expanded on their isolated receptor research.

It would be difficult to envision chemoreceptor biosensors ever making a commercial appearance. The approach would be labor intensive, difficult to reproduce on a large scale, hard to package, and expensive. Also, all the enzyme limitations discussed earlier hold especially for chemoreceptors, and the systems may have poor selectivity, not only because receptors are usually selective for classes of chemical compounds, but also because of the presence of a variety of different receptors in the same tissue. The systems are also difficult to interface to transducer systems while retaining mechanical stability and good functional characteristics.

COMMERCIAL PERSPECTIVES

Biosensors have shown their first real promise in medical applications. Instrumentation for patient monitoring is gaining market share driven by medical, legal, and cost considerations. Timely medical information is essential for effective administration of the patient in the hospital or physicians office (54). To contain costs, home monitoring will become more important in health care, as physiological changes in certain conditions (diabetics, pregnancy, colorectal cancer, and others) can be detected early by the patient (55). Finally, future advances in miniature, implantable drug delivery systems will require accurate, long-lived sensors for closed-loop feedback to help regulate drug delivery rates directly into the bloodstream.

The use of biosensors in clinical testing at a patient's bedside or in a physician's office instead of at centralized laboratories allows rapid therapeutic decisions and thus improves patient care. Many of the major medical laboratory suppliers are developing smaller bench-top general chemistry analyzers designed for use in the physicians' office. Eastman Kodak (Rochester, N.Y.) 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 nitch. The fully automated Kodak Ektachem 400 has been on the market for some time and performs 10 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 physicians' office.

Two technologies have driven the development of these smaller instruments. The first is the development of solid-state stabilized reagents. While 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 have 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.

Several companies are developing the next-generation, hand-held-size, portable instruments 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 be like a menu of the most common tests for a specific organ or disease. Examples are a panel for general chemistry, hepatic, hematology, renal, 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 pO_2, pCO_2, pH, sodium, potassium, calcium, and hematocrit; 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 data on sodium, potassium, chloride, urea nitrogen, glucose, and hematocrit in less than 2 min. The ACCUMETER cholesterol test from ChemTrak is designed for a lipid screen to evaluate cardiovascular disease risk, and ABAXIS' MiniLab MCA will be a general chemistry analyzer that uses electrochemical sensors with a novel fluid handling scheme using a centrifugal analyzer, but it is not currently on the market. While all 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. An overview of these technologies can be found in the literature (56,57).

Home health-care technology has been available since the 1960s, with the development of semiquantitative glucose,

urine, and, finally, 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 sec 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 looked at for measurement in this format are lactate, pyruvate, urea, lactose, galactose, alcohols, and L-amino acids (58).

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 sensor; a number of 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 (35). Unfortunately, long lifetimes have not been established due to 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.

A report published by Frost and Sullivan, titled "The U.S. Market for Smart Sensors" (59), states that while the biosensor industry is small at the moment, commercially speaking, they are expected to grow 45% per year over the 1990–1995 period, resulting in the conversion of much of the $54 billion/year medical laboratory service business into point-of-care analysis. The enthusiasm for the technology is rampant, but 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 capitalist who can accept the risk in view of the growth potential.

CONCLUSIONS

Biosensors are undergoing rapid development. Low equipment and materials costs will provide new opportunities to cut health-care costs. Biosensors are well suited to meet medicine's future requirements because of the almost-limitless range of reactions available from biochemical interactions and the possibility of low cost and rapid response in determining biological compounds. They will play a valuable role in the next generation of clinical instruments.

Despite the tremendous potential of biosensors, researchers face many hurdles. Some of the research opportunities include better methods for immobilizing biosensitive materials onto the sensor structures, developing membrane materials that resist surface occlusion and optimize transport and finding chemistries that are stable through the range of the working environment.

Immunosensors are specific, but most have problems in either binding or sensitivity. A relatively low ligand–receptor binding constant results in reversibility but low sensitivity. A very strong binding constant has a high sensitivity but no reversibility. Means to regulate the binding constant must be found for the construction of most sensor applications.

Enzyme biosensors are affected by ions and temperature. There is a need to develop techniques for packaging biosensors that allow use in these hostile environments or development of artificial enzymes that will overcome some of the problems presented by the complex natural molecules. Finally, enzyme sensors are not sensitive enough for a variety of applications, and the response of the sensor systems needs to be optimized.

Similar limitations hold for chemoreceptors, which may have poor selectivity. Chemoreceptors are also difficult to interface to transducer systems while maintaining mechanical stability and function.

This review conveys the breadth of the research done on biosensors without citing each potential article. If current trends continue, we can expect a productive decade ahead for biosensor research and commercialization.

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