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Introduction to the principles and applications of biosensors

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A biosensor is an analytical device that responds to an analyte in an appropriate sample and interprets its concentration as an electrical signal via a suitable combination of a biological recognition system and an electrochemical transducer. As a result of recent scientific and technological progress, such devices are likely to play an increasingly important role in generating analytical information in all sectors of human endeavour, from medicine to the military. In particular, biosensors will form the basis of cheap, simple devices for acquiring chemical information, bringing sophisticated analytical capabilities to the non-specialist and general public alike. The market opportunities for the rapid exploitation of novel developments in this sector are substantial. Biosensor research is also likely to have a significant impact on the development of modern electronics.

Sensors in analysis

The endeavours of technologically advanced societies are increasingly reliant on the estimation, monitoring and control of chemical species. This requirement for analytical information applies to all sectors of activity, including health care and veterinary medicine, the food, pharmaceutical, bioprocessing and petrochemical industries, environmental monitoring and control, defence and agriculture. A major part of this chemical intelligence is acquired via the operation of sophisticated analytical laboratories, often within centralized facilities, which are intensive of both capital and skilled labour. However, there are many circumstances for which such arrangements are inadequate. For example, in medicine, timeliness in both the testing and monitoring of marker analytes can be critical in the diagnosis and later treatment of disease. This need is being expressed as a decentralization of laboratory analyses into the bedside, surgery and home environments. Many diagnostic tests are already executed in physicians' offices in the U.S.A. in those cases where rapid, simple analytical techniques are readily available. Thus approximately 70% of haematology tests and over 90% of urine analyses are performed in this way. In contrast, where current analytical procedures are more complex, for example in therapeutic drug monitoring and immunology, there remains a substantial dependence on the central laboratory, with its inevitable delay in obtaining the information. Not surprisingly, therefore, only 4% of immunological testing in the U.S.A. is presently performed in general practice facilities.

The concept of bringing the analytical laboratory to the patient is likely to revolutionize clinical practice. Indeed, tests such as those for pregnancy and for urine or blood glucose are already widely available for self-diagnosis, particularly in the U.S.A. However, the full realization of this prospect will require the development of novel chemical sensor technologies

for a wide range of analytes. Such sensors would need to be sensitive, selective, rapid in response, inexpensive and easy to use. These devices would also complement (and sometimes replace) the use of costly machines. In addition, they offer the potential for continuous online analysis in, for example, critical care units and in non-medical applications such as industrial process monitoring and hydroponics. New sensors would also be useful in the replacement of existing bioassays for monitoring explosives and water quality and in those circumstances requiring remote sensing in adverse environments. A plethora of analytes is implicated in these diverse applications; thus, new sensor technology will be required to exhibit the appropriate specificity (Lowe 1984). Figure 1 summarizes the range of analytes and their relevance to different sectors

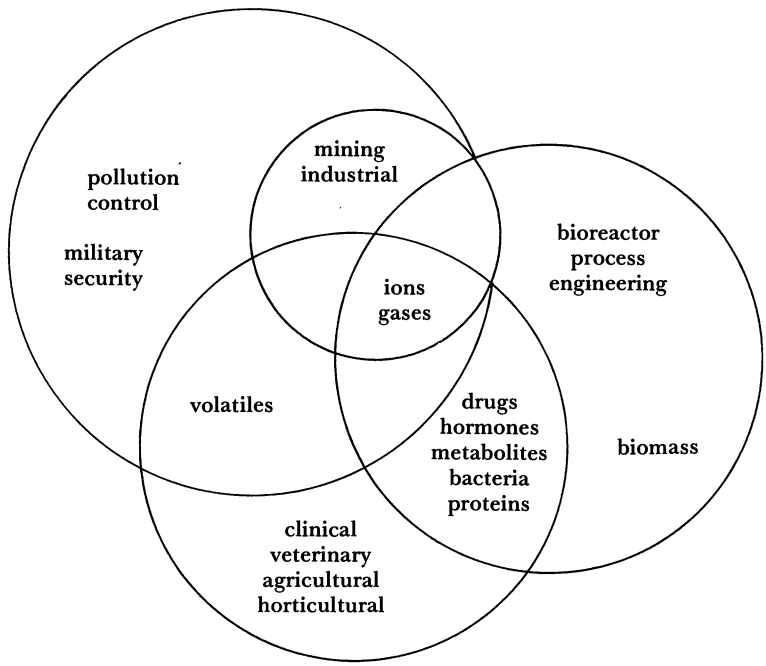
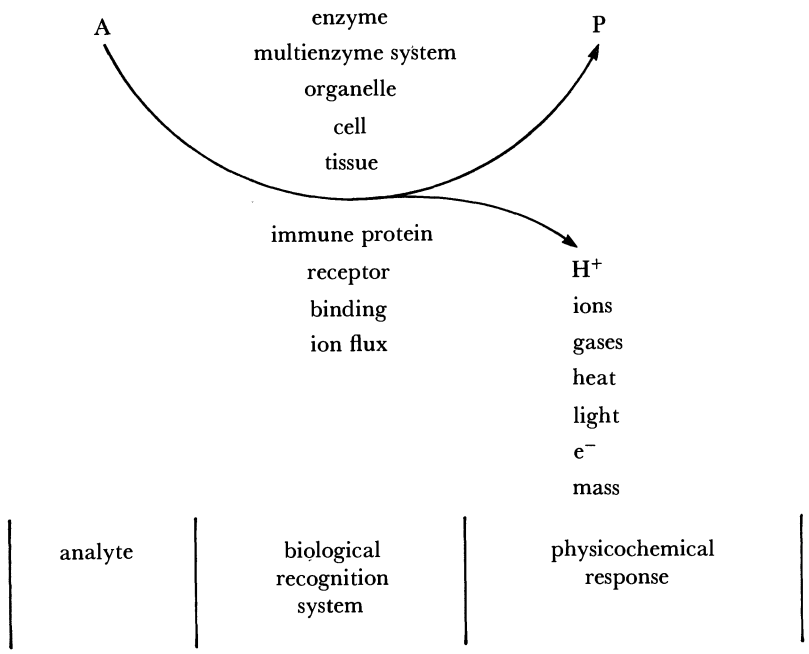


FIGURE 1. Potential analytes and their applications sectors.

of application. An important category of analytes is gases, such as respiratory (O_2, CO_2), toxic (NH_3, Cl_2, CO, H_2S, HCN), nerve, anaesthetic (N_2O, halophane) and flammable (CH_4, butane), which may be present in liquid samples (body fluids), carrier gases or atmospheric samples. Ions such as H^+, monovalent cations (Li^+, Na^+, K^+, NH_4^+), divalent cations (Ca^{2+}, Mg^{2+}), phosphate and heavy-metal ions (Cu^{2+}, Cd^{2+}, Hg^{2+}) provide attractive targets for sensors of appropriate specificity. Similarly, metabolites present at relatively high concentrations (glucose, urea, triglycerides, cholesterol) and those present in trace concentrations (hormones, steroids, therapeutic drugs) provide commercially significant targets for the medical and the agricultural food industries. Some of the trace materials may have concentrations as low as 10^{-18} M. Volatile analytes such as flavours, essences, pheromones, explosives and toxic vapours (benzene, toluene, toluene diisocyanate) also represent acceptable targets for many sectors. Finally, the analysis of specific viruses, bacteria, parasites and non-specific biomass has important implications in the clinical, veterinary, agricultural, horticultural, food processing, engineering and biotechnological sectors.

Fundamental principles of biosensors

A putative sensor must be able to distinguish the target analyte from a host of inert and potentially interfering species (Lowe 1985). For example, the concentration of some proteins in blood serum may be no more than 1 μg l^{-1} in a total protein concentration of approximately 70 g l^{-1}, requiring a discriminating power of 1 in 10^7–10^8 to recognize specifically the target analyte. Thus, particularly with biological samples, the sensor must often display a remarkable degree of specificity for the analyte, while retaining the appropriate sensitivity for monitoring the analyte in the concentration range at which it is found in the sample. Generally speaking, it is only biological molecules that display the required combination of specificity and sensitivity to provide universal recognition systems for incorporation as the essential, sensing elements in electrochemical devices. A biosensor may thus be defined as a device that recognizes an analyte in an appropriate sample and interprets its concentration as an electrical signal, via a suitable combination of a biological recognition system and an electrochemical transducer. Scheme 1 shows that the biological recognition system, typically an enzyme, multienzyme system,



Scheme 1. A generalized biological recognition–response system. Abbreviations: A, analyte; P, product.

organelle, whole microorganism, plant or animal cell, tissue slice or immune, binding or receptor protein, is responsible for the specific recognition of the analyte and subsequent response with a change in one or more physicochemical parameters associated with the interaction. For example, the biological interaction may result in a change of proton concentration, or release or uptake of gases (CO_2, O_2, NH_3), specific ions (NH_4^+, anions), heat or electrons, or it may result in a change in an optical parameter of the system (optical density, refractive index) which, if generated in close proximity to a suitable transducer, may be converted into an electrical signal. Figure 2 illustrates the general principle of a biosensor and

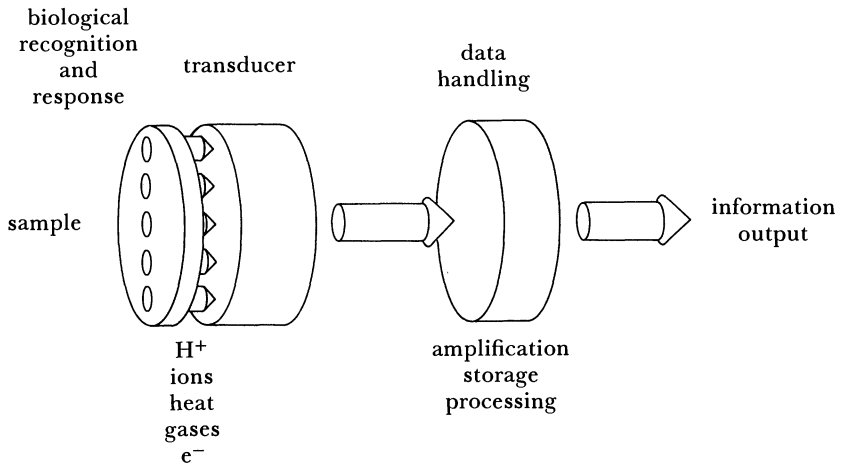


FIGURE 2. Schematic of a generalized biosensor.

emphasizes the importance of the close juxtaposition between the biological recognition–response system and the transducer, which converts the biological signal into an electrical one before the signal is amplified, processed and converted to the desired format. Intimate contact between the biological and electrochemical systems is generally achieved by immobilization of the biosystem on the device surface by chemical cross-linking with a bifunctional reagent, by physical restraint behind a polymer membrane or within a gel matrix, or by direct covalent attachment. With an appropriate combination of these procedures, it is usually possible to construct specific and sensitive biosensors for the quantitation of a number of potential analytes by coupling the recognition powers of biological systems with appropriate transducers.

TRANSDUCER TECHNOLOGIES

Transducers are devices that respond to the products of the biocatalytic or binding process. Table 1 categorizes transducers into four main types: potentiometric, amperometric, optical and other devices. Potentiometric devices function under equilibrium conditions and measure the accumulation of charge density at the electrode surface, encouraged by a selective binding

TABLE 1. CLASSIFICATION OF TRANSDUCERS

class	examples
potentiometric	ion-selective electrode (ISE)
	ion-selective field-effect transistor (ISFET)
	gas-selective electrode
amperometric	metal electrodes
	mediated systems
	conducting organic salts
optical	ellipsometry
	planar waveguide
	fibre optic
	surface plasmon resonance
other	thermistor
	surface conductance
	piezoelectric/surface acoustic wave (SAW)

or catalytic process. For example, ion-selective electrodes (ISEs) monitor ions in solution by sensing changes in electrode potential arising from binding ions to an appropriate selective membrane. More recently, ion-selective membranes have been used to replace the metallized gates of field-effect transistors to generate ion-selective field-effect transistors (ISFETs). Both types of device have now been fabricated with membranes sensitive to H^+, Na^+, K^+, Li^+, NH_4^+, Ca^{2+}, anions, drugs, and gases such as CO_2 and NH_3. In addition, the combination of ion-selective devices with enzyme and immunological recognition–response systems has extended the range of potentiometric devices to enzyme substrates and antigens. Furthermore, biosensors based on integrated solid-state devices (ISFETs, enzyme FETs, immunoFETs) display considerable potential for miniaturization, for multiple homo- and heterosensor configurations and for 'intelligent' functions incorporating interrogation, redundancy and statistical evaluation techniques.

Amperometric or current-measuring sensors monitor faradaic currents that arise when electrons are exchanged between a biological system and an electrode held at an appropriate potential. Biosensors exploiting direct electron transfer from an enzyme catalyst to an electrical system have been reported but have so far remained impracticable. However, various approaches designed to divert electrons from their natural electron acceptors have proved more successful. Most of these exploit electron-transfer mediators to shuttle electrons from the prosthetic group of the enzyme to the electrode. An ideal mediator should accept electrons rapidly from the reduced biocatalyst and exhibit effective electrochemistry at a practical electrode. The mediator should be stable in both oxidized and reduced forms (with the reduced form being unreactive with oxygen), be amenable to immobilization and display a sufficiently low redox potential to obviate interferences due to oxidation of extraneous species at the electrode surface. Mediators such as benzoquinone, 2,6-dichlorophenolindophenol, hexacyanoferrate and ferrocene derivatives have all been used to couple electrochemically the glucose oxidase reaction, for example, to appropriate recipient electrodes.

Similarly, a number of workers have reported the use of modified glassy carbon and graphite electrodes, and, more recently, the conducting salt NMP^+ $TCNQ^-$, for the mediated oxidation of the reduced nicotinamide coenzyme, NADH. Such electrodes convert the ubiquitous coenzyme couple NAD^+–NADH and thereby provide a means of quantifying many oxidoreductase substrates.

End-point detection by amperometry has been applied to the immunoassay of trace metabolites. However, more direct procedures have also been applied to detect the antigen–antibody interaction. For example, evanescent wave immunoassay exploits antibodies or other binding proteins immobilized on the surface of an optically transparent waveguide. Light, internally reflected within a fibre optic or planar waveguide, is affected by adsorption of biological materials at the optical interface. Surface plasmon resonance measures the increase in thickness of a dielectric layer on thin films of metals deposited on glass prisms or diffraction gratings. Both procedures have the potential for use in probes and for the reagentless monitoring of immune reactions. Such direct-reading optical devices could be developed for drug and serum protein assay, blood banking, tissue typing and microbiological testing.

Invariably, biological interactions take place with changes in other physicochemical parameters, notably enthalpy, ionic conductance and mass. Such effects can be exploited by coupling the biocatalytic reaction with appropriate transducers such as thermistors, surface conductance probes and piezoelectric or surface acoustic wave devices.

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Exploitation of biosensors

Biosensors, in the form of fairly complex analytical devices containing immobilized enzymes and microorganisms, are already available. They enjoy a modest level of exploitation in the areas of food and fermentation analysis, health care, and defence and environmental monitoring. For example, probably the largest biosensor business today involves the production of nerve-gas sensors for the military; these devices are based on electrochemical systems that incorporate immobilized acetylcholinesterase and are manufactured in the U.K. by Thorn EMI–Simtec. Although a variety of other biosensors is available, the only devices currently of commercial significance are the various enzyme-based glucose analysers, with an existing market value of several million pounds sterling per year. Most of the existing commercial sensors are indirect enzyme electrodes; for example, most glucose analysers measure the current generated at a high-potential platinum electrode when hydrogen peroxide is generated by glucose oxidase in the presence of its substrate. Much of the complexity and expense of such instruments is caused by the constraints imposed by the properties of the biosensor element, including inappropriate kinetics for use on undiluted samples, sensitivity to oxygen concentration and the need to exclude analytes that may interfere, particularly at the high potentials involved.

There is considerable scope for the further development of such first-generation biosensor devices for a wide range of analytes; these will sometimes involve sophisticated enzymology (for example, enzyme amplification). However, recent scientific advances, particularly in the area of enzyme electrochemistry, offer the prospects of cheap, simple, easily used sensors for a range of analytes based on electron transfer from enzyme redox centres to electrodes composed of modified carbon or conducting salts. These second-generation biosensors are discussed by Frew & Hill and by Albery (this symposium) and are expected to appear on the market over the next year or two.

An interesting example of a potentially important clinical application of a second-generation amperometric sensor for glucose is shown in figure 3. This sensor is based on glucose oxidase immobilized on a low-potential carbon electrode containing an insoluble ferrocene mediator. The technology, originally developed jointly between groups at Cranfield and Oxford (Cass *et al.* 1984) is being used at Guy's Hospital to make small electrodes, which are implanted subcutaneously. The response of the sensor, implanted in pigs, to injection of insulin is compared with the effect on blood glucose concentration, measured in blood samples by means of a first-generation system (Yellow Springs Analyser). Similar electrodes are being tested in humans with a view to developing a hypoglycaemia alarm for diabetics and, eventually, a sensor for use with an artificial pancreas. Third-generation biosensors will involve a most intimate association of the biological element within the electronic device itself, for example incorporation of an enzyme within a conducting polymer or semiconductor material (Foulds & Lowe 1985).

An extremely important area of biosensor research, primarily for medical applications, is immunodiagnostics. Biosensor technology is likely to make a major impact in this area, particularly through the development of simple, cheap, rapid, single-step immunodiagnostic tests. Several approaches are discussed by Aizawa and by Green (this symposium). For example, the development of electrochemical methods that involve the use of mediators is one approach that could simplify existing ELISA techniques by achieving an amperometric endpoint rather than requiring the generation of a coloured product for later measurement. One imaginative

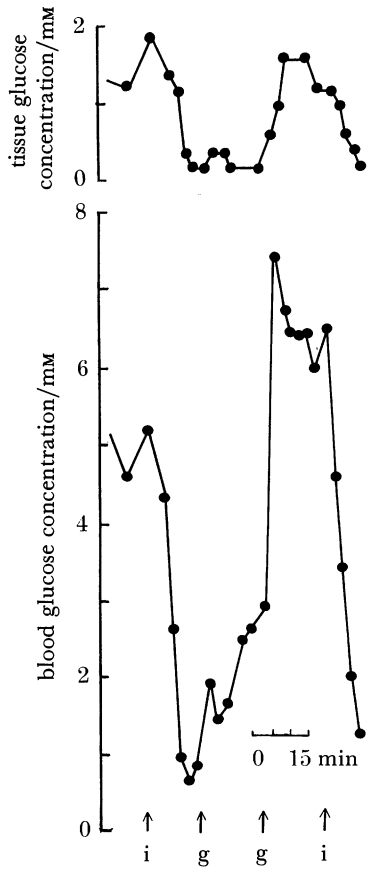

FIGURE 3. Tissue glucose concentrations measured by a subcutaneously implanted ferrocene micro-glucose sensor, compared with concurrently measured blood glucose levels assayed with a Yellow Springs Instruments analyser. Abbreviations: ins, 5 units insulin injected intravenously (i.v.); g, 5 g glucose injected i.v. (Adapted from Claremont & Pickup (1987).)

approach, which may lead to a simple amperometric test strip, involves the detection of a common enzyme label, alkaline phosphatase, by the generation of an electrochemical signal (McNeil & Bannister 1986). The enzyme hydrolyses a phosphate derivative of ferrocene to a phenolic derivative, which has different electrochemical properties and can be detected amperometrically with great sensitivity.

Other promising methods for exploitation of biosensors in immunodiagnostics include optical techniques, a particularly novel example of which is described by Shanks (this symposium). Recently, it has been shown that conductimetry may be applied, although the method shows greater prospects for measuring analytes, such as urea, for which there is not an appropriate redox enzyme.

Over the past few years, biosensor research and development has largely concentrated on the biomedical area. There are several reasons for this: it represents almost one third of the total analytical market; the market is receptive to short-lived or disposable devices and enzyme-based products; and it is large enough in many cases to recover the substantial development costs. The development and adoption of biosensors in other areas is generally slower. This is often because of problems of inadequate stability or inadequate market size to justify the development

costs to a potential biosensor producer. However, the realization that such sensors, when used in, for example, process control, may make substantial improvements in process economics, is now leading to more investment in appropriate research and development by potential users. Prospects for applications in process control are discussed by Clarke (this symposium).

One interesting example of biosensor applications for non-medical purposes is an electrochemical biomass monitor giving approximate microbial numbers in less than one minute. This is based on the principle of using mediators with low molecular masses to connect metabolic electronic activity to a metal anode (Higgins *et al.* 1986). There are many applications for such a sensor, including estimation of microorganisms in milk, food processing and cutting fluids. It could, of course, also find application in clinical analysis and would be particularly useful if it could be developed as a species-specific probe by combining the electrochemical concept with immunological recognition. Another non-medical example employs a multi-electrode glucose sensor to estimate glucose concentrations at different distances from the surface of meat (Higgins *et al.* 1986). The profile is known to indicate the microbiological status of the surface of the meat, and thus such a probe (with an associated microprocessor) could be developed into a pocket device for rapid meat-quality monitoring in, for example, wholesale, distribution and retail businesses.

Future prospects

There are interesting analogies to be drawn between biosensor research and recombinant DNA technology. In particular, there is a close link between existing science (for example, molecular electronics and protein electrochemistry) and valuable commercial products. The market potential for biosensors is considerable in the light of the existing world analytical market, in the region of £15000M per year. Technological development, by making analysis more accessible to the non-specialist and the public, could expand this already massive market substantially.

Many areas of science are involved in this multidisciplinary activity, including chemistry, electronics, enzymology, immunology, microbiology, molecular electronics, physics, recombinant DNA technology and protein engineering. The major limitation on biosensor development is currently lack of availability of an adequate range of biological sensor elements. This is primarily the domain of the enzymologist, microbiologist and immunologist. There is a tendency to underestimate the importance of these subjects in relation to biosensors; for example, relatively little effort is devoted to seeking microbial activities of particular relevance. It is therefore likely that there will be increasing integration of such scientists into biosensor research programmes.

Both simple and sophisticated protein engineering techniques will play a major role in adapting existing biocatalytic activities to the practical requirements of analysis by, for example, changing the kinetic, specificity, electrochemical and stability properties of enzymes or even creating totally novel activities. Recombinant DNA technology and nucleic acid chemistry will also play an increasingly important role as biosensors for genetic analysis are created.

Concerning transducers, it is not clear whether the electrochemical systems will retain their preeminence in practical devices. Some of the increasing research activity with a range of optical devices shows considerable promise, even for disposable sensors. Electrochemical, optical and

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other approaches are likely to benefit from the application of discoveries in molecular electronics.

Increasing application of biosensors for non-medical monitoring and analysis is anticipated in the next few years. Perhaps the most exciting, but least predictable, consequences of biosensor research lie in the area of bioelectronics, the association of biological elements or principles with electronic systems. Much conjecture and doubtless much fiction has already been generated around this subject. We do not wish to add to this, only to provoke thought.

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