Biosensors

A biosensor is an analytical device which converts a biological response into an electrical signal (Figure 6.1). The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly.

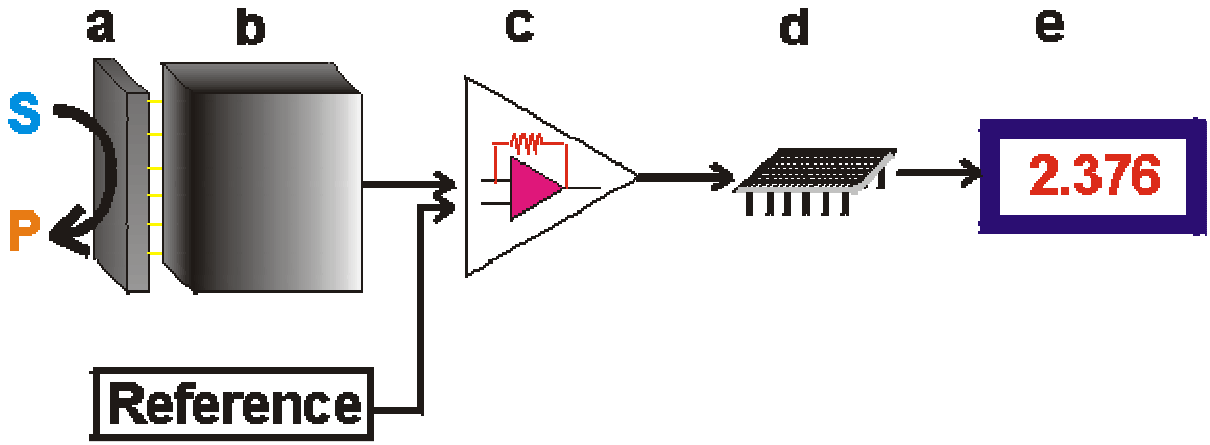

Figure 6.1. Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry (e.g. 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring.

Research and development in this field is wide and multidisciplinary, spanning **biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering.** Most of this current endeavour concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips. However, all the main transducer types are likely to be thoroughly examined, for use in biosensors, over the next few years. A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be **highly specific** for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks (see later), show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as **independent of such physical parameters as stirring, pH and temperature** as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme.
3. The response should be **accurate, precise, reproducible and linear** over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be **tiny and biocompatible**, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable.
5. The complete biosensor should be **cheap, small, portable** and capable of being **used by semi-skilled operators.**

The key part of a biosensor is the transducer (shown as the 'black box' in Figure 6.1) which makes use of a physical change accompanying the reaction. This may be

1. the heat output (or absorbed) by the reaction (**calorimetric biosensors**),
2. changes in the distribution of charges causing an electrical potential to be produced (**potentiometric biosensors**),
3. movement of electrons produced in a redox reaction (**amperometric biosensors**),
4. light output during the reaction or a light absorbance difference between the reactants and products (**optical biosensors**), or
5. effects due to the mass of the reactants or products (**piezo-electric biosensors**).

There are three so-called 'generations' of biosensors:

- **First generation** biosensors where the normal product of the reaction diffuses to the transducer and causes the electrical response.
- **Second generation** biosensors which involve specific 'mediators' between the reaction and the transducer in order to generate improved response
- **Third generation** biosensors where the reaction itself causes the response and no product or mediator diffusion is directly involved.

The electrical signal from the transducer is often low and superimposed upon a relatively high and noisy (i.e. containing a high frequency signal component of an apparently random nature, due to electrical interference or generated within the electronic components of the transducer) baseline. The signal processing normally involves subtracting a 'reference' baseline signal, derived from a similar transducer without any biocatalytic membrane, from the sample signal, amplifying the resultant signal difference and electronically filtering (smoothing) out the unwanted signal noise. The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration.

Calorimetric biosensors

Many enzyme catalysed reactions are exothermic, generating heat (Table 6.1) which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. This represents the most generally applicable type of biosensor. The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment (Figure 6.2). Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream. This may be simply calculated from the enthalpy change and the amount reacted. If a 1 mM reactant is completely converted to product in a reaction generating 100 kJ $mole^{-1}$ then each ml of solution generates 0.1 J of heat. At 80% efficiency, this will cause a change in temperature of the solution amounting to approximately 0.02°C.

Table 6.1. Heat output (molar enthalpies) of enzyme catalysed reactions.

Reactant	**Enzyme**	**Heat output ~H (kJ $mole^{-1}$)**
Cholesterol	Cholesterol oxidase	53
Esters	Chymotrypsin	4 - 16
Glucose	Glucose oxidase	80
Hydrogen peroxide (nadtlenek)	Catalase	100
Penicillin G	Penicillinase	67
Peptides	Trypsin	10 - 30
Starch (skrobia)	Amylase	8
Sucrose	Invertase	20
Urea (mocznik)	Urease	61
Uric acid	Uricase	49

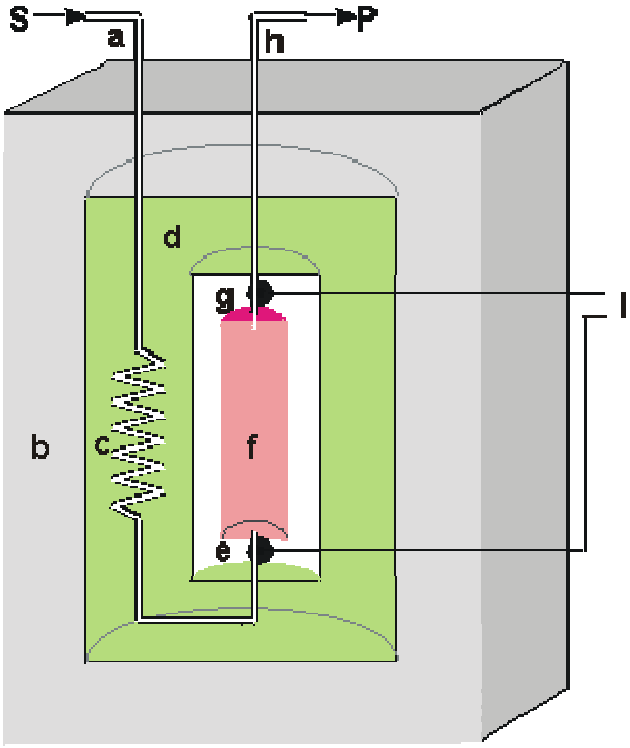

Figure 6.2. Schematic diagram of a calorimetric biosensor. The sample stream (a) passes through the outer insulated box (b) to the heat exchanger (c) within an aluminium block (d). From there, it flows past the reference thermistor (e) and into the packed bed bioreactor (f, 1ml volume), containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor (g) and the solution passed to waste (h). External electronics (l) determines the difference in the resistance, and hence temperature, between the thermistors.

The thermistors, used to detect the temperature change, function by changing their electrical resistance with the temperature, obeying the relationship

$$Ln\left(\frac{R_1}{R_2}\right) = B\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

therefore:

$$\frac{R_1}{R_2} = e^{B\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where R_1 and R_2 are the resistances of the thermistors at absolute temperatures T_1 and T_2 respectively and B is a characteristic temperature constant for the thermistor. When the temperature change is very small, as in the present case, $B(1/T_1) - (1/T_2)$ is very much smaller than one and this relationship may be substantially simplified using the approximation when $x<<1$ that $e^x \cong 1 + x$ (x here being $B(1/T_1) - (1/T_2)$,

$$R_1 = R_2\left\{1 + B\left(\frac{T_2 - T_1}{T_1 T_2}\right)\right\}$$

As $T_1 \cong T_2$, they both may be replaced in the denominator by T_1.

$$\frac{\Delta R}{R} = -\left(\frac{B}{T_1^2}\right)\Delta T$$

The relative decrease in the electrical resistance ($\Delta R/R$) of the thermistor is proportional to the increase in temperature (ΔT). A typical proportionality constant ($-B/T_1^2$) is $-4\%°C^{-1}$.

The resistance change is converted to a proportional voltage change, using a balanced Wheatstone bridge incorporating precision wire-wound resistors, before amplification. The expectation that there will be a linear correlation between the response and the enzyme activity has been found to be borne out in practice. A major problem with this biosensor is the difficulty encountered in closely matching the characteristic temperature constants of the measurement and reference thermistors. An equal movement of only 1°C in the background temperature of both thermistors commonly causes an apparent change in the relative resistances of the thermistors equivalent to 0.01°C and equal to the full-scale change due to the reaction. It is clearly of great importance that such environmental temperature changes are avoided, which accounts for inclusion of the well-insulated aluminium block in the biosensor design (see Figure 6.2).

The sensitivity (10^{-4} M) and range (10^{-4} - 10^{-2} M) of thermistor biosensors are both quite low for the majority of applications although greater sensitivity is possible using the more exothermic reactions (e.g. catalase). The low sensitivity of the system can be increased substantially by increasing the heat output by the reaction. In the simplest case this can be achieved by linking together several reactions in a reaction pathway, all of which contribute to the heat output. Thus the sensitivity of the glucose analysis using glucose oxidase can be more than doubled by the co-immobilisation of catalase within the column reactor in order to disproportionate the hydrogen peroxide produced.

Potentiometric biosensors

Potentiometric biosensors make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal.

Jak wiadomo metal (lub niemetal) zanurzony w roztworze, zawierającym jego jony otrzymuje w stosunku do roztworu pewien potencjał, który w określony sposób zależy od stężenia (aktywności) jonów tego metalu. Tak np. jeśli zanurzymy srebrny drut w wodzie to pewna ilość srebra przejdzie do roztworu, tworząc jony Ag+, a metal otrzyma ładunek ujemny i powstanie w ten sposób różnica potencjałów. Gdy zaś do wody z zanurzonym drutem srebrnym doda się soli srebrowej, wtedy powstanie ciśnienie osmotyczne skierowane w przeciwną stronę i zależne od stężenia jonów srebrowych. Gdy stężenie to jest niewielkie , przeważa prężność roztwórcza i różnica potencjałów pozostanie, chociaż będzie mniejsze niż w przypadku zanurzenia drutu srebrnego w wodzie.

Gdy będziemy zwiększać stężenie jonów srebrowych , doprowadzimy do zrównoważenia potencjałów, wówczas nie będzie żadnego ładunku na metalu. Kiedy zwiększymy je jeszcze bardziej stężenie jonów srebrowych i ciśnienie osmotyczne przewyższy prężność roztwórczą srebra, część jonów rozładuje się na metalu i naładuje go dodatnio. Jak wynika z powyższych rozważań, różnica potencjałów może być miarą stężenia jonów danej substancji w roztworze

In the simplest terms this consists of an immobilised enzyme membrane surrounding the probe from a pH-meter (Figure 6.3), where the catalysed reaction generates or absorbs hydrogen ions (Table 6.2). The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display. Typical of the use of such electrodes is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

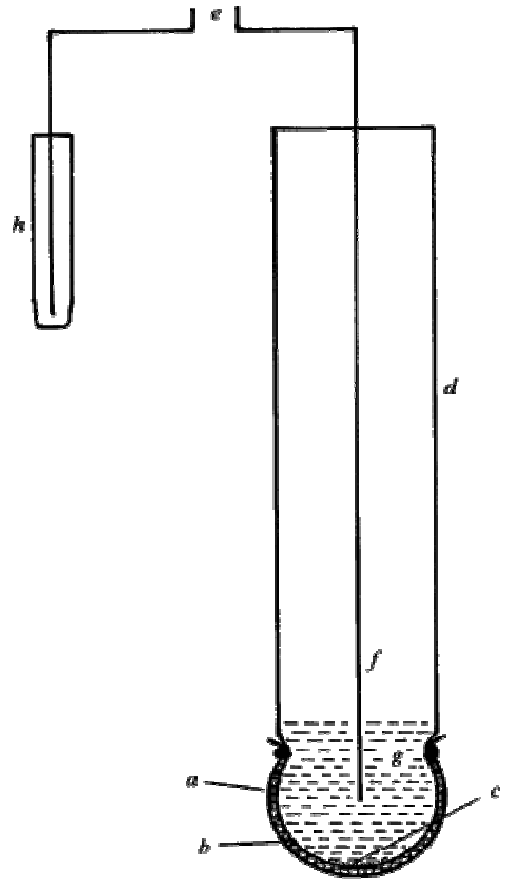

Figure 6.3. A simple potentiometric biosensor. A semi-permeable membrane (a) surrounds the biocatalyst (b) entrapped next to the active glass membrane (c) of a pH probe (d). The electrical potential (e) is generated between the internal Ag/AgCl electrode (f) bathed in dilute HCl (g) and an external reference electrode (h).

There are three types of ion-selective electrodes which are of use in biosensors:

1. Glass electrodes for cations (e.g. normal pH electrodes) in which the sensing element is a very thin hydrated glass membrane which generates a transverse electrical potential due to the concentration-dependent competition between the cations for specific binding sites. The selectivity of this membrane is determined by the composition of the glass. The sensitivity to H^+ is greater than that achievable for NH_4^+,
2. Glass pH electrodes coated with a gas-permeable membrane selective for CO_2, NH_3 or H_2S. The diffusion of the gas through this membrane causes a change in pH of a sensing solution between the membrane and the electrode which is then determined.
3. Solid-state electrodes where the glass membrane is replaced by a thin membrane of a specific ion conductor made from a mixture of silver sulphide and a silver halide. The iodide electrode is useful for the determination of I^- in the peroxidase reaction (Table 6.2c) and also responds to cyanide ions.

The response of an ion-selective electrode is given by

$$E = E_0 + \frac{RT}{zF} Ln([i])$$

where E is the measured potential (in volts), E_0 is a characteristic constant for the ion-selective/external electrode system, R is the gas constant, T is the absolute temperature (K), z is the signed ionic charge, F is the Faraday, and [i] is the concentration of the free uncomplexed ionic species (strictly, [i] should be the activity of the ion but at the concentrations normally encountered in biosensors, this is effectively equal to the concentration).

This means, for example, that there is an increase in the electrical potential of 59 mv for every decade increase in the concentration of H^+ at 25°C. **The logarithmic dependence of the potential on the ionic concentration is responsible both for the wide analytical range and the low accuracy and precision of these sensors**. Their normal range of detection is 10^{-4} - 10^{-2} M, although a minority are ten-fold more sensitive. Typical response time are between one and five minutes allowing up to 30 analyses every hour.

Biosensors which involve H^+ release or utilisation necessitate the use of very weakly buffered solutions (i.e. < 5 mM) if a significant change in potential is to be determined. The relationship between pH change and substrate concentration is complex, including other such non-linear effects as pH-activity variation and protein buffering. However, conditions can often be found where there is a linear relationship between the apparent change in pH and the substrate concentration.

A recent development from ion-selective electrodes is the production of ion-selective field effect transistors (**ISFET**s) and their biosensor use as enzyme-linked field effect transistors (**ENFET**s, Figure 6.4). Enzyme membranes are coated on the ion-selective gates of these electronic devices, the biosensor responding to the electrical potential change via the current output. Thus, these are potentiometric devices although they directly produce changes in the electric current. The main advantage of such devices is their extremely small size (<< 0.1 mm^2) which allows cheap mass-produced fabrication using integrated circuit technology. As an example, a urea-sensitive FET (ENFET containing bound urease with a reference electrode containing bound glycine) has been shown to show only a 15% variation in response to urea (0.05 - 10.0 mg ml^{-1}) during its active lifetime of a month. Several analytes may be determined by miniaturised biosensors containing arrays of ISFETs and ENFETs. The sensitivity of FETs, however, may be affected by the composition, ionic strength and concentrations of the solutions analysed.

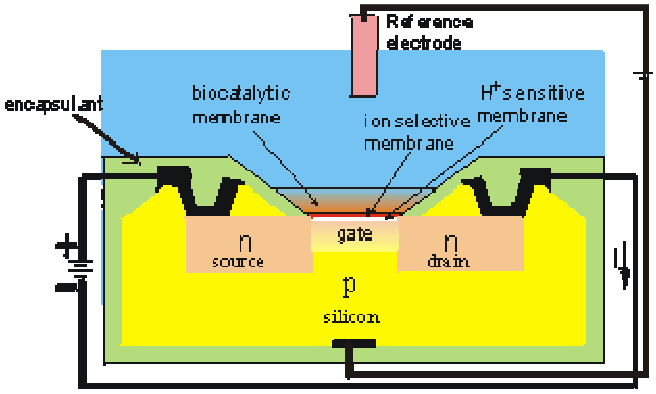

Figure 6.4. Schematic diagram of the section across the width of an ENFET. The actual dimensions of the active area is about 500 □m long by 50 □m wide by 300 □m thick. The main body of the biosensor is a p-type silicon chip with two n-type silicon areas; the negative source and the positive drain. The chip is insulated by a thin layer (0.1 □m thick) of silica (SiO_2) which forms the gate of the FET. Above this gate is an equally thin layer of H^+-sensitive material (e.g. tantalum oxide), a protective ion selective membrane, the biocatalyst and the analyte solution, which is separated from sensitive parts of the FET by an inert encapsulating polyimide photopolymer. When a potential is applied between the electrodes, a current flows through the FET dependent upon the positive potential detected at the ion-selective gate and its consequent attraction of electrons into the depletion layer. This current (I) is compared with that from a similar, but non-catalytic ISFET immersed in the same solution. (Note that the electric current is, by convention, in the opposite direction to the flow of electrons).

Amperometric biosensors

High sensitivity, selectivity, and ability to operate in turbid solutions are advantages of electrochemical biosensors. **Amperometric detection is based on measuring the oxidation or reduction of an electroactive compound at a working electrode** (sensor). A potentiostat is used to apply a constant potential to the working electrode with respect to a second electrode (reference electrode). A potentiostat is a simple electronic circuit that can be constructed using a battery, two operational amplifiers, and several resistors.

The applied potential is an electrochemical driving force that causes the oxidation or reduction reaction. The potential of the reference electrode is well defined through equilibrium, as in the following reaction:

$$Ag_{(Solid)} + Cl^- \rightleftharpoons AgCl_{(Solid)} + e^-$$

Provided Cl concentration is fixed, the subsequent reaction produces a stable potential.

The current response can be defined mathematically using Faraday's law:

$$I = nF\left(\frac{da}{dt}\right)$$

where the current in amperes (I) represents the electrochemical oxidation or reduction rate of the analyte at the working electrode, da/dt is the oxidation or reduction rate in mols[1], F is Faraday's constant, and n is the number of electrons transferred. The reaction rate depends on both the rate of electron transfer at the electrode surface and analyte mass transport.

As the potential is increased, the reaction reaches the point where the rate is limited by the mass transport of reactant to the electrode. When the reaction at the electrode surface is sufficiently fast, the concentration of analyte at the electrode is zero, and a maximum overall rate of reaction is reached. This overall rate is limited by the rate of mass transfer given by the following equation:

$$I = nAFD\left(\frac{dC}{dX}\right)_{x=0}$$

where dC/dX is the flux of C (electroactive species) to the electrode surface, A is the electrode area, and D is the diffusion coefficient. The rate of mass transport to the electrode surface depends on the bulk concentration of analyte, the electrode shape and area, and diffusion and convection conditions.

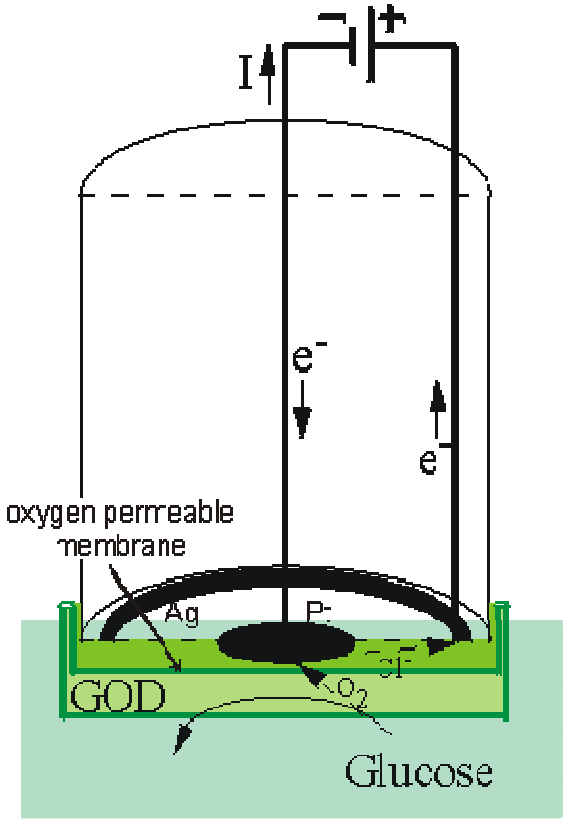

Figure 6.5. Schematic diagram of a simple amperometric biosensor. A potential is applied between the central platinum cathode and the annular silver anode. This generates a current (I) which is carried between the electrodes by means of a saturated solution of KCl. This electrode compartment is separated from the biocatalyst (here shown glucose oxidase, GOD) by a thin plastic membrane, permeable only to oxygen. The analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s) and product(s). This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilising dip-coated membranes.

Elektrodę tlenową uczuloną enzymatycznie zanurza się w roztworze nasyconym tlenem z powietrza i rejestruje prąd początkowy. Po wprowadzeniu do badanego roztworu glukozy część tlenu dyfundującego do katody jest zużywana w reakcji utlenienia glukozy z wykorzystaniem immobilizowanego enzymu zgodnie z reakcją (2). W wyniku tego procesu zmniejsza się koncentracja tlenu w roztworze i maleje strumień jego dyfuzji w kierunku powierzchni katody. Gdy procesy na elektrodzie osiągną stan ustalony, wówczas rejestruje się prąd końcowy. Obserwowany spadek natężenia prądu jest wprost proporcjonalny do stężenia glukozy. Wykonując pomiary natężenia prądu dla różnych koncentracji glukozy wyznacza się krzywą kalibracyjną. Na podstawie znajomości krzywej kalibracyjnej oznacza się stężenie glukozy w badanej próbce.

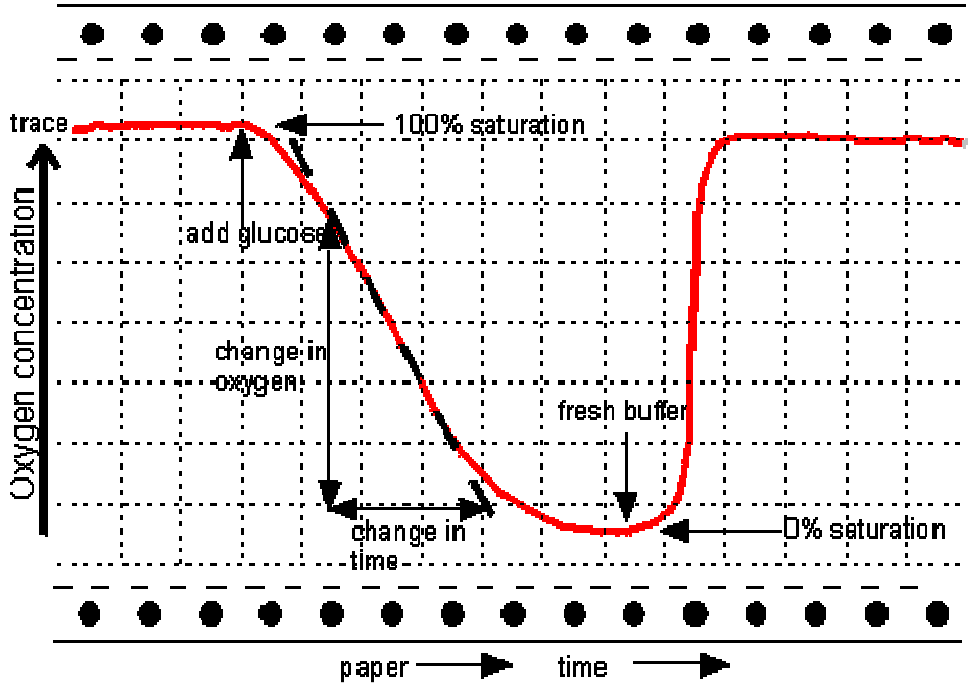

Figure 6.6. The response of an amperometric biosensor utilising glucose oxidase to the presence of glucose solutions. Between analyses the biosensor is placed in oxygenated buffer devoid of glucose. The steady rates of oxygen depletion may be used to generate standard response curves and determine unknown samples. The time required for an assay can be considerably reduced if only the initial transient (curved) part of the response need be used, via a suitable model and software. The wash-out time, which roughly equals the time the electrode spends in the sample solution, is also reduced significantly by this process.

Amperometric biosensors function by the production of a current when a potential is applied between two electrodes. They generally have response times, dynamic ranges and sensitivities similar to the potentiometric biosensors. The simplest amperometric biosensors in common usage involve the Clark oxygen electrode (Figure 6.5). This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode. When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced. Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g. Teflon, polytetrafluoroethylene). The following reactions occur:

Ag anode $4Ag^0 + 4Cl^- \longrightarrow 4AgCl + 4e^-$

Pt cathode $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero. The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration. It is clear that a small, but significant, proportion of the oxygen present in the bulk is consumed by this process; the oxygen electrode measuring the rate of a process which is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions. This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors. A typical application for this simple type of biosensor is the determination of glucose concentrations by the use of an immobilised glucose oxidase membrane.

The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes (Figure 6.6).

Sensory amperometryczne stosuje się do określania poziomu glukozy (w medycynie, biotechnologii i przemyśle spożywczym). Dokładność i szybkość określenia poziomu tej substancji jest szczególnie istotne w przypadku osób chorych na cukrzycę. Stężenie glukozy w analicie można określać metodami optycznym lub elektrochemicznymi. Zdecydowanie szerzej stosowane są różne metody elektrochemiczne takie jak amperometria czy też cykliczna woltamperometria. Jako czujniki do oznaczania glukozy stosuje się między innymi **amperometryczne sensory** uczulane enzymatycznie. Czujnik ten stanowi elektroda tlenowa zawierająca odpowiedni enzym w części receptorowej. Enzymem tym jest β-oksydaza glukozowa, która katalizuje następującą reakcję utleniania glukozy:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2$$

Czujniki te cechuje przede wszystkim wysoka selektywność w stosunku do glukozy.
Na powierzchni elektrody tlenowej znajduje się warstwa immobilizowanego (unieruchomionego) enzymu.

Optical biosensors

There are two main areas of development in optical biosensors. **These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process.** The former usually involve the widely established, if rather low technology, use of colorimetric test strips. These are disposable single-use cellulose pads impregnated with enzyme and reagents. The most common use of this technology is for whole-blood monitoring in diabetes control. In this case, the strips include glucose oxidase, horseradish peroxidase and a chromogen (e.g. *o*-toluidine or 3,3',5,5'-tetramethylbenzidine). The hydrogen peroxide, produced by the aerobic oxidation of glucose, oxidising the weakly coloured chromogen to a highly coloured dye.

$$\text{chromogen(2H)} + H_2O_2 \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O$$

The evaluation of the dyed strips is best achieved by the use of portable reflectance meters, although direct visual comparison with a coloured chart is often used. A wide variety of test strips involving other enzymes are commercially available at the present time.A most promising biosensor involving luminescence uses firefly luciferase (*Photinus*-luciferin 4-monooxygenase (ATP-hydrolysing), EC 1.13.12.7) to detect the presence of bacteria in food or clinical samples. Bacteria are specifically lysed and the ATP released (roughly proportional to the number of bacteria present) reacted with D-luciferin and oxygen in a reaction which produces yellow light in high quantum yield.

$$\text{ATP} + \text{D-luciferin} + O_2 \longrightarrow \text{oxyluciferin} + \text{AMP} + \text{pyrophosphate} + CO_2 + \text{light (562 nm)}$$

The light produced may be detected photometrically by use of high-voltage, and expensive, photomultiplier tubes or low-voltage cheap photodiode systems. The sensitivity of the photomultiplier-containing systems is, at present, somewhat greater ($< 10^4$ cells ml^{-1}, $< 10^{-12}$ M ATP) than the simpler photon detectors which use photodiodes. Firefly luciferase is a very expensive enzyme, only obtainable from the tails of wild fireflies. Use of immobilised luciferase greatly reduces the cost of these analyses.

Piezo-electric biosensors

Piezo-electric crystals (e.g. quartz) vibrate under the influence of an electric field. The frequency of this oscillation (f) depends on their thickness and cut, each crystal having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorb from the surface of the crystal, obeying the relationshipes

$$\Delta f = \frac{Kf^2 \Delta m}{A}$$

where Δf is the change in resonant frequency (Hz), Δm is the change in mass of adsorbed material (g), K is a constant for the particular crystal dependent on such factors as its density and cut, and A is the adsorbing surface area (cm^2). For any piezo-electric crystal, the change in frequency is proportional to the mass of absorbed material, up to about a 2% change. This frequency change is easily detected by relatively unsophisticated electronic circuits. A simple use of such a transducer is a formaldehyde biosensor, utilising a formaldehyde dehydrogenase coating immobilised to a quartz crystal and sensitive to gaseous formaldehyde. The major drawback of these devices is the interference from atmospheric humidity and the difficulty in using them for the determination of material in solution. They are, however, inexpensive, small and robust, and capable of giving a rapid response.

Immunosensors

Nową grupą biosensorów, które mają duże znaczenie w ocenie stanu środowiska naturalnego oraz w medycynie są bioczujniki immunologiczne (immunosensory)[1]. Biosensory te służą do oznaczania różnych substancji posiadających znaczenie immunologiczne. Substancjami tymi są proteiny, surowice, hormony, herbicydy i inne. **Immunosensory charakteryzują się na ogół dużą czułością, dużą szybkością odpowiedzi, małym zakresem detekcji oraz dają możliwość automatyzacji.**

Immunosensors transduce antigen-antibody interactions directly into physical signals. Biosensors may be used in conjunction with **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssays (**ELISA**). The principles behind the ELISA technique is shown in Figure 6.9. ELISA is used to detect and amplify an antigen-antibody reaction; the amount of enzyme-linked antigen bound to the immobilised antibody being determined by the relative concentration of the free and conjugated antigen and quantified by the rate of enzymic reaction. Enzymes with high turnover numbers are used in order to achieve rapid response. The sensitivity of such assays may be further enhanced by utilising enzyme-catalysed reactions which give intrinsically greater response; for instance, those giving rise to highly coloured, fluorescent or bioluminescent products. Assay kits using this technique are now available for a vast range of analyses.

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[1] **Immunologia** to nauka zajmująca się wpływem czynników chorobotwórczych na odporność organizmu.
Enzym – białko katalizator przeprowadzający reakcje chemiczne w organizmach żywych
Antygen - generator przeciwciał, może być każda substancja, która wykazuje dwie cechy: immunogenność, czyli zdolność wzbudzenia przeciwko sobie odpowiedzi odpornościowej swoistej, oraz antygenowość, czyli zdolność do reagowania z przeciwciałam

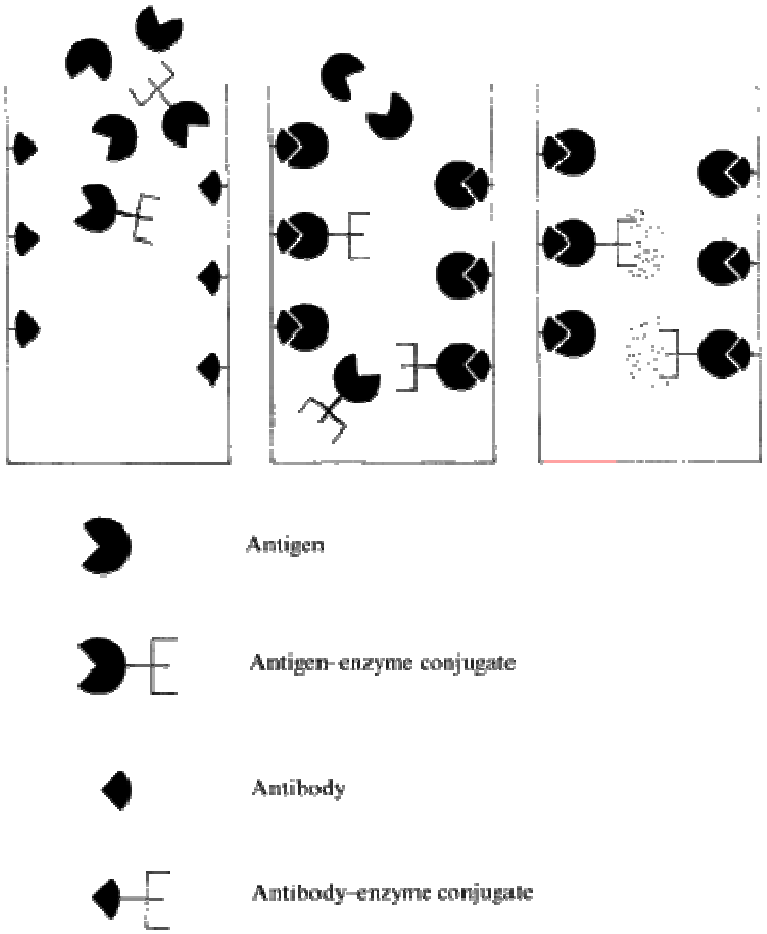

Figure 6.9. Principles of a direct competitive ELISA. (i) Antibody, specific for the antigen of interest is immobilised on the surface of a tube. A mixture of a known amount of antigen-enzyme conjugate plus unknown concentration of sample antigen is placed in the tube and allowed to equilibrate. (ii) After a suitable period the antigen and antigen-enzyme conjugate will be distributed between the bound and free states dependent upon their relative concentrations. (iii) Unbound material is washed off and discarded. The amount of antigen-enzyme conjugate that is bound may be determined by the rate of the subsequent enzymic reaction.

Recently ELISA techniques have been combined with biosensors, to form **immunosensors**, in order to increase their range, speed and sensitivity. A simple immunosensor configuration is shown in Figure 6.10 (a), where the biosensor merely replaces the traditional colorimetric detection system. However more advanced immunosensors are being developed (Figure 6.10 (b)) which rely on the direct detection of antigen bound to the antibody-coated surface of the biosensor. Piezoelectric and FET-based biosensors are particularly suited to such applications.

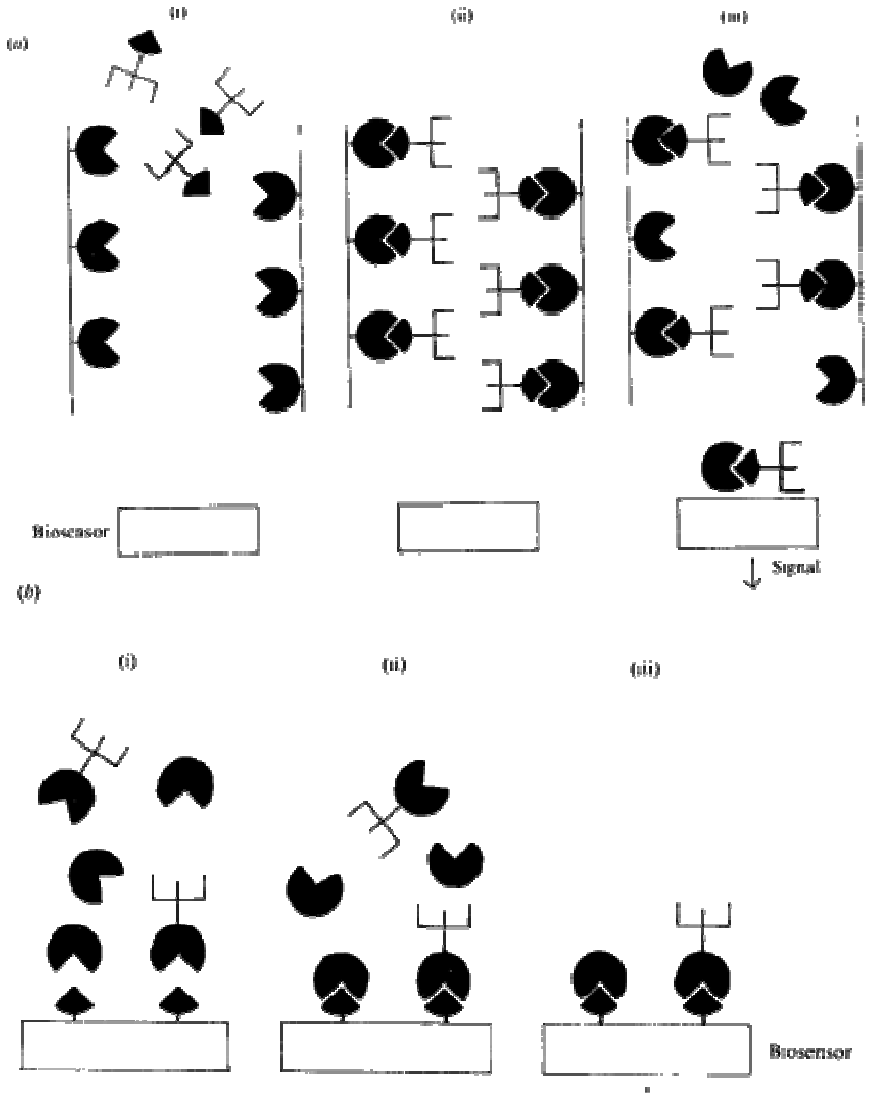

Figure 6.10. Principles of immunosensors. (a)(i) A tube is coated with (immobilised) antigen. An excess of specific antibody-enzyme conjugate is placed in the tube and allowed to bind. (a)(ii) After a suitable period any unbound material is washed off. (a)(iii) The analyte antigen solution is passed into the tube, binding and releasing some of the antibody-enzyme conjugate dependent upon the antigen's concentration. The amount of antibody-enzyme conjugate released is determined by the response from the biosensor. (b)(i) A transducer is coated with (immobilised) antibody, specific for the antigen of interest. The transducer is immersed in a solution containing a mixture of a known amount of antigen-enzyme conjugate plus unknown concentration of sample antigen. (b)(ii) After a suitable period the antigen and antigen-enzyme conjugate will be distributed between the bound and free states dependent upon their relative concentrations. (b)(iii) Unbound material is washed off and discarded. The amount of antigen-enzyme conjugate bound is determined directly from the transduced signal.

The electrode surface is modified by immobilized antigen as shown in Fig. 6.11(Fig.1) and the paroxidase antibody-conjugate associates with antigen on the electrode surface. Once added to the media and on reaching the electrode surface the antibody-conjugate peroxidase starts to catalyze electroreduction of hydrogen peroxidase which results in an increase in the electrode potential. When the target analyte is added displacement occurs resulting in a decrease in electrode potential.

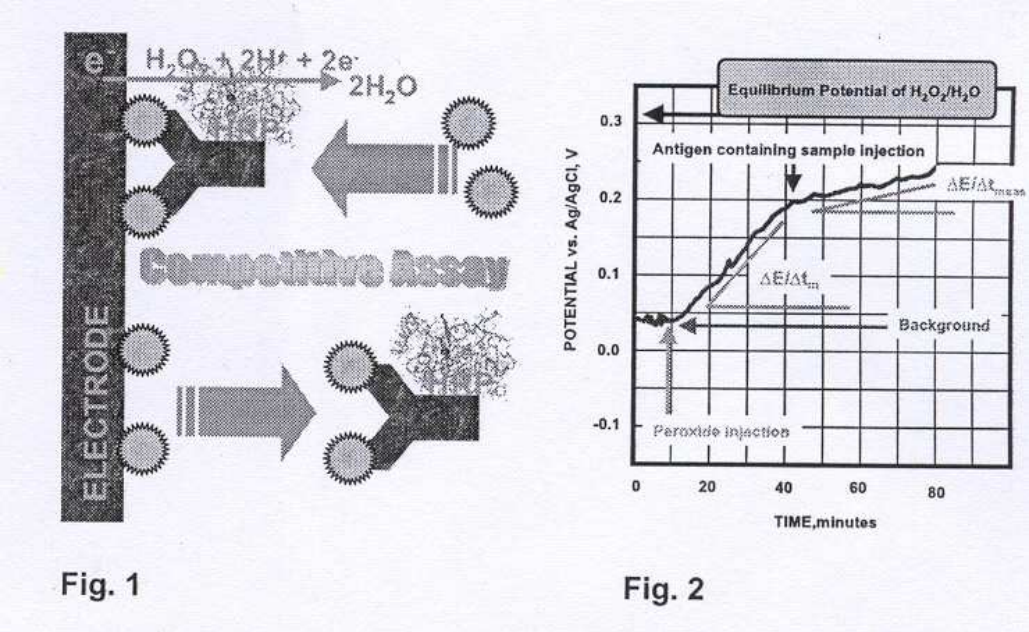

Fig. 6.11 Principle of operation of immuno-sensor (Fig.1) and its electrical characteristic (Fig. 2)

Fig. 6.12 presents the protocol for measurement of potential change (E) due to consecutive addition of conjugate into the cell. In a presence of hydrogen peroxidase, a background electrod epotential is established. The addition of solution containing anti-bodies labeled with perixidase (conjugate) results in an increase of electrode potential. **The increase of conjugate concentration leads to an increase in the rate of electrode potential change (dE/dt)**. **The immunointeraction can be detected directly by recording the potential shift.** It suggests a single stage analysis scheme and the signal can be detected continuously with increasing and decreasing concentration of analyte. The basic detection device is high input impedance voltmeter.