Biosensors for microbial detection

Anil Deisingh discusses the use of biosensors for the detection of bacteria

THE CENTERS FOR DISEASE CONTROL in the USA has estimated that 76 million people suffer foodborne illnesses annually, with 325 000 being admitted to hospitals of whom more than 5000 die. Furthermore, it has been estimated that the yearly cost of these illnesses is US$ 5-6 billion in direct medical expenses and lost productivity. *Salmonella* infections account for $1billion of these costs. *E. coli* O157 :H7 causes 20 000 illnesses and 500 deaths per year in the USA. In the UK, the Public Health Laboratory Service has indicated that in 2001, there were 85 468 food poisoning notifications which represent a 600% increase from 1982. In the last decade, therefore, increased efforts have been made towards the development of new approaches for the rapid detection of microbes in food and other environments. These include immunological assays, MALDI TOF-MS (see SFAM News, September 2002) and biosensors, among others. In this article, we discuss the use of biosensors for the detection of bacteria and, to a lesser extent, viruses.

A biosensor can be defined as *'a compact analytical device incorporating a biological or biologically-derived sensing element (such as an enzyme, antibody, microbe or DNA) either integrated within or intimately associated with a physicochemical transducer'* (Turner *et al.*, 1987). Upon interaction with a chemical species, the physicochemical properties of the sensing layer (mass, optical properties, resistance etc) change and this is detected by the transducer. The changes are then converted into an electrical signal which is then processed. The transducer may be optical (e.g., optical fibre), electrochemical (e.g., ion- selective electrodes), heat-sensitive (e.g., calorimetric) or piezoelectric (e.g., acoustic wave). The table on page 30 gives further examples of the various transducers. The main parts of a typical biosensor are shown in Figure 1 on the next page. The objective of any biosensor is the production of either discrete or continuous electronic signals which are proportional to a single analyte or a related group of analytes (Turner *et al*, 1987).

There are many advantages associated with the use of biosensor technology as a sensitive detection method. These include:

- Specificity as a result of using biological sensing elements which can distinguish between the analyte under investigation and similar molecule.
- Rapid response times, usually with results being obtained as real-time measurements.
- Simplicity of construction as both transducer and the region for selective chemistry are located on a single platform. This makes it possible to have reagentless measurements and also for on-the-spot analyses
- The ability to provide continuous data with minimal perturbation of the analyte. The biological element can be re-used for different analyses which provides biosensors with a major advantage over immunoassays.

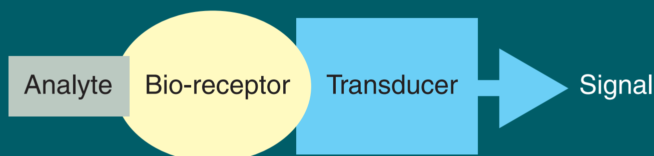
Applications of Biosensors

In general, the discussion will centre around the types of transducers listed in the Table on page 30. Some types, such as electrochemical, optical and piezoelectric, will be given priority as they are having great impact upon the detection of microbes.

Electrochemical

Potentiometric biosensors are usually based on ion-selective electrodes.

Figure 1. The main parts of a typical biosensor



Transducers used in Biosensor development

Category	Principle	Examples
Electrochemical	(a) potentiometric: depends on changes in potential of a system at a constant current (I=0) (b) amperometric: detects changes in current as a function of concentration of electroactive species	Ion-selective electrodes, ion-selective field effect transistors, LAPS Solid electrolyte gas sensors, electronic noses
Optical	Link changes in light intensity to changes in mass or concentration, therefore, fluorescent or colorimetric molecules must be present	Optical fibres, surface plasmon resonance, absorbance luminescence
Piezoelectric	Sensitive to changes in mass, density, viscosity and acoustic coupling phenomena	Surface acoustic wave sensors
Thermal	Detect changes in temperature	Calorimetric sensors

These devices measure the change in ion concentration during a reaction. Generally, a simple sensor consists of an immobilized enzyme membrane surrounding the probe of a pH meter where the catalysed reaction will generate or absorb hydrogen ions. This leads to a change in pH which can be easily read. Three main types of ion-selective electrodes are often used in biosensors: normal glass pH electrodes, glass pH electrodes coated with a selective gas-permeable membrane and solid-state electrodes consisting of a thin membrane of a specific ion conductor. It is also possible to use metal oxide semiconductors (MOS) which can be used to measure charge on a surface which will cause a current flow proportional to the charge. MOS devices are small and so they have fast response times due to reduced diffusion. However, the sensitivity of these can be affected by the ionic strength and concentrations of the solutions being analysed.

Potentiometric biosensors have been widely used for bacterial analyses. Examples include the detection of bacterial contamination in milk using an L-lactate biosensor, bacterial growth and sequence-specific biosensing of DNA. Electrochemical detection of DNA hybridization involves the monitoring of a current under controlled potential conditions. The hybridization is detected via increased current of a redox indicator or by changes in conductivity or capacitance.

In this article, however, we would like to concentrate on the use of light-addressable potentiometric sensors (LAPS) which are proving popular as a platform for detecting microbes. These are semiconductor-based systems with an electrolyte-insulator-semiconductor (EIS) structure. When a current is applied across the EIS region, a depletion layer is formed at the insulator-semiconductor interface. The capacitance of the depletion layer changes with the surface potential which is a function of the ion concentration in the electrolyte. In order to determine the capacitance, the semiconductor is illuminated by modulated light and the current is measured. LAPS have several advantages when compared with other sensors: the surface is flat, there is no need for wires or passivation and they can measure pH and concentration.

Researchers at the USDA have used a LAPS system in combination with an immunoligand assay (ILA) to detect live *E. coli* O157:H7. They have reported that both live and dead bacteria can be detected in 30-45 minutes. In this system, bacteria are captured onto a filter membrane by using specific antibodies. A silicone-based sensor is then placed adjacent to the membrane and, upon illumination, small changes in acidity are detected. The signal is proportional to the number of bacteria present and it was possible to detect 2 000 dead or 25 000 live *E. coli* O157:H7 organisms/ ml (Gehring *et al.*, 1998).

In a recent development, a LAPS approach was used to detect *E. coli* in drinking water (Ercole *et al.*, 2002). An immunoassay was developed such that there was specificity to a particular capsular protein present in the bacterium. The transducer, based on the LAPS principle, was able to detect the production of ammonia by a urease- *E. coli* antibody conjugate. It was claimed that 10 cells /ml were detected in 1.5 hours.

Generally, amperometric biosensors work by enzymatically generating a current between two electrodes. They have fast response times, dynamic ranges and sensitivities similar to potentiometric biosensors. Many amperometric biosensors depend on dissolved oxygen concentration which can pose a major problem. To overcome this situation, mediators are employed. These transfer electrons directly to the electrode thereby eliminating the need for the reduction of an oxygen co-substrate. The most commonly used mediators are the ferrocenes. Amperometric biosensors have been used for the detection of *E. coli* in water (screen-printed electrodes), bacterial vaginosis, studies of bacterial contamination, detection of agents of biological warfare (e.g. anthrax) and detection of *E. coli* heat-labile enterotoxin and other neurotoxins. Amperometric biosensors have also been used to study bacterial luciferase reactions, nanoscale bacterial surface proteins and growth and viability of bacterial populations.

Optical

Optical biosensors are usually based upon optical fibres or surface plasmon resonance (SPR), although it is common to find luminescence, fluorescence and absorbance also being used. Optical fibres are long, thin strands of pure glass

which can transmit light over long distances. Advantages to their use include cheaper cost, thinness, reduced interference by other signals, low power usage, lightness and flexibility. They are ideal as biosensors because they give rapid signals with high specificity for the organism of interest. Reported uses of these biosensors include the detection and quantification of bacteria in meat and poultry e.g. *Salmonella*, *E. coli* and *Listeria*. Many of these are based on the use of antibodies for the specific recognition of the pathogen. By immobilizing several antibodies on different fibre probes, it is possible to detect several bacterial species simultaneously. These are array biosensors which are now widely researched. It is common to obtain results in less than 2 hours, although preparation time may be longer depending on the need for incubation. It is possible to obtain detection limits as low as 100 cfu /ml.

An interesting recent development has been described by Walt's group at Boston University (Epstein *et al*, 2002). They have described the use of a fibre optic biosensor microsphere array which is capable of zeptomole (10^{-21} mol or ~ 600 DNA molecules) detection limits. This method has the potential to provide high-throughput DNA analysis of bacteria with the advantages of small size, flexibility and a detection limit which is two orders of magnitude lower than other reported values.

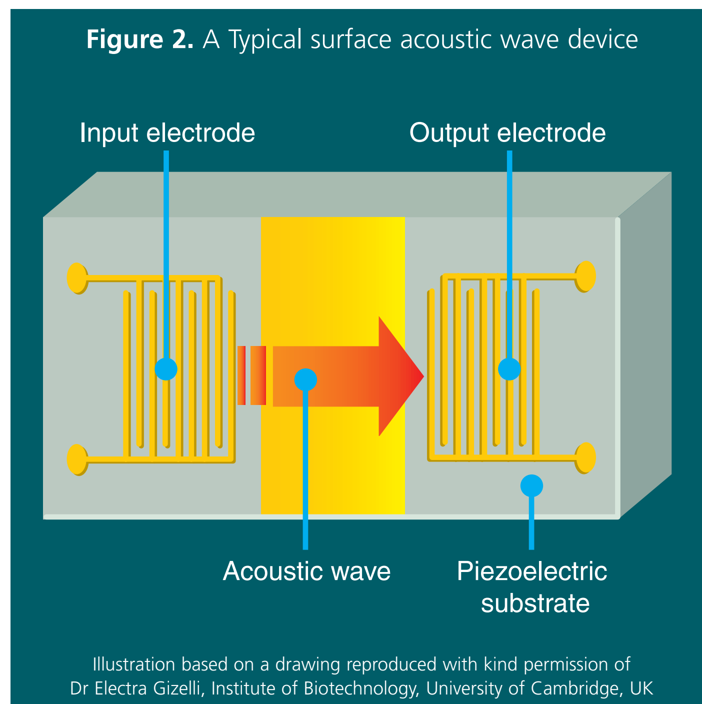
Surface plasmon resonance (SPR) techniques are having a major impact on the development of new optical biosensors. SPR occurs when light is reflected off thin metal films in such a way that a fraction of the light incident at a defined angle can interact with the delocalised electrons in the metal film (plasmon). This leads to a decrease in the light intensity. The change in the SPR signal is directly proportional to the immobilized mass on the metal film. SPR can measure, in real-time, the interactions of biomolecules with interfaces as a result of changes in the refractive index. A commercially available immunosensor SPR system is the *BIACore* apparatus which was jointly developed by Professor Lundstrom of Linkoping, Sweden and Pharmacia Corporation. This is now used in some academic and industrial laboratories.

SPR biosensors have been used for the real-time detection of *E. coli* O157:H7 using antibodies bound to the sensor surface, for detecting bacteria and viruses in the marine environment, classification of polycyclic aromatic hydrocarbon (PAH) toxicity using immobilized luminescent bacteria, determination of denitrifying bacteria in soil and the sequence-specific binding of human immunodeficiency virus type I. These examples show the wide range of analyses which may be performed with SPR technology.

Several configurations of SPR sensors are in development and these include SPR fibre optic probes, SPR planar probe sensors, multichannel sensing devices and combination of SPR sensors with other methods such as anodic stripping voltammetry and critical angle refractometry.

Conventional SPR systems are expensive and large with the result that many laboratories are unable to obtain such specialized equipment. Texas Instruments have developed and commercialized a miniature SPR sensor called the Spreeta™. This costs about US $50 and includes all the required components such as light source, polarizer, prism, sensing area and angle detector in an area of about 2 cm^2. The sensor can measure properties such as refractive index changes, avidin-biotin binding, antibody-antigen dissociation kinetics, specific detection of small molecules, protein binding and attachment of DNA complements. This device may prove very useful in the detection of microbes.

Figure 2. A Typical surface acoustic wave device



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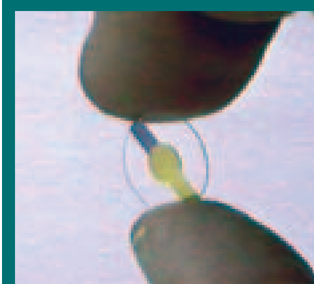
Piezoelectric-based acoustic wave devices

Acoustic wave devices have been commercially used for more than 60 years with the telecommunications industry being the largest consumer, primarily in the mobile phone sphere. These devices are sensitive to changes in mass, density, viscosity and acoustic coupling phenomena. As the acoustic wave propagates through or on the surface of the material, the velocity and /or amplitude of the wave are changed. Changes in the velocity can be monitored by measuring the frequency of the sensor which can then be related to the physical parameter under consideration. Piezoelectric acoustic wave sensors apply an oscillating electric field to create a mechanical wave which can propagate through the substrate and is then re-converted to an electric field to allow for measurement.

Quartz is the most frequently used piezoelectric crystal because it can act as a mass-to-frequency transducer. AT-cut crystals (+ 35° 15´ orientation of the plate with respect to the crystal plane) are favoured because of the excellent temperature coefficients in the range 10 - 50°C. One of the first piezoelectric sensors was the

Figure 3. A quartz crystal containing gold electrodes



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thickness-shear mode (TSM) sensor which, if the substrate is quartz, may commonly be termed the quartz crystal microbalance (QCM) or bulk acoustic wave (BAW) sensor. A typical surface acoustic wave device is shown in Figure 2 while Figure 3 is a representation of a quartz crystal containing gold electrodes. Other piezoelectric substrates include lithium tantalite, lithium niobate, silicon carbide and gallium arsenide.

Several researchers have reported on the use of acoustic wave biosensors to detect microbes. Sequences of *E. coli* O157:H7 have been successfully detected using a PCR-acoustic wave sensor combination (Deisingh & Thompson, 2001). A DNA sequence unique to *E. coli* O157:H7 was amplified by PCR. Immobilization of a probe for the bacterium on the sensor by the biotin-neutravidin interaction was used to detect hybridization of the sequence generated by PCR. This approach can be used to detect the organism in food, water and clinical samples. Pathirana and co-workers (2000) have developed a biosensor for *Salmonella typhimurium* based on the use of a polyclonal antibody immobilized on the surface of a QCM acoustic wave device. The sensor had a detection limit of about 350 ± 150 cells /ml and the response was linear between bacterial concentrations of 10^2 and 10^7 cells/ ml. Cambridge investigators have developed a sensitive detection of the herpes simplex virus type 1 (HSV 1) by using the interaction between the virus and specific antibodies attached to a QCM. The QCM was used to detect the acoustic noise produced when the interactions were broken as the oscillation was increased (Cooper *et al*, 2001). The method, termed rupture event scanning (REVS), is quantitative over at least six orders of magnitude.

Electronic nose

An electronic nose may be defined as 'an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours' (Craven *et al*, 1996). Figure 4 shows the generalized structure of an electronic nose.

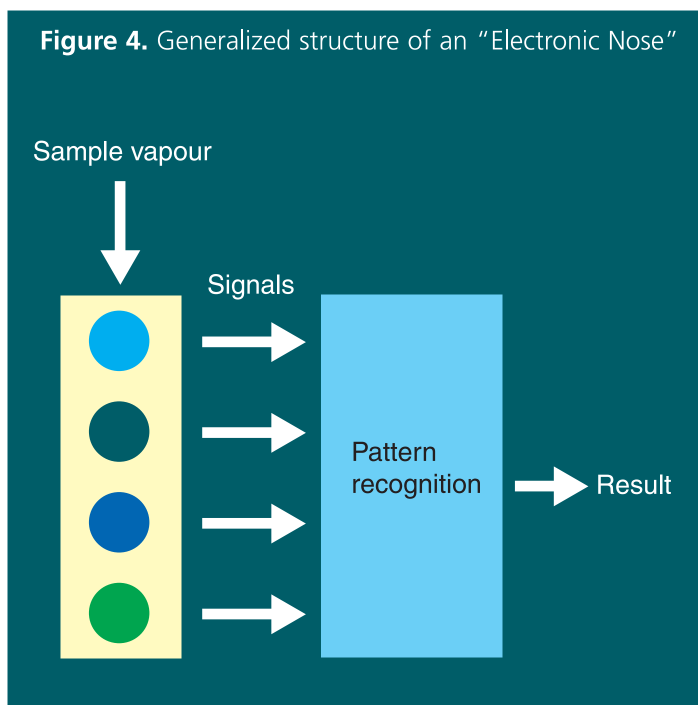

Figure 4. Generalized structure of an "Electronic Nose"

Pattern recognition techniques include principal components analysis (PCA), artificial neural network (ANN), discriminant function analysis and fuzzy logic. The sensor array will sense the vapours from a sample and provide a series of measurements; the pattern recognition system will then compare the measurement pattern with known patterns (standards).

The electronic nose provides a low-cost alternative for analyzing volatile organic compounds when compared with GC-MS. Other advantages, when compared with a human nose, include the ability to detect toxic and odourless compounds and the inability to become tired. However, for all its perceived advantages, commercial success has not been easy. This is mainly due to poor reproducibility and stability, calibration problems and difficulty with interpreting the results. There are several examples of the use of electronic noses in microbiological analysis. Many species such as *E. coli*, *Proteus*, *Pseudomonas* and *Staphylococcus* have been correctly identified by neural network techniques. Additionally, *Helicobacter pylori*, implicated in ulcer formation, has been diagnosed by breath tests, as was infection due to tuberculosis by analyzing the odours generated from sputum samples. Further applications include the quality classification of stored grain, freshness of fish, process control of bread and cheese and bacterial growth on meat and vegetables.

Conclusion

Biosensors are making a great impact on the development of rapid, sensitive assays for the detection of microorganisms. Although much success has been achieved in terms of research, commercial development has been slow. Kits are now available for several organisms such as *E. coli* O157:H7 and *Salmonella typhimurium* and it is hoped that more will become available shortly. New developments include integrated systems (see "lab on a chip" article in the March issue of *Microbiologist*), the use of molecular beacons and nanosensor production. These should ensure even more rapid and specific detection.

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