A detailed analysis of this situation is complicated, although this is not needed for understanding the operation of a biosensor. The effect of this reaction is that the redox process cycles round many times. The reverse oxidation of $R$ is not seen but the forward reduction peak is enhanced many times (see Figure 2.17).

In this figure, the reversible cyclic voltammogram is of ferrocene (dicyclopentadieneiron(III)). The catalytic wave is caused by interaction with glucose oxidase in the presence of glucose (described in more detail below in Chapter 5).

**SAQ 2.11**

What is the essential difference between a catalytically limited wave and a kinetically limited wave?

## 2.4 Conductivity

Conductivity is the *inverse of resistance*. It is a measure of the ease of passage of electric current through a solution. Ohm's law gives the following relationship:

$$E = IR$$

and for the conductance, $L$ [in siemens (S), where 1 S = 1 ohm$^{-1}$]:

$$L = 1/R$$

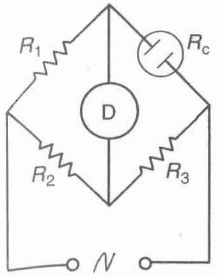
and therefore:

$$E = I/L$$

Conductance is related to the dimensions of a cell in a similar way to resistance. For a cell of length $l$ and cross-sectional area $A$, the conductance $L = \kappa A/l$, where $\kappa$ is the specific conductivity (S cm$^{-1}$). This is often further normalized by dividing by the molality of the solute to give the molar conductivity, $\Lambda = \kappa/C$ ($C$ in mol cm$^{-3}$), so the units of $\Lambda$ are S mol$^{-1}$cm$^{-2}$.

Conductivity is fairly simple to measure, being directly proportional to the concentration of ions in the solution. Figure 2.18 shows a general conductivity bridge circuit. In the traditional bridge, the resistance $R_3$ is adjusted to balance the bridge and a cell constant is then used to convert conductance into (specific) conductivity. In modern instruments, this is carried out automatically to give a digital read-out.

The conductivity varies according to the charge on the ion, the mobility of the ion and the degree of dissociation of the ion. These all introduce complications. In itself the technique has no selectivity. It can be used in controlled situations but really needs to have selectivity superimposed by means of a membrane or coating.



$$R_c = R_3 \times \frac{R_1}{R_2}$$

$$L_c = \frac{1}{R_c} = \frac{R_2}{R_1 R_3}$$

**Figure 2.18** A conductivity bridge. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

The measurement of conductance involves an alternating current, as in the classical conductance bridge. Varying the frequency of the alternating current can extend this. The quantity measured is then the *admittance* = 1/*impedance*), which not only depends on simple conductance but also on the capacitance and inductance of the system. These components can be separated as imaginary components, in particular by using a frequency response analyser, and then displaying in an *Argand diagram*, as shown in Figure 2.19. Such diagrams are sometimes called *admittance (impedance) spectra*. This approach has not so far been used to any great extent in developing sensors and biosensors, but is now receiving increased attention.

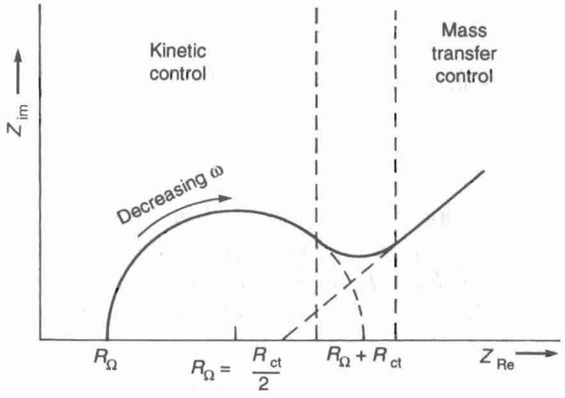


**Figure 2.19** A typical Argand diagram, showing the frequency dependence of the 'imaginary' impedance against the 'real' impedance. From Bard, A. J. and Faulkner, L. R., *Electrochemical Methods: Fundamentals and Applications*, © Wiley, 1980. Reprinted by permission of John Wiley & Sons, Inc.

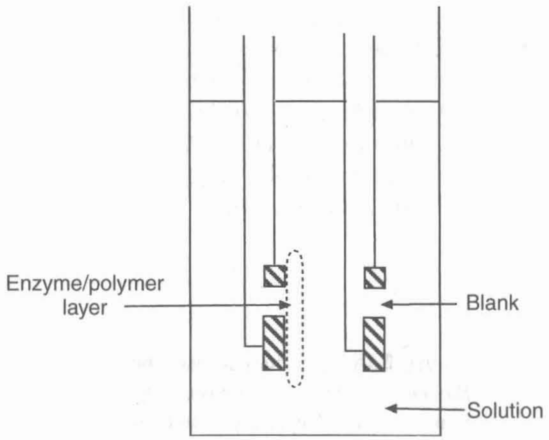


**Figure 2.20** Schematic of a differential type of conductivity cell, as used in biosensors. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

**SAQ 2.12**

Why can direct current not be used in a conductivity bridge?

In principle, a change in conductance can be used to follow any reaction that produces a change in the number of ions, the charge on the ions, the dissociation of the ions or the mobility of the ions. Usually a differential type of cell is used, as shown in Figure 2.20.

**DQ 2.6**

Discuss factors, which would enable one to use conductance devices as transducers.

*Answer*

*Any reaction or change that involves a change in the number of ions, the charge on the ions or the mobilities of the different ions will produce a change in the conductivity of the solution, which could therefore be used as the transducer. This is a relatively simple, although a somewhat under-used method.*

## 2.5 Field-Effect Transistors

Field-effect transistors (FETs) are devices in which a transistor amplifier is adapted to be a miniature transducer for the detection and measurement of potentiometric signals, produced by a potentiometric sensor process on the gate of the FET. A separate reference electrode is also needed. Circuit wiring is minimized, so that in addition to miniaturization, electronic noise is greatly reduced and sensitivity is increased. The FET device can be part of an integrated-circuit system leading to the read-out, or to the processing of the analytical data. However, as yet, no particularly satisfactory miniaturized reference electrodes exist. According to Janata (see Bibliography), most of the proposed versions violate some of the basic principles of reference electrodes. Despite this, a number of possibilities have been proposed and used, varying from a 'pseudo-reference electrode', consisting of a single platinum or silver wire, to the screen-printed type made with silver–silver chloride ink. Perhaps a more satisfactory approach is to avoid the problem by operating in a differential mode with two FETs, i.e. one being a blank with a gate having negligible response to the analyte and the other coated with the analyte-selective membrane.

### 2.5.1 *Semiconductors – Introduction*

Materials can be classified as metals, non-metals or semiconductors. Generally metals are good conductors of electricity, while non-metals are bad conductors i.e. they behave as insulators. Semiconductors come somewhere in between. The differences can be seen in the way that they form energy levels. Non-metal atoms form discrete bonding and anti-bonding molecular orbitals when they combine to form molecules. The bonding orbitals contain the bonding electrons, while the anti-bonding orbitals are empty, unless electrons are promoted into these by excitation. The energy space between these levels is 'forbidden' and is therefore unoccupied. In metals, there are overlapping energy bands and so there is no forbidden region. Electrons can move freely throughout the bands, thus leading to their high conductivities. Semiconductors form energy bands, although in this case they are separated by a forbidden region. The lower band is known as the *valence band* (VB), while the upper band is called the *conduction band* (CB). The energy gap between the two is called the *band gap*. These features are shown in Figure 2.21.

If small amounts of dopants are added to a semiconductor, it may acquire an excess of electrons to give a p-type semiconductor, or a deficit of electrons (excess of holes) giving an n-type semiconductor. Fifth-row elements (in the Periodic Table), such as arsenic, form p-type semiconductors, while third-row elements, like gallium, will form n-type semiconductors.

The Fermi level ($E_F$) is the point where the probability of filling the (energy) band is 0.5. For an undoped (intrinsic) semiconductor, this will be half-way between the VB and the CB, while for a doped semiconductor, $E_F$ lies nearer to the VB in p-doped materials and nearer to the CB in n-doped materials.

A common arrangement of semiconductors for sensor applications is the metal–insulator–semiconductor (MIS) system. If no potential is applied, the Fermi level is the same across the metal to the semiconductor. However,

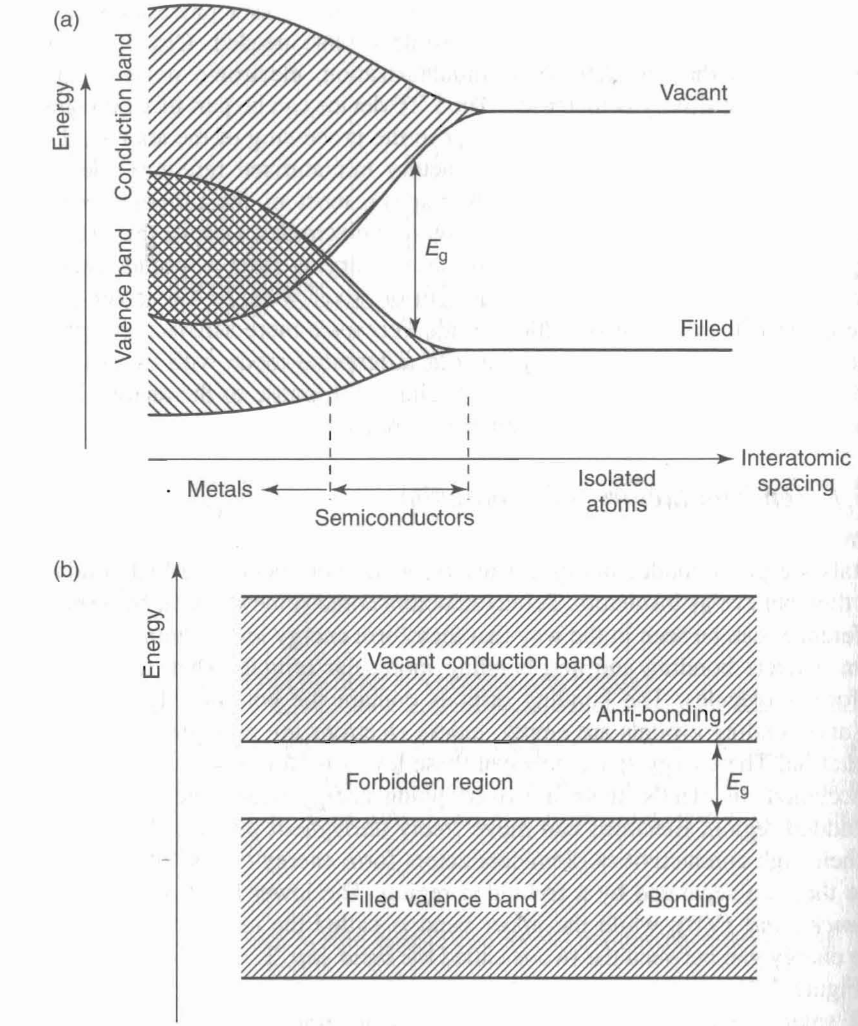


**Figure 2.21** (a) Classification of a material according to energy bands and interatomic spacing. (b) The semiconductor band gap energy model. From Hall, E. A. H., *Biosensors*, Copyright 1990. © John Wiley & Sons Limited. Reproduced with permission.

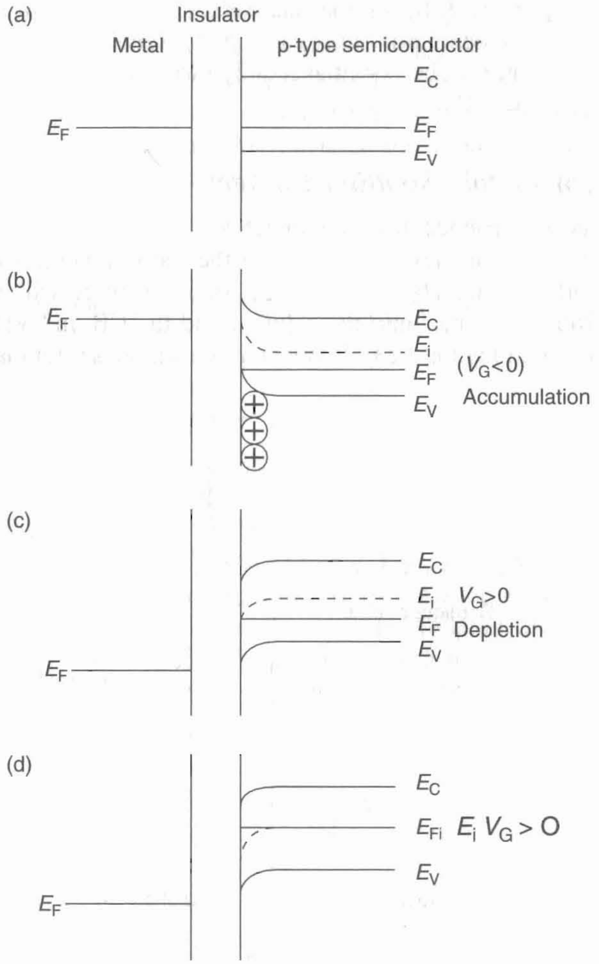


**Figure 2.22** Energy bands through an MIS system as a function of the applied voltage $V_G$. From Hall, E. A. H., *Biosensors*, Copyright 1990. © John Wiley & Sons Limited. Reproduced with permission.

when a potential is applied the levels on the two sides separate. The system then behaves like a capacitor and charges build up on each side. Figure 2.22(a) shows the energy levels across a p-type semiconductor. It also shows the effect of applying a potential (a gate voltage, $V_G$) across the MIS system. With a small negative potential ($V_G < 0$) Figure 2.22(b), there is an accumulation of electrons at the metal/insulator (M/I) interface, and of holes (positive charges) at the semiconductor/insulator (S/I) interface. $E_F$ is shifted towards the VB lower than the value in the metal by an amount equal to $V_G$ and the energy levels near the semiconductor become bent upwards to compensate for this. With a small positive potential ($V_G > 0$) Figure 2.22(c), there is a depletion effect as positive holes are repelled from the S/I interface. In this case, the VB and CB bend downwards to compensate for this. If $V_G$ is further increased, eventually the hole and electron concentrations near the interface become equal. Now, the Fermi level is again midway between the VB and the CB – equivalent to the intrinsic level (Figure 2.22(d)). Further increases in potential beyond this lead to an excess electron concentration, thus causing the semiconductor to invert and become n-type in nature. The potential required to cause inversion is known as the threshold potential ($V_T$).

### 2.5.2 Semiconductor–Solution Contact

When an n-type semiconductor is in contact with a solution containing a redox couple (Ox/R), the Fermi level is related to the redox potential $E^0$. If the $E_F$ of the semiconductor lies above that of the solution, there will be a net flow of electrons from the former into the solution and the CB and VB will be bent upwards (as shown in Figure 2.23). If the interface region is illuminated with light of energy greater than the band-gap energy ($E_G$), there will be a separation of the electron–hole pairs. The holes migrate to the surface with a potential equivalent to the VB and cause oxidation of R to Ox. The electrons move into the bulk semiconductor and to the external circuit or react with an electron-acceptor (Ox) species, thus casing reduction. This phenomenon is known as photocatalysis. Titanium dioxide is used extensively as a photocatalyst material.

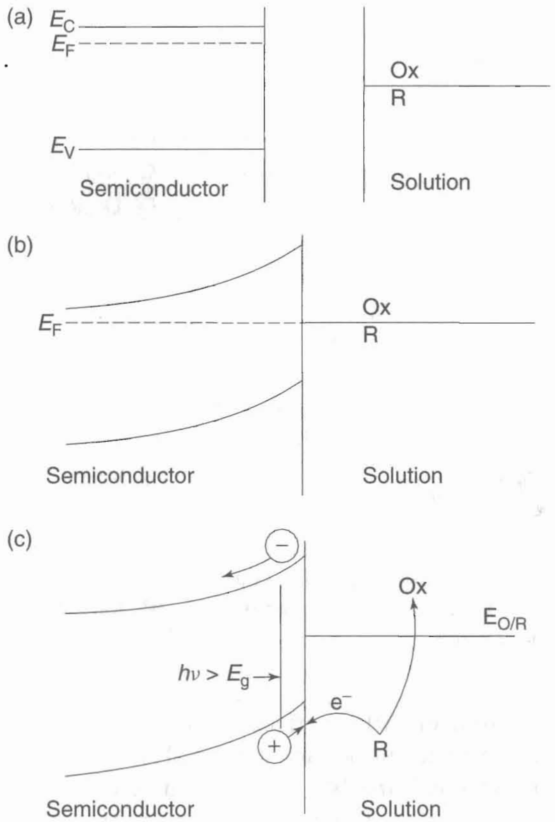


**Figure 2.23** Formation of a junction between an n-type semiconductor and a solution containing a redox couple Ox/R: (a) before contact; (b) at equilibrium in the dark; (c) after irradiation, where $h\nu > E_G$. From Hall, E. A. H., *Biosensors*, Copyright 1990. © John Wiley & Sons Limited. Reproduced with permission.

**SAQ 2.13**

Explain how inversion occurs in a field-effect transistor.

### 2.5.3 Field-Effect Transistor

This is an arrangement to monitor and control changes in the MIS system. Inversion at a p-type S/I system can be monitored by two n-type sensors placed on either side of the p-type layer. The basic type of field-effect transistor (FET) is the insulated-gate FET (IGFET). This is shown in Figure 2.24. A source region (4), consisting of n-type silicon, is separated from a similar drain region (5), also of n-type silicon, by p-type silicon (1), with the insulator (2) consisting of silicon dioxide. The source is electrically biased with respect to the drain by the applied potential, $V_D$. The gate (3) is a metal. insulated from the rest, so that it

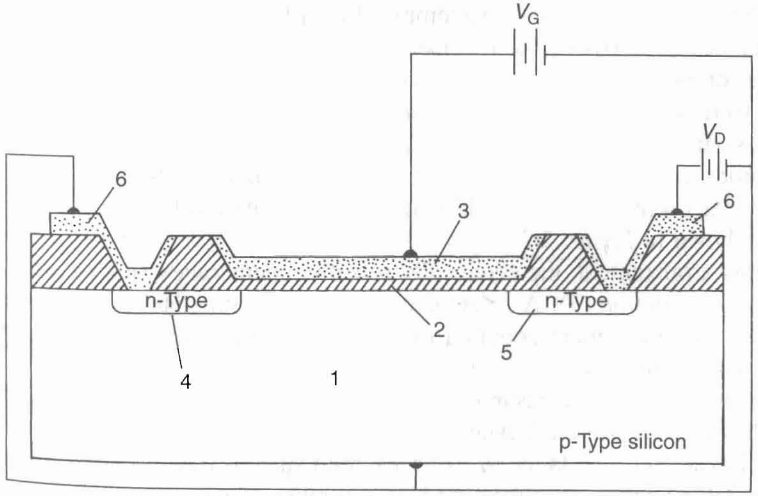


**Figure 2.24** Schematic of the insulated-gate field-effect transistor (IGFET): 1, p-type silicon substrate; 2, insulator; 3, gate metal; 4, n-type source; 5, n-type drain; 6, metal contacts to source and drain. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

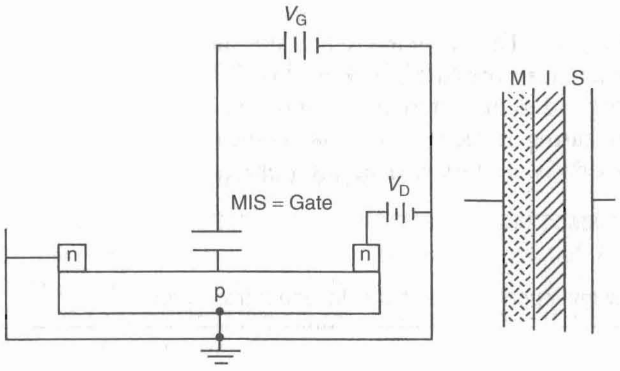


**Figure 2.25** Schematic of the gate in an IGFET: M, metal; I, insulator; S, semiconductor. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

forms a capacitor sandwich, a metal/insulator/semiconductor (MIS) arrangement, as shown in Figure 2.25

This gate region is charged with a bias potential $V_G$,. The current from the drain (5) to the source (4), $I_D$, is measured. There is also a threshold potential, $V_T$, at which silicon changes from p-type to n-type, and inversion occurs. With a small positive $V_D$ and $V_G < V_T$, silicon (1) remains in the p-state, and there is no drain current; n-Si is biased positive with respect to p-Si. When $V_G > V_T$, there is surface inversion, and p-Si becomes n-Si. Now current can pass from drain to source, without crossing the reversed-bias p–n junction. $V_G$ now modulates the number of electrons from the inversion layer and so controls the conductance. $I_D$ flows from source to drain, and is proportional to both the electrical resistance of the surface inversion layer and $V_D$.

In order to convert this device into a sensor, the metal of the gate is replaced by a chemically sensing surface. This general conformation is known as a CHEMFET and is shown in Figure 2.26.

In this arrangement, the chemically sensitive membrane (3) is in contact with the analyte solution (7). A reference electrode (8) completes the circuit via the $V_G$ bias. The membrane potential minus the solution potential has the effect of correcting for this bias.

The current may be measured directly at constant $V_G$ by using a circuit such as that shown in Figure 2.27. Alternatively, one can keep $I_D$ constant by changing $V_G$ and measuring the latter by using a circuit such as the arrangement shown in Figure 2.28. Such a system is used in a number of sensor modes. The general CHEMFET mode has already been mentioned. A further mode is the ion-selective mode (ISFET), which uses the FET as an ion-selective electrode. Following on from this, the ENFET is a form of biosensor in which the gate contains an enzyme system.

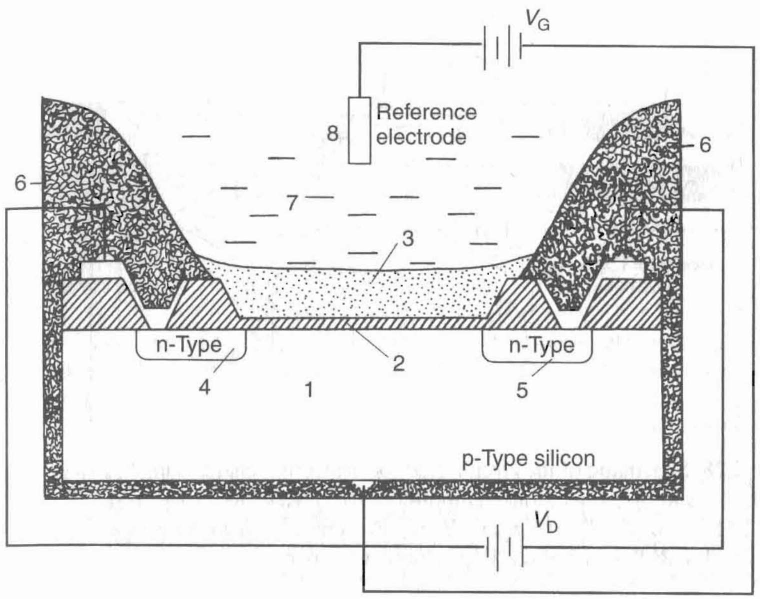


**Figure 2.26** Schematic of a field-effect transistor with a chemically sensing gate surface (CHEMFET): 1, silicon substrate; 2, insulator; 3, chemically sensitive membrane; 4, source; 5, drain; 6, insulating encapsulant; 7, analyte solution; 8, reference electrode. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

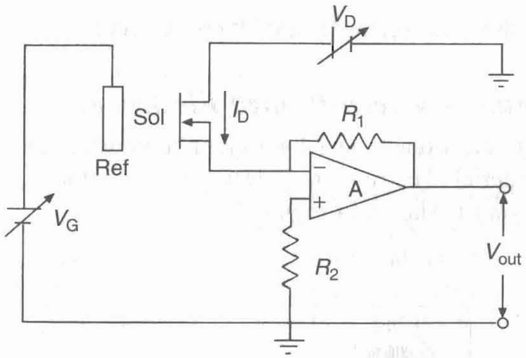


**Figure 2.27** Schematic of the circuit used for measuring $I_G$ at a constant gate voltage: A, operational amplifier; $R_1$, 1 kΩ; $R_2$, 470 Ω. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

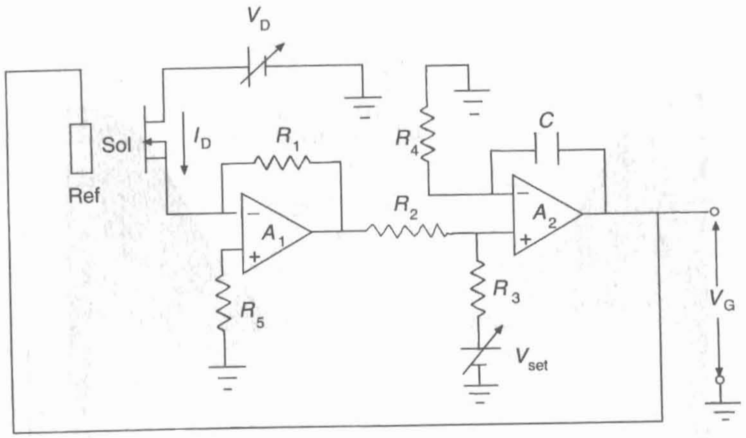


**Figure 2.28** Schematic of the circuit used for measuring changes in $V_G$ at a constant drain current: $A_1$ and $A_2$, operational amplifiers; $R_1$, 1 KΩ; $R_2 = R_3$, 100 kΩ; $R_4$, 20 KΩ; $R_5$, 470 Ω; $c$, 10 pF. From Eggins, B. R., *Biosensors: An Introduction*, Copyright 1996. © John Wiley & Sons Limited. Reproduced with permission.

## 2.6 Modified Electrodes, Thin-Film Electrodes and Screen-Printed Electrodes

Modified electrodes will be discussed in detail in Chapter 3.

A major aspect in the manufacture of sensors is miniaturization. Three developments, which have assisted this, are thick-film electrodes formed by screen-printing, thin-film electrodes and microelectrodes.

### *2.6.1 Thick-Film – Screen-Printed Electrodes*

Here, the working electrode is usually a graphite-powder-based 'ink' printed on to a polyester material. The reference electrode is usually silver–silver chloride ink. A typical layout is shown in Figure 2.29.

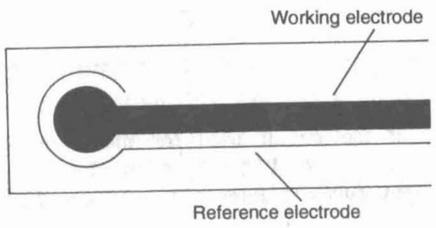


**Figure 2.29** A screen-printed electrode. From Wang, J., *Analyst*, **119**, 763–766 (1994). Reproduced with permission of The Royal Society of Chemistry.

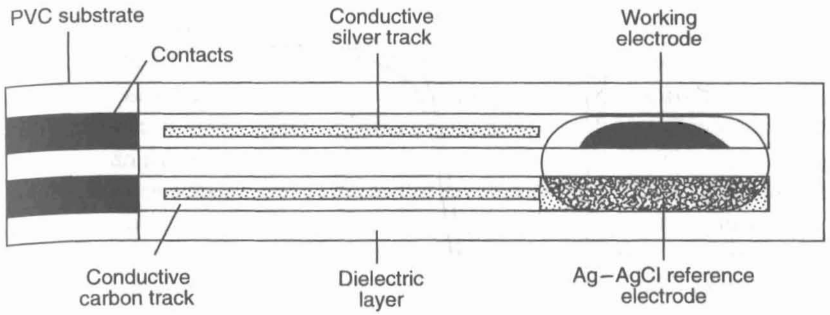


**Figure 2.30** Schematic of the ExacTech biosensor disposable electrode strip. From Hilditch, P. I. and Green, M. J., *Analyst*, **116**, 1217–1220 (1991). Reproduced with permission of The Royal Society of Chemistry.

Appropriate modifying components can be incorporated into the carbon ink, such as gold, mercury, chelating agents (for use in stripping voltammetry), mediators such as phthalocyanines and ferrocenes to catalyse electron transfer, or enzymes such as glucose oxidase, ascorbic acid oxidase, glutathione oxidase or uricase. The procedure has the advantages of miniaturization, versatility and cheapness, and in particular lends itself to the mass production of disposable electrodes. A version is marketed commercially in the 'ExacTech' biosensor for glucose (Figure 2.30).

**SAQ 2.14**

How could a screen-printed electrode be made by using a plant tissue material such as that of a banana?

### *2.6.2 Microelectrodes*

Microelectrodes, also called ultra-microelectrodes, having dimensions in the range 1–10 μm, have greatly extended the range of sample environments and experimental time-scales that can be used for electroanalysis. Such electrodes have surface areas which are many times smaller than the cross-sectional area of a human hair. They operate with small currents in the pA to nA range, and have steady-state responses and short response times. Electrodes have been made in the form of discs, bands, cylinders, rings and arrays. A simple disc can be made by embedding a platinum wire or carbon fibre in glass or epoxy resin and exposing the (cross-sectional) disc to the solution.

Because of their small dimensions, the double-layer capacitance is low, so that the faradaic current is large when compared to the background capacitive current. Due to the small current magnitudes, the $IR$ drop is very much reduced (or