Biosensors: Fundamentals and Applications

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Biosensors have emerged recently as a vibrant technique for qualitative and quantitative determination of various analytes for environmental, clinical, agricultural, food, and defence applications. Many biosensors have shown excellent characteristics for synthetic samples and pristine laboratory specimens. However, these interesting devices are not yet sufficiently robust for real-world application. The existing limitations are related directly to the operational and long-term stability of the biological receptor and physical transducer. Some of the other limitations could be attributed to poor reproducibility between sensors and selectivity in complex matrices. For practical applications, the most important obstacles are encountered once the sensor is used outside laboratory conditions and is applied for \textit{in situ} monitoring of real samples. The areas of development that are expected to have an impact on biosensor technology are immobilisation techniques, nanotechnology, miniaturisation, and multi-sensor array determinations. In this book, the basic concepts of biosensors are presented pertaining to fabrication and their wide range of applications in different fields.

In \textbf{Chapter 1}, the fundamentals of biosensors are discussed. Although many new types of transducers are being developed continuously for use in biosensors, optical (i.e., luminescence, absorption, and surface plasmon resonance), electrochemical and mass (i.e., surface acoustic wave, and microbalance) transduction methods are discussed in detail because these are traditional methods. The core part of biosensing lies in molecular recognition. In \textbf{Chapter 2}, efforts are made to describe different biological recognition elements that can be used for the development of biosensors and their interactions for the detection of analytes. In the development of a whole range of biosensors, the
immobilisation of bioreceptors has a key role because it ensures the high reactivity, orientation, accessibility and stability of the surface-confined probe and avoids non-specific binding. Many methods, such as absorption, electrochemical entrapment, biotin–avidin coupling, and covalent binding, can be used for biomolecule immobilisation, and selection of these methods is reliant on the choice of the matrix.

In Chapters 3 and 4, the applications of various conducting polymers and nanomaterials are summarised. Biosensors based on highly sensitive and precise nanomaterials have opened up the possibility of creating novel technologies for rapid, early-stage detection and the diagnosis of diseases. Different characteristic properties of nanomaterials have paved the way for the fabrication of a huge range of biosensors with improved analytical capacities. Chapter 5 describes the broad range of application of biosensors for the detection of various analytes. Special efforts have been made on the application of biosensors for the detection of cancer biomarkers.

In Chapter 6, we explore the potential of biosensors for the design and development of ‘smart’ sensing technologies using novel sensing strategies, such as ‘lab-on-a-chip’, paper-based sensors, and wearable sensors.

We hope that this book will cater to the urgent requirement of students and senior researchers venturing into this field of interdisciplinary research that has enormous practical applications. We thank Professor Yogesh Singh (Vice Chancellor, Delhi Technological University, Delhi, India) for his interest in this work.

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1 Fundamentals of Biosensors

1.1 Introduction

The role of biological and biochemical processes is paramount in clinical diagnostics, medical applications, bioreactors, food quality control, agriculture, control of industrial waste water, mining, and the military defense industry [1, 2]. However, the conversion of biological data to measurable electrical signals is currently a tedious and time-consuming process [3]. In this context, biosensors have been explored widely because they can be used to convert a biochemical process into a measurable signal [4, 5]. The basic difference between the biosensor and physical/chemical sensor is that its recognition element is biological [6]. With advances in device technology, the use of biosensors has increased and they can be used to detect what many others traditional sensing systems cannot. Nowadays, many biosensors are being produced industrially and are being utilised to develop large-scale multi-valued sensing systems [7]. Much research is being conducted in the field of biosensors, with an estimated 60% annual growth rate, with the major contribution coming from the healthcare industry [8].

The history of biosensors began through the development of an enzyme electrode by Clark [9]. Thereafter, researchers from various fields (physics, chemistry, and material science) have come together to develop more sophisticated, reliable, and mature biosensing devices [10]. Biosensors can be used in the fields of medicine, agriculture, biotechnology, defence as well as the war against bioterrorism [11]. Depending on the area of application, various definitions and terminologies are being used to define a biosensor. The most
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common cited definitions are those by Higson (a chemical sensing device in which a biologically derived recognition entity is coupled to a transducer, to allow the quantitative development of some complex biochemical parameter) and Frazerare [an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and a physical element (that transduces the recognition event)] [9]. In general, a biosensor is an analytical device that incorporates a biological sensing element integrated with a physico-chemical transducer that measures the sensitivity and specificity of a biochemical reaction to deliver complex bioanalytical measurements with a simple, easy-to-use format. For the fabrication of a biosensor for non-specialist markets, the following conditions are required [3, 5]:

- The desired analyte should be specific and stable under a normal storage condition.
- The sensor should be accurate, precise and show high sensitivity in a reproducible way, and linearity must be obtained with different concentrations.
- Physical parameters such as pH, temperature should be optimised, which will lead to sample analysis with minimal pre-treatment.
- The biosensor should be small and biocompatible so that it can be used for invasive monitoring in clinical diagnostics.
- The fabricated biosensor should be portable, cost-effective, small, and capable of being used by semi-skilled operators.
- The biosensor should provide real-time analysis so that it can be employed for rapid measurements of analytes from human samples.

Biosensors are composed mainly of two elements: bioreceptors and transducers (Figure 1.1).
• Bioreceptors are biological recognition elements that consist of an immobilised biocomponent that can detect the specific target analyte (e.g., enzyme substrate, complementary DNA, antigen).

• The second and the most important part of the biosensor is the transducer, which converts a biochemical signal into an electrical signal, resulting from the interaction of the analyte with the bioreceptor. The intensity of signal arising as a result of the biochemical reaction is directly or inversely proportional to the analyte concentration.

1.2 Developments in Biosensors

For the development of biosensors, the selection of suitable transducers, immobilisation methods, and bioreceptors are crucial [6]. There is enough scope relating to the innovation in the fabrication of a biosensor for application in clinical diagnostics. Further, this multi-disciplinary field of science and technology is predicted to result in miniaturised, cheaper, and faster biosensors that not only
provide accurate information but also feedback to the real world for necessary actions [12]. Further, on the basis of the transducing elements, biosensors can be classified into four types: electrochemical (EC), optical, piezoelectric, and thermal sensors (Figure 1.2).

**Figure 1.2** Schematic of a biosensor (ISFET: ion-selective field-effective transistor and MOSFET: metal oxide semiconductor field-effect transistor)

### 1.2.1 Electrochemical Biosensors

EC biosensors have been explored widely because they allow analysis of biomolecules with high specificity, sensitivity, and selectivity, have low response time and are cost-effective [13]. An EC biosensor can be used for clinical analyses, in online control processes for industry or environment, or even *in vivo* studies. According to a recommendation from the International Union of Pure and Applied Chemistry in 1999, ‘An electrochemical biosensor is a self-contained integrated...
device, that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is kept in direct spatial contact with an electrochemical transduction element’ [14]. ‘EC sensing’ usually requires a working electrode (WE), reference electrode (RE), and a counter (or auxiliary) electrode (CE). In the EC biosensor, the reactions are detected only in proximity to the electrode surface. Thus, the electrode has a significant role in the overall performance of EC biosensors. Further, the detection ability of the biosensor is based on the properties of the electrode (electrode material, its surface modification or its dimensions) [15].

The transduction element in the EC system is the WE, where the biochemical reaction takes place. The CE (conductive and steady) acts as a connection to the electrolytic solution for applying current to the WE. For the RE, silver/silver chloride has been commonly used, which is kept at a distance from the reaction site to maintain a desirable and stable potential (Figure 1.3). During the redox reaction of the molecule at the electrode surface, electrons are transferred from the analyte to the WE, or from the electrode to the analyte.

![Figure 1.3 Assembly of different electrodes in an EC biosensor](image)
The direction of flow of electrons depends on the properties of the analyte, which can be controlled by applying the electric potential to the WE [16]. An oxidation reaction is said to occur if the WE is driven to a positive potential, where the flow of current depends on the concentration of the analyte (electroactive species) diffusing to the surface of the WE. On the other hand, if the WE is driven to a negative potential, then a reduction reaction occurs. The third electrode (i.e., CE or the auxiliary electrode) is often used to measure the current flow because it functions as a cathode whenever the WE is operating as an anode and vice versa. The surface area of the CE is often larger than that of the WE, and the half-reaction at the CE should be rapid so that it may not limit the process at the WE. The auxiliary electrode is adjusted so that it can balance the reaction occurring at the WE, and this configuration allows the potential of the WE to be measured against a known RE. The commonly used auxiliary electrodes are fabricated from electrochemically inert materials such as gold, platinum or carbon.

EC biosensors measure the current produced as a result of the oxidation and reduction reactions. The three electrodes are connected to a potentiostat, which controls the potential of the WE and measures the resulting current. In an EC reaction, a potential is applied to the WE and the resulting current is measured versus time. In a solution, the equilibrium concentrations of the reduced and oxidised forms of a redox couple are linked to the potential ($E$) via the Nernst equation, Equation 1.1:

$$ E = E_0 + \frac{RT}{nF} \ln \frac{C_{\text{oxi}}}{C_{\text{red}}} $$

where $E_0$ is the standard half-cell potential, $F$ is the Faraday constant, $T$ is absolute temperature, and $C_{\text{oxi}}$ and $C_{\text{red}}$ are concentrations of the oxidation and reduction centres, respectively. When the potential is applied to the WE, the redox couples present at the electrode adjust their concentration ratios according to the Nernst equation. The
resulting electrical signal is related to the recognition process by the target analyte and is proportional to the analyte concentration. An EC biosensor has many advantages such as speed, simplicity, low cost, high sensitivity, and relatively simple instrumentation [17, 18]. The EC sensor, where the electrode is used as the transduction element, represents an important subclass of sensors and is based on the nature of the EC changes detected during a biorecognition. EC biosensors fall into one of four categories: amperometric, potentiometric, impedance and conductometric [19].

1.2.1.1 Amperometric Sensors

Amperometric sensors (AS) measure the current change resulting from the oxidation and reduction of an electroactive species while a constant potential is being applied. The change in the current is related to the concentration of the species in solution. In an AS, all the three types of the electrode (WE, RE and CE) are employed [20]. The WE used in AS applications are gold, indium-tin-oxide coated glass substrate, carbon, and platinum, whereas silver/silver chloride is used as the RE, which has a fixed potential that controls the potential of the WE. Along with the RE, the CE can also be used to measure current flow. During oxidation and reduction of the molecule at the inert metal electrode, electrons are transferred from the analyte to the WE, and the flow of the electrons is governed by the properties of the analyte. An oxidation reaction occurs when the WE is driven to a positive potential, whereas a negative potential results in the reduction reaction [21]. Different analytes [enzyme, nucleic acid(s) (NA), antibodies] can be integrated with the amperometric transducer for clinical diagnostics [22]. However, the major limitation in using this transduction is that electroactive interference in a sample matrix can result in the generation of false current reading. To overcome this limitation, various methods, such as dilution of the sample, changing the medium of the analyte, coating the electrode with various conducting polymers, or the addition of a suitable mediator, have been adopted [23].
1.2.1.1 Cyclic Voltammetry

In cyclic voltammetry (CV), the WE potential is swept at a specific sweep rate (in volts/second), and the resulting current is recorded versus time. Usually, the sweep is reversed at a specific switching potential. Hence, it is named ‘CV’ [3]. The sweep rate is constant and the initial and switching potentials are known, so the time can be easily converted to potential, and current versus applied potential can be recorded (Figure 1.4). CV is applied to gain information on the EC reactions of electroactive species having a known redox potential. The current is monitored at the WE (because the CE conducts electricity from the signal source to the WE) during a potential scan against a constant RE potential. The electrolytic solution is used to provide ions to the electrodes during a redox process [24].

![Cyclic voltammogram of a bare gold electrode in phosphate buffer saline (100 mM, pH 7.4, 0.9% sodium chloride) solution containing 5 mM [Fe(CN)₆]³⁻/⁴⁻ at a scan rate of 30 mV/s (E_PA: potential of the anodic peak current; E_PC: potential of the cathodic peak current; I_PA: anodic peak current; and I_PC: cathodic peak current)](image_url)
Figure 1.4 describes a model CV of a bare gold electrode where the resulting current versus applied potential curve is predicted for an ideal, reversible system. The peak current in this voltammogram is given by the Randles–Sevcik equation (Equation 1.2):

$$I_p = 2.69 \times 10^5 A n^{3/2} D^{1/2} \nu^{1/2} C$$

(1.2)

where $I_p$ is peak current (A), $n$ is electron stoichiometry (eq/mol), $A$ is the electrode area (cm²), $D$ is diffusion coefficient (cm²/s), $C$ is concentration (mol/cm³), and $\nu$ is the scan rate (V/s). The potential at the mid-point of the two peaks in the voltammogram can be represented by Equation 1.3:

$$\frac{(E_{anodic} + E_{cathodic})}{2} = E^o + \frac{RT}{nF} \left( \ln \frac{D^1_{R}}{D^0_{O}} \right)$$

(1.3)

where $E^o$ is the redox potential and $D^0_{O}$ and $D^1_{R}$ are the diffusion coefficients for the oxidised and reduced halves of that redox couple, respectively. Finally, the separation between the two peaks of the voltammogram is given by Equation 1.4:

$$\Delta E_p = E_{anodic} - E_{cathodic} = 2.3 \frac{RT}{nF} = \frac{59}{n} \text{mV (at 298 K)}$$

(1.4)

CV is a versatile diagnostic tool used to assess the reversibility of the EC reaction and its diffusion-controlled character. Other than sensing applications, it can be used to characterise the processes that take place at the sensing electrode. Hence, by conducting CV of an electrode, one could easily deduce the concentration, diffusion coefficient, the number of electrons per molecule of analyte oxidised or reduced, and the redox potential for the analyte.

1.2.1.2 Potentiometric Sensor

Potentiometric transduction was first reported in the year 1969 where the enzyme-based sensor was used for detection of urea [25].
It relies on the utilisation of an ion-selective electrode (ISE) and ion-sensitive field-effect transistor (ISFET) for obtaining the analytical information. In these sensors, the biological recognition element converts the recognition process into a potential signal to provide an analytical signal. The potentiometric biosensor consists of two electrodes: an indicator electrode (which is used to develop a variable potential from the recognition process) and an RE (usually silver/silver chloride, which provides a constant half-cell potential) [26]. In an EC cell during the recognition process, the transduction of the potentiometric biosensor depends on the accumulation of a potential difference between the indicator electrode and the RE. High impedance voltammetry is used to measure the potential difference or the electromotive force between the two electrodes. The WE consists of a permselective ion-conductive membrane which is sometimes called an ISE [12]. The measurement of the potential response of a potentiometric device is governed by the Nernst equation, in which the logarithm of the concentration of the substance being measured is proportional to the potential difference.

1.2.1.3 Conductometric Sensors

A conductometric sensor measures the change in the electrical conductivity of the cell solution using two electrodes that are separated by a certain distance or by a medium [27]. The conductometric sensor has been used widely in enzyme sensors in which the enzymatic reaction results in a change of ionic strength and the conductivity of a solution between two electrodes. An alternating current (AC) source is used across the electrode for conductivity measurement and, thus, the change in the ionic composition provides a conductance which is measured using an ohmmeter. Recently, micro-/nanoelectronic devices (field-effect transistor) based on conductometric transduction have been used for the detection of NA hybridisation, and immunosensors [3]. The advantages of the conductometric device are that no RE is
required, it is inexpensive, and there is possibility of miniaturisation or direct electrical response. In spite of these interesting features, this transduction method is less sensitive compared with the other EC methods, and is strongly dependent on the response to buffer capacity [28]. However, conductometric-based transduction has received a great deal of attention and the sensitivity problem has been overcome. Subsequently, some research has been directed towards the improvement of performance and sensitivity.

1.2.1.4 Electrochemical Impedance Spectroscopy

Impedance can be defined as the ability of the circuit to resist the flow of electrical current. In EC impedance, the current of the cell is measured by an AC potential to an EC cell. The impedance is a measure of the impeded flow of ions through solutions, interfaces, and coatings [29]. When a sinusoidal potential is applied, the response to this potential results in the generation of an AC signal which can be considered to be a sum of sinusoidal functions. The impedance can be calculated by setting the input potential and measuring the induced current. Impedance methods are powerful because they are capable of sampling electron transfer at high frequency and mass transfer at low frequency [30]. Impedance measurements are made at an open circuit potential in which the current flows in an electrified interface resulting from an EC reaction [31]. The electrons are transferred across the electrified interface as illustrated in Figure 1.5A and this process always contains a non-Faradaic component, which is expressed as Equation 1.5:

\[ [O] + ne^- \rightarrow [R] \]  \hspace{1cm} (1.5)

where \( n \) is the number of electrons transferred, \( O \) is the oxidant and \( R \) is its reduced product. The charge transfer has Faradaic and non-Faradaic components. The Faradaic component arises from the
electron transfer via a reaction across the interface that overcomes an appropriate activation barrier, namely the polarisation resistance ($R_p$) along with the uncompensated solution resistance ($R_s$) [32]. The non-Faradaic current results from charging the double-layer capacitor ($C_{dl}$). When the charge transfer occurs at the interface, the mass transport of the reactant and product has a major role in determining the rate of electron transfer, which depends on the consumption of the oxidants and the production of the reductant near the electrode surface. The mass transport of the reactants and the products provides another class of impedance, Warburg impedance ($Z_w$), which can be explored electrochemically and can be shown in the form of a peak current in a voltammogram [33]. The electrochemical impedance circuit (EIC) shows that each circuit component corresponds to each interfacial part (Figure 1.5B). The EIC first proposed by Randles displays the high-frequency components ($R_s$) and low-frequency components (e.g., $Z_w$). The left-to-right arrangement of the EIC is necessary because the impedance data are typically displayed in this manner. Moreover, the activation barrier at a particular potential can be represented by the $R_p$ but, at the standard (or formal) electrode potential, the barrier becomes the charge-transfer resistance ($R_{ct}$). A Faradic impedance spectrum (Nyquist plot) includes a semicircle region observed at a higher frequency corresponding to an electron-transfer limited process on the $Z'$ axes, and is followed by a linear straight line at 45° to the real axes at lower frequencies, revealing a diffusion-limited electron-transfer process, as shown in Figure 1.6 [34].

The complex impedance plot can be represented as a sum of the real ($Z'$) and imaginary ($Z''$) components that mainly originate from the resistance and capacitance of the cell and, for the parallel circuit, it can be calculated using Equations 1.6 and 1.7:

$$Z = Z' + jZ'' = R_s + \frac{R_p}{1 + \omega^2 C_p^2 R_p^2} + \frac{\omega C_{dl} R_p^2}{1 + \omega^2 C_p^2 R_p^2} \quad (1.6)$$
Figure 1.5 A) The electrified interface in which the electrode is negatively charged; counter cations are aligned along the electrified surface and B) the electrode–solution interface can be modelled by an equivalent circuit (Randles circuit)

Figure 1.6 Nyquist plot with a depressed arc where the polarisation is due to a combination of kinetic and diffusion processes
C_d = \frac{1}{2\pi f_{\text{max}} R_{ct}} \quad (1.7)

In electrochemical impedance spectroscopy (EIS) the $Z_w$ represents the bulk properties of the electrolyte solution and can be estimated from the Nyquist plot to describe the electrical response at the electrode. It can be expressed as an intercept of the straight line having a slope of 45° using Equations 1.8 and 1.9:

$$Z_w(\omega) = W_{int} \frac{R_p \lambda}{(2\omega)^{1/2}} \left[i - j\right] \quad (1.8)$$

$$W_{int} = R_s R_p - R_p^2 i C_d \text{ where, } i = \frac{K_f}{D_o^{1/2}} + \frac{K_b}{D_k^{1/2}} \quad (1.9)$$

where, $\lambda$ is defined as the Warburg coefficient, $K_f$ and $K_b$ are forward and backward electron transfer rate constants, respectively, and $D_o$ and $D_k$ are diffusion coefficients of the oxidant and reductant, respectively.

EIS has been used widely by many researchers to detect cancer/tumour cells, viruses, bacteria and pathogens. The EIS-based biosensor could become a powerful tool for clinical diagnostics in the near future, and can be used for real-time monitoring because it can provide label-free detection.

### 1.2.2 Optical-based Biosensor

During the last decade, much research has been conducted in the field of optical biosensors, especially in the area of food safety, security, life science, environmental monitoring and medicine [35]. The high sensitivity, small size, and cost-effectiveness of optical biosensors may provide an alternative tool to other conventional analytical methods. The first optical chemical sensor was developed for measurement
of the concentration of carbon dioxide and oxygen, and was based on analyses of the changes in the absorption spectrum. After that, a large variety of optical sensors, such as ellipsometry, interferometry, spectroscopy (luminescence, phosphorescence, fluorescence, Raman), optical waveguide structures, and surface plasmon resonance (SPR) have been used in biosensor applications [36–38]. Among them, the most commonly used optical biosensors are based on SPR or fluorescence integrated with the optical fibres [39]. These sensors rely on a change in the refractive index, absorbance and fluorescence properties of analyte molecules or a chemo-optical transducing medium. In the last decade, there has been enormous growth in the research and technological development of optical biosensors because they provide direct, real-time and label-free detection of many chemical and biological substances. Despite these technological developments, the commercialisation of this sensor for field application has been slow. The major problem with this transduction is related to the interaction of the biological molecule with the transducer surface, and the integration of optical biosensors into a miniaturised device. Second, the cost is presently a major hurdle for realistic mass production of biosensors.

1.2.2.1 Surface Plasmon Resonance

SPR biosensors utilise surface plasmon waves (SPW, which are electromagnetic) that detect changes when the target analyte interacts with a biorecognition element on the sensor surface [40]. During the interaction of the target analyte with the immobilised biomolecule on the sensor surface, there is a change in the refractive index at the sensor surface. The excitation of SPW by an optical wave results in the resonant transfer of energy into the SPW, and SPR manifests itself by resonant absorption of the energy of the optical wave. This change produces a variation in the propagation constant of the SPW, which is detected using a spectrophotometer [40, 41]. The high signal from the electromagnetic field in the dielectric, the propagation constant of the SPW, and consequently the SPR condition, are very sensitive
to variations in the optical properties of the dielectric adjacent to the metal layer supporting the SPW (transducing medium) [42]. Therefore, by monitoring the interaction between the SPW and the optical wave, the change in the optical parameters of the transducing medium can be measured. The building blocks of an SPR instrument are shown in Figure 1.7. The two most common detection approaches used in SPR sensors are (a) the measurement of the intensity of the optical wave near the resonance and (b) measurement of the resonant momentum of the optical wave, including angular and wavelength interrogation of SPR [43, 44].

![Figure 1.7 Basic components of an instrument for SPR biosensing. A glass slide with a thin gold coating is mounted on a prism. Light passes through the prism and slide, reflects off the gold, and passes back through the prism to a detector. Reproduced with permission from M.A. Cooper, Nature Reviews Drug Discovery, 2002, 1, 515. ©2002, Nature Publishing Group [45]]
Various biorecognition elements, such as proteins, antibodies, NA, and enzymes, have been integrated with SPR biosensors. The important feature of a SPR biosensor is that it can provide label-free sensing without radioactive and fluorescence tagging, which makes it highly attractive for real-time monitoring [46]. Also, SPR-based transduction can be used for interaction without exhibiting any unique properties of fluorescence or characteristic absorption and scattering bands. However, the major drawback with this transduction is its specificity due to non-specific interaction with the biorecognition element. Second, SPR is not suitable for studying small analytes because they yield inadequate responses, and the SPR measures the mass of the material binding to the sensor surface. SPR biosensors have been used to detect the binding of an analyte as small as $\approx 2 \text{ kDa}$, but direct detection is not possible because these molecules generate insufficient changes in a bound mass [6]. With recent improvements in the signal-to-noise ratio, it has been possible to measure the binding of such small analytes. To date, SPR has been used widely in fundamental biological studies, health-science research, drug discovery, clinical diagnostics as well as environmental and agriculture monitoring.

### 1.2.2.2 Chemiluminescence

Chemiluminescence is the energy of a chemical reaction which produces an emission of light, usually described as luminescence. During a chemical reaction, when the atom or molecule relaxes from its excited state to its ground state, luminescence is produced as a side product of the reaction [47]. In chemiluminescence biosensor, light is generated as a result of the binding event between the analyte and the immobilised biomolecule which is detected using a photomultiplier tube. This transducer-based platform has been widely used to identify specific biochemical reactions and their property. The chemiluminescence-based biosensor has been widely used for immuno-sensing and NA hybridisation studies as it provides enhanced sensitivity along with simple instrumentation, fast dynamic response properties, and a wide calibration range [48].
the interesting features, chemiluminescence transduction has a few drawbacks, such as a low quantitative accuracy due to short lifetime and its application in real time monitoring.

1.2.2.3 Fibre Optic Biosensor

The optical fibre or the optrode have found considerable interest in the fabrication of ultrasensitive biosensor. It consists of a light source, a biorecognition element and, an optical fibre which is used to transmit light and acts as the substrate, and a detector (e.g., spectrophotometer) [49]. The optical fibre-based biosensor comprises of a flexible optical fibre consisting of small wires that are made up of glass/plastic having different geometrical morphology. These fibres transmit light signals over long distances with minimum lost value. Due to their remarkably strong, flexible and durable structures, it can be conveniently used in harsh and hazardous conditions. These optical fibres also permit the transmission of multiple signals synchronously and by this means it can obtain various capabilities for sensing of the analyte. The high quality and low cost of the optical fibres make them a suitable transducer for sensing applications. In principle during the interaction of the analyte with the biorecognition elements at the surface of the fibre, a biochemical reaction takes place which results in a change of the optical properties which is correlated to the analyte concentration [50]. Due to their high sensitivity, fast response, high selectivity, and low detection limits, these optoelectronic are consistently being used for miniaturised high-performance sensor and small fibre optical sensors. Compared to the EC biosensing approach, optical fibres-based biosensor provides higher sensitivity, safety, freedom from electromagnetic interference and can be used for real-time monitoring. This transduction has been used for remote sensing and single molecule detection as it is reagent-less and flexible [3]. In spite, of these interesting, features the poor stability of biorecognition element and sensitivity to ambient light are certain disadvantages associated with optical fibre-based transduction.
1.2.3 Piezoelectric Sensors

Piezoelectric sensors are sensitive mass-to-frequency transducers that are of interest for potential applications in analytical chemistry. A piezoelectric device is sensitive to changes in the mass, density, or viscosity of samples in contact with its active surface. The piezoelectric effect is observed when the pressure applied to a dielectric material deforms its crystal lattice. The piezoelectric transduction provides mechanical and electrical forces to a biological medium usually in the form of progressive or standing acoustic waves, which may be of different types [51]. Understanding of the properties is crucial for the selection of the perfect acoustic wave for a given analyte. The mechanical force causes the separation of the cationic and anionic centres and hence changes the dipole moment of a molecule. There are two types of piezoelectric sensors: i) bulk wave devices and ii) surface acoustic wave (SAW) devices [52]. The SAW can be compared with bulk wave devices that are capable of fundamental oscillations at higher frequencies. Current research indicates that SAW devices are capable of lower detection limits [53]. Therefore, to develop viable, practical piezoelectric biosensors, it is important to consider a complete sensor-development process from day one of the sensor-design process. Extensive research conducted over the decades in the area of piezoelectric biosensors has led to the development of theoretical and experimental knowledge. However, efforts are underway for the commercialisation of this transduction-based biosensor. Recent research on piezoelectric sensors has shown their potential use in medical diagnostic tools and laboratory experiments [51]. However, the main advantage of using this type of transduction is that it provides a multi-domain sensing mechanism and has good temperature stability in comparison with EC and optical biosensors [18, 54]. For development of the complete device, it is essential to devise specific design concepts that relate to the nature of a piezoelectric transducer-biological film-enclosure interface. In a piezoelectric sensing mechanism, the mechanical character of the piezoelectric material, the enclosure of a biosensor as well as
sample handling systems may interfere with the sensing mechanical motion [55]. This may lead to a decrease in the sensor performance. The quartz crystal microbalance (QCM) is a good example of a piezoelectric biosensor in which measurement of the mass of a thin-surface coating is based on the ‘piezoelectric effect’ (Figure 1.8) [56]. The QCM biosensors are compatible with the protocol of biopolymer polymerisation that provides patterned thin layers of a certain mass and thickness. The Langmuir–Blodgett films and polyelectrolyte complexes obtained by consecutive deposition of the oppositely charged polyionic components offer similar prospects for biosensor assembly [57]. Piezoelectric transducers have been applied widely for immunosensing applications, especially for the detection of cholera toxin, hepatitis B, hepatitis C and food-borne pathogens. According to the literature, this transduction has been used for detection of DNA and protein with a detection limit of 1 ng/cm² [58].

Figure 1.8 (a) Gold crystals along with the QCM holder and (b) set-up for in situ QCM measurement. Reproduced with permission from Z. Matharu, A.J. Bandodkar, G. Sumana, P.R. Solanki, E.M. Ekanayake, K. Kaneto, V. Gupta and B. Malhotra, The Journal of Physical Chemistry B, 2009, 113, 14405. ©2009, American Chemical Society [58]

1.2.4 Calorimetric-based Biosensor

With the introduction of enzyme-based biosensors by Clark and Lyons in 1962, efforts have been made towards the fabrication of calorimetric-based transduction [59, 60]. The fundamental principle
of a calorimetric biosensor relates to the measurement of the changes in temperature in the reaction between the biorecognition element and a suitable analyte. The calorimetric biosensors measure heat evolution (enthalpy change) following biochemical reactions [61]. This change in temperature can be correlated to the number of reactants consumed or products formed. In a calorimetric device, the heat change is measured using a thermostat (usually a metal oxide) or thermopile (usually ceramic semiconductor) [62]. Previously, calorimetric transduction was employed for enzyme-based sensors, but nowadays they are being used for detection of immunosensors and cells. The advantage of using this type of transduction is stability, enhanced sensitivity, and its application in point-of-care diagnostics. Calorimetric sensors are easy to miniaturise and can be integrated with microfluidic devices for enhanced sensitivity. Recent studies indicate that this sensor is capable of detecting NA hybridisation rapidly, and has been used in the food industry and environmental monitoring [54, 63].

1.3 Conclusions

A biosensor device consists of a biological sensing element intimately connected or integrated within a transducer. The fundamental principle of a biosensor is to produce an electronic digital signal that is proportional to the concentration of the particular analyte. The arena of expertise required for biosensor development can be sustained via collaboration from many areas of academia and industry. This emerging field offers new and powerful tools for the exciting alternative to traditional methods, thereby allowing rapid and multiple detections and the diagnosis of any analyte. Thus, biosensor technology presents an opportunity for the development of robust, low-cost, accurate detection of analytes. The future depends on the development of new sensing elements and transducers.
References


2 Biorecognition Elements in a Biosensor

2.1 Introduction

The biological recognition element is an important part of a biosensor. Initially, biosensor recognition elements were isolated from living systems. However, many biosensor recognition elements are being synthesised in the laboratory [1, 2]. The interest in the sensing of the desired target analyte is already being influenced by the materialisation of engineered binding proteins [3, 4]. With the development in the design of nanostructures and new interface materials, these recognition elements will have a leading role in the development of advanced biosensor devices. In the process of biosensing, receptors are appealing because of their generic ‘receiving’ as well as ‘sending’ functions. Receptors are the transmembrane (plasma and intracellular membranes) soluble proteins which bind to the specific molecules (ligands), resulting in a specific cellular response due to the binding event [5]. The receptors act as mediators of physiological processes and serve as natural targets for various biomolecules. Valdes and co-workers first described the necessity of receptor preparations as biosensor sensing elements for multiple ligands of interest [6–8, 9]. Receptor preparations are attractive in biosensor recognition elements due to their high specificity and affinity for ligands. However, their instability, low yield, labour-intensive isolation, lengthy purification protocols of membrane-associated proteins, as well as transduction make them difficult to use in receptor-mediated sensing [3, 10, 11].

It is possible to generate many receptor proteins using recombinant techniques and a multitude of expression systems. The direct monitoring of receptor–ligand interaction may be difficult due to the absence of a signal amplification associated with other sensor biorecognition
elements (enzyme recognition elements). The next section focuses on the different immobilisation methods and principles of biorecognition and different molecular recognition (MR) elements that are being used currently in biosensor developments.

2.2 Immobilisation Methods

For efficient performance of a biosensor, the biological components should be attached appropriately to the transducers. Biosensors are usually designed with high loading of biomolecules to ensure sufficient biocatalyst activities and, to further sustain the biological activity, an appropriate molecular environment should be provided [12, 13]. The local chemical and thermal environment can have profound effects on the stability of the biomolecule. The choice of immobilisation method depends on factors such as the physico-chemical properties of the analyte, nature of the biological element, type of transducer used, and the operating environment of the biosensor [14]. Also, it is crucial that the biological element should exhibit maximum activity in its immobilised microenvironment [15, 16]. There are four methods for biomolecule immobilisation that are being used presently (Figure 2.1) [17, 18].

2.2.1 Adsorption

Adsorption is the simplest method for the immobilisation of biomolecules. However, the bonding is weak and the lifetime of an electrode is short. The adsorption process can be divided further into two classes: physical and chemical. Physical adsorption is weak and occurs mainly via van der Waals forces, whereas chemical adsorption is stronger and involves the formation of covalent bonds.

2.2.2 Covalent Bonding

Covalent bonding involves bonding between a functional group in the biomaterial to the supporting matrices. Covalent
immobilisation requires mild conditions such as low temperature, low ionic strength, and pH in the physiological range, under which reactions are undertaken [19]. The functional groups that are involved in covalent bonding are -NH$_2$, -COOH, -OH, C$_6$H$_4$OH and -SH.

### 2.2.3 Crosslinking

Crosslinking is the bonding of two or more molecules by covalent bonds. For this method, usually, the biomaterial is chemically bonded to solid supports or another supporting material, such as a crosslinking agent, to increase the attachment significantly. In the crosslinking process, bifunctional agents such as glutaraldehyde are used to bind the biological materials [20].
2.2.4 Entrapment

In the entrapment method, solutions of polymeric materials are prepared to contain biological materials that are to be ‘entrapped’ onto the working electrode. The solution is coated on the electrode by various methods. However, this method can give rise to barriers to the diffusion of the substrate, leading to a delay in the reaction. Also, a loss of bioactivity may occur through pores in the gel. The gels commonly used include starch gels, nylon and conductive polymers such as polyaniline (PANI) or nafion [16].

2.3 Principles of Biorecognition

The molecules in solution collide billions of times per second and, in most cases, the ‘complexes’ formed by these collisions are weak, short-lived and non-specific [21]. However, if the surface properties of one molecule are complementary to the other (i.e., the attractive forces generated by the interactions of the features compensate the repulsive forces and entropic costs of bringing them together), then stronger, stable, and specific interactions will result [22, 23]. The formation of specific complexes plays a vital part in biological and technological processes. Thus, MR can be defined as the process of specific binding between molecules (macromolecule or molecular assembly), and the other analyte/target molecule (small molecule or another macromolecule) [24, 25]. In traditional terminology, with roots in the study of biosignaling, the macromolecular component of an interaction is called a ‘receptor’ and a small molecule that, upon binding to its receptor, elicits a typical biological response is known as a ‘ligand’ [21]. There may be a different definition of MR in terms of the process and analyte. For example, an organic chemist may describe the MR of cholesterol by cyclodextrin as the formation of a ‘host–guest complex’ whereas a biochemist may describe the MR of cholesterol by the enzyme cholesterol oxidase as ‘substrate binding’. Similarly, in the language of an immunologist, membrane-bound receptors are called ‘receptors’, whereas soluble receptors are called ‘antibodies’, and anything that binds to antibodies is called
an ‘antigen’. In analytical chemistry, the interaction partner that is designed to bind the analyte is often called a ‘molecular sensor’ or ‘biosensor’; molecules that interact with the biosensor may also give false-positive readings or act by preventing the analyte binding, leading to false-negative readings [21].

Due to the interdisciplinary nature of research in MR, it is important to be familiar with the terminology used in other fields. For example, if one were designing a biosensor for the detection of a sugar, the considerable body of work that has been done to elucidate the mechanisms of carbohydrate-modifying enzymes may be of interest. Typically, MR arises due to the contributions of many weak, reversible interactions. For the rational design of biomolecules and materials, it is crucial to understand the driving force behind the formation of such complexes and how those effects are related. Fortunately, even though our understanding of these interactions is currently limited to render them \textit{ab initio} (from basic principles), and the design of MR systems is a challenge. Moreover, the physical principles underlying them are sufficiently straightforward to achieve at least semi-quantitative description of the key players. Specifically, only six key forces dominate MR in biological systems. On the basis of the different recognition behaviour of biomolecules, biorecognition elements are (in general) classified into three categories: natural, semi-synthetic and synthetic (Figure 2.2). Each of these recognition elements are discussed in detail in the next section.

\subsection*{2.3.1 Natural Biorecognition Elements}

\subsubsection*{2.3.1.1 Enzymes}

In biosensor application, the use of catalytic enzyme-based sensor recognition elements has been explored widely because they offer various measurable reaction products (protons, electrons, light, and heat) that arise from the catalytic process. The ability of an enzyme to specifically recognise its substrates and to catalyse their transformations makes them efficient biocatalysts. In enzyme-based biosensors, there
is an intimate association of a biocatalyst-containing sensing layer with a transducer, and the working principle is based mainly on the catalytic action and binding capabilities for specific detection. The mechanism for the enzymatic reaction can be explained by the ‘lock and key’ and ‘induced fit’ hypotheses that are highly specific for this type of biosensor. In an enzymatic biosensor, the particular catalytic reaction of the enzyme results in the detection of much lower limits compared with that of traditional binding techniques, and the process can be explained using the Michaelis–Menten equation. Further, components such as the substrate concentration, temperature, pH and presence of a competitive and non-competitive inhibitor may influence the actions of the catalytic enzyme. To overcome these barriers, several approaches, such as the use of permselective membranes (nafion), preliminary oxidation of interferons, or the use of self-referencing electrodes, have been adopted [26]. Recently, electrochemical (EC) enzyme biosensors based on various novel electrode materials and immobilisation strategies have been used because they provide efficient
and stable attachment of the enzyme, biocompatibility and enhance the surface area and EC response [27, 28]. All developments have been based on optimising and improving the performance of the enzyme sensor rather than developing new sensing principles. Further, efforts are being made to improve the electronic communication between the electrode surface and redox centre of the enzyme for the fabrication of third-generation biosensors which can be used for in vivo monitoring. The fundamental principle of operation of an enzyme electrode is shown in Figure 2.3, in which the substrate to be determined diffuses into the enzyme layer, where the enzymatic reaction occurs (Equation 2.1):

\[ S + R_{\text{enzyme}} \rightarrow P + R' \]  

(resulting in a product (P or R') or consuming co-reactant (R) which can be measured by transducers. The most commonly used enzyme biosensors in the clinical sector have been designed for urea, lactate, glucose, glutamate, and cholesterol. Among the various enzyme-based sensor recognition elements, urease has been used widely in medical and environmental applications for the detection of urea.

Figure 2.3 Schematic illustration of enzymatic biosensors based on (A) mediated electron transfer and (B) direct electron transfer
Many types of glucose and glutamate sensors have been developed. The key challenges are to minimise the interference and provide sufficient selectivity in physiological conditions [29]. Recently, many researchers have been working on enzyme-based biosensors that can be used for the detection of cholesterol [30], food pathogens, heavy metals and pesticides [31, 32]. Among various reported enzyme recognition element-based biosensors, the glucose biosensor has been studied widely [33]. The medical need for the monitoring of glucose has led to the development of different glucose sensors. Nowadays, efforts are being focused more on the fabrication of bloodless glucose sensors, implantable EC glucose sensors [3, 34, 35], microfabricated electrophoresis chips [36]; and needle microsensing [37, 38]. The basic principle of glucose measurement depends on interaction with one of three enzymes: hexokinase, glucose oxidase (GOx) or glucose-1-dehydrogenase [39]. These enzymes have different redox potentials, cofactors, turnover rate and selectivity for glucose. GOx is considered to be the standard enzyme for biosensors because it has a relatively higher selectivity for glucose [40]. Further, it is cheap, readily available and it can withstand greater extremes of pH, ionic strength, and temperature compared with other enzymes. In the case of EC-based glucose biosensors, the immobilised GOx catalyses the oxidation of β-D-glucose by molecular oxygen and results in the production of gluconic acid and hydrogen peroxide [41]. To work as a catalyst, GOx requires a redox cofactor – flavin adenine dinucleotide (FAD) – which is reduced to FADH₂ by working as an initial electron acceptor. The cofactor is further regenerated by reacting with oxygen and, in the process, there is formation of hydrogen peroxide, which is oxidised at a catalytic (classically platinum) anode. The electrode quickly recognises the number of electrons transferred, and it is proportional to the number of glucose molecules present in the blood [33]. The complete reaction for the glucose biosensor can be summarised in Equations 2.2–2.4:
Glucose + GOx − FAD⁺ → Gluconolactone + GOx − FADH₂ (2.2)  

$$\text{GOx} - \text{FADH}_2 + \text{O}_2 \rightarrow \text{GOx} - \text{FAD} + \text{H}_2\text{O}_2$$ (2.3)  

$$\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}^-$$ (2.4)

Enzyme-based EC biosensors offer several significant advantages for chemical sensing. This biosensor technology is growing rapidly due to the increasing availability of new enzymes and alternative biocatalysts, as well as advances in biomaterials and EC techniques. Consequently, new biosensors with enhanced measuring capabilities continue to be developed. Although a variety of nanosensor technologies have been developed using EC and fluorescent methods, there is still a need to integrate such sensing systems with *in vivo* measurements. The implantation of EC sensing chips and optical interrogation through the skin will be of great interest. In addition, a method to eliminate or reduce the need for patients to take blood samples is promising with the miniaturisation of sensing systems for point-of-care (POC) in the future. Amplification strategy is another significant area in the development of enzymatic glucose biosensors. This could be achieved by the incorporation of glucose-related enzymes or other detection molecules into nanomaterials.

### 2.3.1.2 Antibodies

Antibody-based biosensors were investigated first in the 1950s, after which the possibility for immunosensors emerged [42]. Antibodies are some of the most common bioreceptors used in biosensors. On the basis of their elective properties and the synthesis protocol, they are classified as ‘polyclonal’, ‘monoclonal’ or ‘recombinant’ [43]. The antibody is a ‘Y’-shaped immunoglobulin that consists of two heavy chains and two light chains. Sometimes, the disulfide bonds in protein, and an extra protein called the ‘joining’ or ‘J-chain’ may result in
the formation of dimeric and pentameric structures in humans [44]. The heavy and light chains in an antibody comprise of a constant and variable part. The latter binds with a corresponding antigen that is highly specific and selective [45, 46] (Figure 2.4). Thus, an immunosensor consists of an antibody which acts as a bioreceptor and can bind with the corresponding antigen. Thus, an antibody is highly specific, stable, and versatile. The specificity of the antibody towards the corresponding antigen depends on the amino acids present in the antibody [47, 48].

Figure 2.4 Scheme showing the structure organisation of antibody

(C_H: constant regions; C_L: constant light region; F_ab: fragment antigen-binding; F_C: fragment crystallisable region; V_H: variable regions; and V_L: variable light region)

The immunoassay technique is a widely explored analytical method used for the detection and quantification of biomolecules. It takes advantage of the affinity binding between antibodies and the corresponding antigens, thereby allowing the detection of antigen at very low concentrations and detection of the antigen in complex biological matrices (whole blood, serum, and other biological fluids) [49, 50]. The best approaches in which an antigen and an antigen-specific antibody interact are similar to a lock-and-key model. The interaction between a specific antibody and its unique antigen is highly stereoselective, which results in the formation of three-dimensional (3D) structures [51]. Due to this 3D configuration
and the diversity inherent in an individual antibody, it is possible to develop an antibody that can recognise and bind with any one of a large variety of molecular shapes. This unique property of antibodies and their ability to identify molecular structures allows researchers to develop antibodies that bind specifically to chemicals, biomolecules, and microorganisms [52]. The binding reaction can be measured by monitoring the changes in the different physical phenomena, the configuration of the assay and the labels used. Immunosensors can be categorised based on the mode of signal transduction as ‘direct’ or ‘indirect’ [53]. A direct immunosensor follows the actual binding event, whereas the interaction between antigens and antibodies is usually continuous and in real-time [54]. An indirect immunosensor measures the result of a binding event, i.e., an increased/decreased amount of the bound label (enzyme, electroactive indicator) [55, 56]. Further, to suit the purpose of the particular assay, antibodies can be modified covalently in many ways. Immunological methods are used to ‘tag’ enzymes, biotin, fluorophores and radioactive isotopes to antibodies, which thus provides a higher detection signal in biological assays [57, 58]. Antibody labelling can be done in two ways: (i) the direct method (a labelled antibody reacts directly with the antigen of interest); and (ii) the indirect method. In the latter, two steps are involved. First, an unlabelled primary antibody (first layer) reacts with the antigen and a labelled secondary antibody (second layer) reacts with the primary antibody [59, 60]. Several modes of transduction have been used for the fabrication of immunosensors and, among these, optical and EC methods have been used widely [61, 62]. However, the sensitivity of optical-based immunonosensors is poor in comparison with EC methods, which provide fast, simple, and economical detection [63]. Immunosensors have been envisioned to have a vital role in the improvement of public health by providing rapid detection, high sensitivity, and specificity in clinical chemistry, food quality, and environmental monitoring [30]. Developments in immunosensors have led to increased interest in POC measurements. Immunosensors have become promising tools for detection of the early stages of cancer because traditional diagnostic methods have poor sensitivity, selectivity and are time-consuming.
2.3.2 Semi-synthetic Biorecognition Element

2.3.2.1 Nucleic Acids

The analysis of nucleic acids (NA) has become a valuable tool for genetic diagnostics, recognition of disease-causing microorganisms in the human body, food, and environment, as well as for assessment of medical treatment. In 1944, Oswald Avery presented evidence regarding the involvement of NA in the storage and transfer of the genetic information needed for protein synthesis [64]. A triplet of nucleotides constitutes codons, and a set of codons encodes instructions for sequences of amino acids during proteins synthesis. In 1953, James Watson and Francis Crick proposed a structure for deoxyribonucleic acid (DNA) that explains how DNA stores genetic information [64].

The principle of DNA sensing is to detect single-stranded deoxyribonucleic acid (ssDNA) fragments by utilising their hybridisation with complementary probe sequences [65]. The specific binding of a surface-confined probe with its complementary target strand results in generation of a useful electrical signal. Further, the extent of hybridisation determines the presence or absence of complementary sequences in the sample. Many reviewers have cited recent increases in the use of electrical transducers in combination with DNA-based detection [66]. Hybridisation is an inherent property of NA, so it is employed widely in biomedical assays for selective detection of a complementary sequence that is specific to a particular target gene corresponding to a particular disease. NA-based biosensors have been found to provide sensitive, selective, straightforward and economical detection of NA hybridisation. In a typical configuration, a single-stranded probe sequence is immobilised within the recognition layer, where base-pairing interactions recruit the target DNA to the circuit [67]. The bonding is specific (i.e., adenine and guanine purines are bonded with thymine and cytosine pyrimidines, respectively) and the bonding can easily be broken by heat or high pH to produce its denatured form (Figure 2.5) [68]. The molecule re-anneals into
a double-stranded configuration on removal of the heat source or pH extreme, and the hybridisation proceeds *via* interaction among specific complementary bases, as shown by the Chargaff rule. The dynamics of target capture take place at this interface to generate the recognition signal; therefore, immobilising NA probe sequences in a predictable manner while maintaining their inherent affinity for target NA is crucial for overall device performance.

![DNA hybridisation](image)

**Figure 2.5** DNA hybridisation (schematic)

The characteristics of a genosensor rely on the assembly of a NA MR layer onto the transducer surface. The major critical challenge in surface-based NA biosensors lies in the reduced accessibility of target molecules to the NA probes immobilised onto heterogeneous surfaces, especially when compared with probe–target recognition in homogeneous solutions. Understanding the physical structure of the NA probe immobilised on a surface is critical in applications using NA as a MR element. Structurally, it consists of a pair of anti-parallel oriented polynucleotide chains that are linked *via* hydrogen bonds, except in a few viruses that are known to have ssDNA as their
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genome. DNA-based biosensors have been immensely applied for the detection of pathogens, where the ssDNA probe is immobilised onto a transducer surface to recognise its complementary target sequence via hybridisation. The major advantages of a genosensor include high chemical stability and the convenience for synthesis and modification of the NA sequence. For this, the DNA sensor has been most studied among all genosensors. It has been reported that the attached DNA becomes stretched into the solution on application of repulsive electrostatic fields to the surface [69] or a high surface density of DNA [70]. One report has shown that, at low densities, thiolated DNA probes lay nearly flat on the substrate, which anchors predominantly at its high densities. Another report by the same research team suggested that DNA bases in short probes tend to orient parallel to the surface, whereas long DNA probes form disordered films similar to polyelectrolyte brushes. The literature clearly indicates that short DNA probes in densely packed films may take upright conformations, which nevertheless prohibits efficient target accessibility [71].

Among the various transducers used for NA detection, the ability of EC sensors to identify NA directly in complex samples is a valuable advantage [54]. A typical EC DNA sensor consists of an electrode, capture probe, reporter probe (RP) and the target DNA [72]. The capture probe is an element used to recognise and bind to the target DNA, which is usually immobilised onto a solid substrate (electrode surface) or on nanomaterials or other biomolecules. The RP is a molecule that generates the EC signal in response to the EC reaction. The capture probe and RP are created with high specificity to the target DNA. Other components, such as electrode coatings and intermediate molecular linkers, are also commonly integrated for improved sensor performance [66]. EC DNA sensors can detect specific genes of specific bacteria with high sensitivity because these sensors have greater specificity regarding the hybridisation between the probe and complementary target sequence. Biosensors based on direct EC detection of target NA molecules have been demonstrated in various approaches by linking DNA or ribonucleic acid (RNA) hybridisation events onto an oligonucleotide-modified electrode surface [67].
2.3.2.2 Aptamers

In 1990, two research teams independently discovered an in vitro selection and amplification method for isolation of the RNA sequences that bind specifically to their target molecules [73, 74]. These functional RNA oligonucleotides are termed as ‘aptamers’ taken from the Latin word *aptus*, meaning ‘to fit’. Subsequently, DNA and RNA aptamers were identified that could bind tightly with a broad range of targets such as proteins, peptides, amino acids, drugs, metal ions and even whole cells. Nowadays, research is more focused on the improvement of aptamer technology by using rapid, automated, selection technologies so that they can be utilised in scientific and industrial research [75, 76].

Aptamers are small ssDNA or RNA molecules (<100 bases) selected from a random oligonucleotide library [77]. These molecules bind specifically to their respective targets with high affinity. Compared with antibodies, aptamers are much smaller and can be produced readily by synthetic means. The major advantage of an aptamer is that it can undergo multiple denaturation/regeneration cycles because of its oligonucleotide structure, whereas regeneration of antibody-based biosensors is difficult. The simple structure of aptamers allows them to be selected against any target analyte regardless of their antigenicity or toxicity [78].

Aptamers are produced in vitro by an evolutionary method called systematic evolution of ligands by exponential enrichment (SELEX), which does not involve the selection of a living organism (Figure 2.6) [79]. Aptamers can be selected for any desired target, even non-immunogenic or toxic proteins. For the design of aptamers, the selection of ligands beyond natural systems originates from an oligonucleotide library that is produced chemically. The length of the variable region governs the number of variations, and it has been estimated that 425 different oligonucleotide sequences are possible with the variable region of 25 oligonucleotides. The amplification steps of target binding oligonucleotides and the broad range of the oligonucleotide database available facilitate selection of the highest affinity ligands compared with natural selection. The SELEX process can be carried out in parallel in the assay for which the aptamer has been designed. This
results in the generation of stable aptamers that are highly functional and will not dissociate or change their characteristics during the bioassay. Once the aptamer has been selected, it can be synthesised with high reproducibility and purity from commercial sources. To add further desired features to an aptamer, the SELEX conditions can be modified further. This is not possible during the production of antibodies. Further, the structure can be tailored readily so that it can be used for direct reporting of analyte binding, thus obviating the need for the secondary labels. The high affinity of the aptamer against its target is derived from its ability to fold upon binding with its target molecule. Because of these advantages, many aptamer-based biosensors (‘aptasensors’) have been developed [40] and some recent reviews have focused on MR and the sensing aspects of aptamers. With regard to the binding affinity of aptamers, it is equal to that of monoclonal antibodies, and the aptamers provide more accurate results compared to antibodies.

**Figure 2.6** Isolation of aptamers using SELEX. In this example, the target molecule is immobilised on a bead to achieve separation (PCR: polymerase chain reaction). Reproduced with permission from W. Zhou, P-J. Jimmy Huang, J. Ding and J. Liu, *Analyst*, 2014, 139, 2627. ©2014, Royal Society of Chemistry [79]
Aptamers are more resistant to denaturation and degradation, and their binding affinities and specificities can be easily manipulated. They can be modified readily with a functional group to improve the rational design, so that these can be used directly for immobilisation on biochips. Aptamers can also distinguish between chiral molecules, and they can recognise a distinct epitope of a target molecule. In a biosensing application, the primary advantage of using aptamers instead of antibodies is to overcome the non-specific adsorption phenomena that are observed less on NA-originated surfaces compared with protein-derived ones. Also, DNA aptamers are usually highly chemically stable. Hence, aptamers have become increasingly important molecular tools for diagnostics and therapeutics. In particular, aptamer-based biosensors possess unique advantages compared with biosensors, which rely on natural receptors (antibodies and enzymes). In spite of these rapid advances, aptamer-based bioassays are at the infant stage due to the limited availability of aptamer types and the relatively poor knowledge of surface-immobilisation technologies for aptamers. Many aptamers have been used for foodstuff analyses but commercial applications are lacking. Aptamer technology trails immunotest development by about two decades, so the key application areas for rapid tests are already occupied by antibody-based detection systems exhibiting a very high degree of optimisation. Hence, in-depth understanding of aptamers in terms of their conformational and ligand-binding properties may lead to a range of bioassay methods that rely on aptamer receptors.

2.3.3 Synthetic Recognition Elements

2.3.3.1 Imprinted Polymers

Over the past decade, the introduction of specific binding domains within synthetic polymers by template-directed crosslinking of functional monomers [molecular imprinting (MI)] has attracted considerable attention [80, 81]. MI involves arranging polymerisable functional monomers around a template followed by polymerisation and template removal (Figure 2.7). The arrangement is typically
achieved by non-covalent interactions or reversible covalent interactions. An appropriately designed MIP can then bind the template or structurally similar analytes [82]. Roy and co-workers electrochemically fabricated MIP film of polyaniline (PANI; thickness, ≈100 nm) onto indium-tin-oxide (ITO)-coated glass plates using ascorbic acid (AA) as a template molecule. This AA-selective MI-PANI electrode has been developed via over-oxidation of AA-doped PANI electrode, which leads to the removal of AA moieties from the PANI film [83].

![Image: Schematic representation of the MI process for the fabrication of an AA-MIP/ITO electrode and hydrogen-bonding interactions that help in the incorporation of AA moieties in the PANI matrix.](image)

**Figure 2.7** Representation of the MI process for the fabrication of an AA-MIP/ITO electrode and hydrogen-bonding interactions that help in the incorporation of AA moieties in the PANI matrix. Reproduced with permission from A.K. Roy, N.V.S. Chetna Dhand and B.D. Malhotra, *Journal of Molecular Recognition*, 2011, 24, 700. ©2011, John Wiley & Sons, Ltd [83]

In the case of a generic biosensor, an immobilised biorecognition element such as an antibody, aptamer, cell, DNA oligonucleotide,
or enzyme, serves to recognise the target analyte selectively. The binding leads to the generation of an optical, mass, thermal, or EC signal that is related to the analyte concentration in the sample. The biosensor concept has been developed parallel to the development of elegant biosensor arrays for simultaneous multi-analyte detection. In spite of the considerable progress made towards the development of biosensors and microarrays based on biological recognition elements, there are limitations associated with many types of biorecognition elements (antibodies, enzymes) [81]. Aptamers are not yet available for a wide range of analytes, and they can be costly to produce for most analysts. Though biologically based recognition elements are clearly important, a compelling case can be made for developing robust, cost-effective and reusable alternatives for labile biorecognition elements. MIP-based biosensors have the following advantages compared with other biorecognition elements [84].

- The binding affinities are comparable with those of a biological recognition element;
- MIP are robust and stable under a wide range of chemical and physical conditions; and
- They can be designed as recognition sites for analytes that lack suitable biorecognition elements.

MIP have been synthesised for a large variety of molecules and macromolecules: amino acids, mycotoxins, nucleotide bases, pesticides, pharmaceuticals, proteins and vitamins [85]. A primary concern in the development of MIP-based biosensors is signal transduction.

MIP have great application potential in sensor technology, but considerable efforts are needed to produce recognition systems which are optimised for each analyte. For example, in the imprinting of larger biomolecules, there is a need for materials that have a higher degree of flexibility [86]. Another challenge is the development
of membranes imprinted with viruses so that they can be used in diagnostics and treatments. Further research in this area will benefit from the integration of different disciplines. To optimise the choice of template-functional monomer couples and to predict the actions of the imprinted material at a molecular level, molecular modeling and combinatorial chemistry can be interesting tools. Additional studies are needed to fabricate reliable MIP-based biosensor devices for healthcare, environmental protection and industrial development.

2.4 Conclusions

Biomolecular recognition is the process by which the biomolecules recognise and bind specifically to their molecular targets with high affinity. MR is a cornerstone for a wide range of diagnostic and synthetic technologies. Many advances have been made in the development of biosensors, but the world market is dominated by the glucose sensor. Other recognition biorecognition elements, such as antibodies and NA, are based on affinity assays and show high sensitivity and selectivity in the affinity to target molecules. However, the major challenge lies in their sensitivity towards pH, temperature, short shelf-life and facile degradation which, ultimately, limits their repeatable usage. Compared with antibodies, NA are being explored increasingly for fabrication of a stable and reproducible sensor owing to their more robust character. With recent developments in nanotechnology, there is a high possibility for the development of in vivo sensors that could be ingested or injected at the target site. Moreover, the clinical significance arising from constant, real-time metabolic monitoring via sensor-based ligand-specific biorecognition elements is immense. Current technologies such as microstructure fabrication, surface modification, and integration of detection and optimisation of chemistry are not competent with current, well-established detection instrumentation. However, there is an urgent need for high-throughput diagnostic/detection methods to overcome the limitation of established instrumentation techniques. Efforts
should be made towards the fabrication of array-based technology for commercialising sensor platforms that can utilise various biorecognition elements for clinical diagnostics, food monitoring and environment monitoring.

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3 Nanomaterial-based Biosensors

3.1 Introduction

Biosensors based on nanomaterials utilise the unique biological and physical properties of nanomaterials to facilitate recognition of the target molecules, resulting in a measurable change of the electronic signal that can be detected using transducers [1, 2]. With recent advances in nanotechnology, nanomaterials have received much interest for application to biosensors [3]. These materials have been found to result in improved mechanical, electrochemical (EC), optical and magnetic properties of biosensors, leading to the development of single-molecule biosensors and high-throughput biosensor arrays [4]. A wide variety of nanomaterials and composites have been optimised on different sensing platforms using different preparation methods [5]. Further, novel functional nanomaterials and new synthetic methods are being explored continuously to achieve sensors with ultra-high sensitivity. Significant advances have been made in synthetic protocols to prepare various nanomaterials with controlled size, shape, surface charge and physico-chemical characteristics [6–8].

Nanomaterials have at least one dimension in the range, 1–100 nm [9]. Nanomaterials have been shown to exhibit superior properties in terms of reactivity, greater sensing capability and increased mechanical strength compared with the bulk counterpart [10, 11]. The most significant property of nanomaterials is a high ratio of surface area:volume. This results in the generation of many unusual physical and chemical properties, such as enhanced chemical and biological activities, high-molecular adsorption, large catalytic
effects, and extreme mechanical strength [12]. Moreover, the quantum size effect of nanomaterials leads to their discrete electronic band structure, which is similar to that of biomolecules [13, 14]. This quantum property of nanomaterials has been predicted to play a crucial part in the fabrication of ultrasensitive biosensors that have been found to have applications in diagnostics. It has been projected that the coupling of materials science and biology at the nano-size will have a remarkable effect in many areas of science and technology, especially in the field of biosensors because many relevant biomacromolecular structures are in the range 1–1,000 nm [14]. The application of nanomaterials in biosensing has been associated with the necessity to control specific molecules present in the environment or human body. This includes the possibility to improve the quality of life by the development of efficient biosensing devices [15]. Thus, it is a challenge to develop more sensitive and selective biosensors that could detect small quantities of molecules utilising efficient transducing elements and specific recognition materials for biosensing [10, 16]. The advantages of using nanomaterials in biosensors relate to rapid response, high sensitivity, portability and easy miniaturisation compared with existing bulk electrodes. Further, the integration of nanomaterials and transduction devices on a single chip provides many advantages for point-of-care (POC) devices [10]. The unique properties of different nanoparticles (NP) have been predicted to have various roles in biosensors. Metal NP, carbon-based nanostructures, and magnetic NP have been observed to participate as carriers for signal amplification. Metal NP are often used as components of ‘electronic wires’ whereas oxide NP are applied to immobilise biomolecules and semiconductor NP are used as labels or tracers [17–20]. Among other nanomaterials, carbon-based materials and their composites are being explored widely because they promote direct transfer of an electron between biomolecules and the electrode surface [21–23]. In this chapter, we discuss the unique physico-chemical properties of nanomaterials and recent progress in selected nanomaterials and their influence in biosensing.
3.2 Metal Nanoparticle-based Biosensors

Noble metal nanoparticles (MNP) are important nanomaterials that are being used for the development of biosensors and biomedical devices. To meet the increasing demand for more precise and highly sensitive biomolecular diagnostics, MNP can play a crucial role in enhancement of the characteristics of existing biosensing techniques. MNP provide unique physico-chemical properties at the nanoscale that may lead to the development of a wide variety of nano-biosensors for POC diagnostics, *in vivo* sensing, imaging, cell tracking and monitoring disease pathogenesis and other nanotechnology tools [18, 24, 25].

The simplicity, physiochemical malleability, and high surface areas of MNP enable them to be widely applicable in nanotechnology-based approaches for the fabrication of biosensors. MNP can be of different shape, and the size may vary from 1 nm to 100 nm in diameter [3, 11]. The properties of these MNP are known to be dependent on size, which is quite distinct from the properties demonstrated by bulk materials [26, 27]. Further, MNP may be composed of one or more inorganic compounds, such as noble metals, heavy metals, or iron. The different size and composition of MNP may result in new, interesting properties, such as quantum confinement (semiconductor nanocrystals), surface plasmon resonance (SPR) (metal NP) super-paramagnetism (magnetic materials) [28, 29]. Several techniques, including chemical and photochemical reduction, co-precipitation, thermal decomposition, hydrolysis, vapour deposition, laser ablation, and grinding, have been used to synthesise MNP [30–34]. The ultimate goal of these techniques is to obtain MNP with an apparent homogeneity and to provide excellent control over size, shape, and surface properties so that their unique physico-chemical properties can be used for biosensing. MNP such as gold (Au), silver (Ag), platinum (Pt) and palladium (Pd) have laid the foundations for numerous techniques related to molecular diagnostics, imaging, drug delivery and therapeutics [35–37]. Among the various MNP
used as components in biosensors, gold nanoparticle(s) (AuNP) have received maximum attention because they show several interesting properties. AuNP can be synthesised in an aqueous solution by reducing chloroauric acid with a reducing agent, wherein the Au\(^{3+}\) ions are reduced to neutral Au atoms [38, 39]. With the formation of more Au atoms, the solutions become supersaturated, resulting in the precipitation of Au in the form of sub-nanometre particles. Thus, the synthesised AuNP, having a diameter of 1–100 nm, provide a high surface-to-volume ratio with high surface energy that helps in the oriented immobilisation of many biomolecules [40]. Moreover, AuNP can permit fast and direct electron transfer among a wide range of electroactive species and electrode materials. These can be conjugated with biomolecules, thus maintaining the biochemical activity of the tagged biomolecules, and hence can be excellent transducers for several biorecognition applications (Figure 3.1) [41, 42]. The light-scattering properties and the enhancement ability of the local electromagnetic field enable AuNP to be used as signal-amplification tags in various biosensors [43]. Other properties, such as an electron-dense core, highly resonant particle plasmons, direct visualisation of single nanoclusters by scattered light, and catalytic size enhancement by Ag deposition, have made AuNP attractive materials for several applications in biotechnology [20, 44, 45].

AuNP have been studied for bioanalysis using SPR transduction whereby the change in the dielectric constant of the environment of the propagating surface plasmon (changes of the angle, intensity, or phase of the reflected light) of Au films can be measured. AuNP can amplify the SPR signal [46, 47]. Lin and co-workers utilised the localised-SPR effect of AuNP to develop a fibre-based biosensor for determination of organophosphorus pesticides. It was reported that the activity of acetylcholinesterase (AChE) to hydrolyse acetylcholine chloride was inhibited, resulting in a change of the light attenuation due to a local increase in the refractive index. The concentration of AChE was determined based on the correlation between the inhibition rate and light attenuation [48]. Further, a comparative study of the fabricated fibre sensor with and without AuNP suggested that the sensor in the presence of AuNP showed an enhanced response.
AuNP, when used in a ‘sandwich’ configuration with an Au film [49], led to apparent enhancement of the SPR signal. This is because the surface plasmons on AuNP could have initiated a perturbation of the evanescent field of the Au film in addition to the immobilised biomolecules (Figure 3.2). To achieve such enhancement in the SPR signal, the size of the AuNP should be <40 nm and its distance from the Au film surface should be 5 nm [50]. AuNP (60 nm) have also been used as robust transduction platforms for single-molecule detection using the refractive-index sensing of localised SPR coupled with an enzyme-linked immunosorbent assay [51]. AuNP have been used to tag oligonucleotide probes for deoxyribonucleic acid (DNA) hybridisation detection using SPR to recognise surface-confined target DNA selectively via sequence-specific hybridisation. The sensitivity was greater than 1,000-fold over that for unamplified methods [52]. Recently, AuNP-based SPR biosensors have been fabricated into an array format, which enables rapid, high-throughput screening of biomolecular interactions [53, 54]. Several reports have been published relating to immobilisation of AuNP on an optically transparent substrate for application in an optical chip-based biosensor [55, 56]. Silver nanoparticles (AgNP) have proven to be one of the most influential groups of nanomaterials for biosensing approaches, as well as in other biomedical therapeutic applications. Highly sensitive and specific sensors based on noble AgNP have opened up the possibility of creating new diagnostic platforms for disease markers, biological and infectious agents in the early-stage detection of disease, and other physiological threats. The application of AgNP has been found to result in increased EC activity (because they exhibit higher catalytic efficiencies per gram than their bulk material counterparts), good performance, enhancement of mass transport and excellent biocompatibility [57, 58].

The hybrids of MNP have been found to display interesting properties [59]. Efforts are being made to explore new structures of MNP. Attempts have recently been made to obtain an improved analytical performance of a biosensor by preventing non-specific
Figure 3.2 Fabrication of AuNP-tagged antibody for immunosensor application cysteamine-functionalised gold nanoparticles (C-AuNP) and 4-mercaptobenzoic acid (MBA). Reproduced with permission from A. Sharma, Z. Matharu, G. Sumana, P.R. Solanki, C.G. Kim and B.D. Malhotra, *Thin Solid Films*, 2010, 519, 1213. ©2010, Elsevier [43]
adsorption of biomolecules onto MNP [60, 61]. However, for real-world application, this rapidly expanding area is still in its infancy, and widespread practical application of MNP-based biosensors has not yet become possible. To understand the potential applications of MNP, attention should be paid to the design of MNP-based biosensors with high throughput and multiplexed identification of biomarkers. The size and nanostructure of the MNP significantly affects the biosensing properties, so research and development should be focused more on the design and synthesis of MNP that are stable in various environments and which have well-defined geometry and properties [62].

3.3 Nanostructured Metal Oxide-based Biosensors

Many techniques have been proposed to design a material at the nanoscale for the fabrication of highly sensitive, selective and efficient biosensors [19]. In this context, nanostructured metal oxides (NMO) have recently aroused much interest as immobilising platforms in biosensors because of their unique physical, chemical and catalytic properties [63]. Different NMO of zirconium, zinc, titanium, iron, cerium, tin, metal and magnesium have been found to reveal fascinating nano-morphological, functional biocompatible, non-toxic and catalytic properties (Figure 3.3) [64]. NMO facilitate enhanced electron-transfer kinetics and high adsorption capability, thus providing suitable microenvironments for the immobilisation of biomolecules, resulting in improved biosensing characteristics. Further, the controlled preparation of an NMO is considered to play a pivotal part in the development of biosensors. Different morphologies of NMO have been synthesised using various methods, including soft templates (nanorods and nanofibres), sol–gel technique (three-dimensional ordered nanostructures), radio-frequency sputtering and hydrothermal methods [65–68]. These controlled methods may lead to the fabrication of novel biosensing devices having enhanced signal amplification for bio-affinity assays, efficient charge transfer with redox species, and
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<td><strong>Figure 3.3</strong> Representative NMO and their biosensing applications (ChOx: cholesterol oxidase; CNT: carbon nanotube(s); CS: chitosan; HRP: horseradish peroxidase; IEP: isoelectric point; IgG: immunoglobulin G; and Urs: urease). Reproduced with permission from P.R. Solanki, A. Kaushik, V.V. Agrawal and B.D. Malhotra, <em>NPG Asia Materials</em>, 2011, 3, 17. ©2011 Macmillan Publishers Ltd [19]</td>
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immobilised biomolecules [69]. Moreover, NMO provide an excellent pathway for immobilising the desired biological recognition molecules with an electronic signal transduction that leads to the design of a new generation of bioelectronics devices that may exhibit novel functions. NMO show exceptional optical and electrical properties due to electron and phonon confinement, high surface-to-volume ratios, surface reaction activity, catalytic efficiency and strong adsorption ability [70]. These features allow the possibility to use NMO in many new signal transduction technologies in biosensors for rapid \textit{in vivo} analysis. For the fabrication of an efficient biosensor, it is desirable to select suitable NMO for the immobilisation of biomolecules as the binding between an NMO and a biomolecule (at the interface) is known to affect the performance of a biosensor) [71].

Bio-interface properties are known to depend on the effective surface charge and area, functional groups, energy, roughness, porosity, valence/conductance states, and hygroscopic nature of the NMO. The binding of biomolecules with an NMO can be accomplished \textit{via} chemical bonding or physical adsorption. The covalent bonding of a biomolecule depends on the availability of functional groups, which can be tailored by appropriate chemical reactions, whereas the physical adsorption of a biomolecule occurs mainly due to weak interactions (e.g., physisorption, van der Waals, electrostatic) [72, 73]. These interactions depend on the surface morphology, reaction medium and net surface charge of the NMO. Moreover, NMO with a high isoelectric point (IEP) interact electrostatically with biomolecules because they possess a low IEP [74, 75]. Other features, such as short-range forces arising \textit{via} charge as well as steric, depletion, and solvent interactions also play a significant part in the preparation of a nanobio-interface [71]. Thus, designing a suitable NMO-based bio-interface provides a biocompatible microenvironment that helps a biorecognition element to retain its biological activity with high stability. Various surface architectures have been used to fabricate a wide number of EC biosensing devices that show improved sensitivity and selectivity. In this context, attempts have been made to immobilise GOx on NMO for the fabrication of a glucose biosensor [76]. Zinc oxide (ZnO) nanofibres
Nanomaterial-based Biosensors

are used for the fabrication of a highly sensitive amperometric biosensor for continuous monitoring of glucose. The reported biosensor has been shown to have high and reproducible sensitivity due to its high enzyme loading and high surface area, thus providing a microenvironment that helps GOx retain its bioactivity. The nanostructured cerium(IV) oxide (CeO$_2$) (IEP of 9.4) was deposited on Pt-coated glass plates for the immobilisation of GOx, resulting in a glucose biosensor with a linear response to glucose and the Michaelis constant value as low as 1.01 mM. The sol–gel method was reportedly used to fabricate a nanostructured CeO$_2$ film for the fabrication of a glucose biosensor [77, 78]. Several researchers have observed improved sensitivity and linearity of glucose biosensors using many NMO (e.g., zinc, zirconium, iron oxide) and their composites with the desired biopolymers [79–83]. These NMO were not only used to fabricate enzyme biosensors, but were also employed in immunosensors [84] DNA hybridisation detectors [85] and whole-cell biosensors (Figure 3.4). Further, to enhance the optical, electrical and magnetic properties of NMO, several other conducting NP (CNT, graphene, Au, polyaniline) were utilised to obtain improved biosensor characteristics.

NMO-based biosensors provide a new perspective for development of numerous clinical and non-clinical diagnostic tools. The unusual properties of NMO are likely to provide a new path for future interdisciplinary research, resulting in the generation of EC biosensors ranging from enzyme electrodes to genoelectronics. It is expected that a wide variety of new NMO and new synthetic strategies for designing one-dimensional (1D) NMO are likely to produce new bioelectronic sensing applications. Moreover, the functionalisation of NMO with different functional groups to bind target biomolecules, and further doping with electronically active materials, will result in enhancement of charge transfer and may pave the path for new methods for biosensor development.

Understanding the interfacial properties of various biomolecular-transducers using the NMO is very important. Research in this area will lead to the development of highly sensitive biodetection protocols. This may lead to the evolution of new efficient biosensors
that can be utilised for the diagnosis of disease markers, pathogens and other infectious agents of disease and threats. Attempts are being made on understanding the interfacial properties of the various biomolecule–transducer interactions using these NMO. It is expected that new, highly sensitive detection protocols and biosensors will soon emerge for the detection of disease markers, pathogens and other infectious agents of disease and threats. It should be interesting to explore the dependence of size and toxicity of NMO in various new applications, such as NP-based DNA barcodes, embedded particle carriers, NP-based SPR and chemiluminescence detection, and for amplified assay studies using biocatalytic metallisation. These NMO can be fabricated and tested in desired patterns, such as sensor arrays, for the development of functionally integrated devices. The interface of NMO-based devices can be used for parallel real-time monitoring of multiple analytes. Also, it should be interesting to focus on new techniques to fabricate innovative biosensor arrays with desired properties for health care. Such arrays would ‘incarcerate’ different biomolecules onto closely spaced 1D nanostructures based on NMO. Also, efforts should be focused on the prospects and future challenges of NMO for developing biosensing devices that may lead to the evolution of new strategies for bio-affinity assays and efficient electrical communication.

3.4 Carbon Nanotube-based Biosensors

With the emergence of nanomaterials having unique physico-chemical properties, a new class of biosensors (nano-biosensors) based on NP and nanotubes has been developed. CNT have been considered to be the best-suited nanomaterials for transduction of the signals associated with the biorecognition analytes, metabolites, or disease biomarkers, and can serve as effective matrices for the immobilisation of biomolecules in biosensors. CNT have several exceptional physical, chemical, electrical, and optical characteristics properties that render their suitability in biosensing applications. A CNT is a hollow carbon structure, with one or more walls, having a nanometre-scale diameter and an approximate length. It is a well-ordered arrangement of carbon
atoms linked via \( sp^2 \) bonds, which gives it the stiffest and strongest fibres. In 1991, Iijima and co-workers were the first to report multi-walled carbon nanotubes (MCWNT). In 1993, these researchers reported single-walled carbon nanotubes (SWCNT) [86, 87]. After this discovery, CNT have emerged as one of the most investigated nanostructured materials, and each year thousands of studies are being published on the different promising applications of these fascinating nanomaterials in academic and industrial areas. The CNT skeleton comprises of a seamless hollow tube composed of a ‘rolling’ graphite sheet and, according to the number of graphitic layers, the CNT can be classified as a SWCNT or a MWCNT [88, 89]. In short, SWCNT is a single molecular nanomaterial composed of a single layer that rolls a single sheet of graphite into a seamless molecular cylinder [90]. The diameter is 0.75–3 nm whereas the length is 1–50 nm [91]. On the other hand, MWCNT have more than two layers of a ‘curly’ graphite sheet, with a diameter of 2–30 nm (some may be >100 nm), and the distance between each layer is \( \approx 0.42 \) nm. CNT possess an ultra-high specific surface area, and outstanding electrical, mechanical and EC properties which make them very sensitive to exposure to biomolecules, leading to the fabrication of ultrasensitive biosensors [92]. Compared with other nanomaterial-based biosensors that use metal oxides, silicon, and other materials, CNT-based biosensors have the following advantages [93]:

- Due to their large surface area and hollow structure, CNT can provide a high biological activity that helps in the efficient immobilisation of biomolecules, resulting in high sensitivity.

- The outstanding ability of CNT to mediate fast electron-transfer kinetics results in a fast response time.

- CNT have less surface fouling effects and a lower potential of redox reaction that makes them durable and highly stable.

- Compared with other nanomaterials, CNT conduct increased electricity (\( \approx 100 \) times greater than copper wires), which results in the production of excellent electric signals generated upon recognition of a target.
Figure 3.5 Application of CNT in the detection of various analytes in a (A) carboxylated multi-walled carbon nanotube (c-MWCNT)-based biosensor for an AFB$_1$ biosensor; and (B) CNT-based genosensor (dsDNA: double-stranded deoxyribonucleic acid; EPD: electrophoretic deposition; and ftDNA: fragmented-DNA). (A) Reproduced with permission from C. Singh, S. Srivastava, M.A. Ali, T.K. Gupta, G. Sumana, A. Srivastava, R.B. Mathur and B.D. Malhotra, Sensors and Actuators B: Chemical, 2013, 185, 258. ©2013, Elsevier [95]. (B) Reproduced with permission from M.K. Patel, M.A. Ali, S. Srivastava, V.V. Agrawal, S.G. Ansari and B.D. Malhotra, Biosensors and Bioelectronics, 2013, 50, 406. ©2013, Elsevier [108].
• The thermal conductivity of CNT is higher than that of diamond, and their strength is \( \approx 100 \)-times greater than that of steel.

• CNT can have endohedral functionalities because their shells can be opened easily and filled without losing their stability.

• Compared with other mono-conjugated biosensor species, CNT-based biosensors are constituted of scaffolds/platforms that can be multi-functionalised via conjugation of several entities, thereby potentially enhancing recognition and signal-transduction processes.

CNT-based biosensors can be used for the detection and quantification of clinically relevant metabolites such as DNA [94], aptamers, antibodies [95], peptides, proteins, or enzymes [96] (Figure 3.5). Depending on the mechanism of target recognition and the transducer used, these biosensors can be classified as ‘EC’, ‘immunosensors’, ‘electronic transducers’, or ‘optical’ CNT-based biosensors. Among them, EC CNT-based biosensors can be utilised to detect ions, metabolites and protein biomarkers [97]. For example, several CNT glucose biosensors based on the conjugation of GOx have been fabricated. Patolsky and co-workers reported that the structural alignment of SWCNT acts as an electrical connector between enzyme redox centres (GOx) and the electrode [98]. SWCNT act as conductive nano-needles that electrically wire the enzyme redox-active site to the transducer surface. Li and co-workers reported ultrasensitive cholesterol biosensors that consist of modified screen-printed electrodes with cholesterol esterase, peroxidase, oxidase and MWCNT for quantifying total cholesterol in the blood. EC biosensors based on functionalised CNT have also been developed for the detection of environmental toxins and neurotoxic agents [99]. Zhang and co-workers used single-stranded DNA oligonucleotide adsorbed and wrapped around SWCNT to selectively detect cellular nitric oxide [100]. A ribonucleic acid (RNA) aptasensor on alumina electrode has been proposed for the detection of disease-related glycoproteins in blood by coating SWCNT wrapped with protein-specific RNA aptamers [101].
the past 2 years, several amperometric, impedimetric, and field-effect transistor (FET) CNT-biosensors have been used for the detection of cancer biomarkers and cells [102, 103]. For instance, amperometric biosensors based on CNT arrayed on a chip have been reported for the detection of several cancer biomarkers (prostate-specific antigen-monoclonal antibodies and human chorionic gonadotropin). An FET-CNT-based immunosensor was developed for detection of a biomarker for prostate cancer (osteopontin) by linking a genetically engineered single chain-variable fragment protein [104]. Abdolahad and co-workers used a photolithographic process on Ni/SiO$_2$/Si layers to fabricate a vertically aligned CNT-based impedimetric biosensor for the detection of SW48 cells isolated from grade-IV human colon tumours [105, 106]. Although much has been reported in the field of CNT-based biosensors, there are many challenges for further improvement in material performance. In this regard, the development and characterisation of new materials with CNT at the molecular level is essential, and is a key scientific challenge that must be addressed. Simultaneously, efforts should be made to develop molecular modelling to predict the performance of new materials for given biomolecules that will result in faster evaluation of new materials. Further, the chemical and physical properties of functionalised CNT strongly depend on the ambient conditions (temperature and pH). Hence, work should be done to enhance the thermal stability and lifetime of CNT-based biosensors. In this context, the use of nanocomposite materials combined with CNT and a metal (e.g., Au, Pt) are likely to lead to improved properties, such as the thermal tolerance, long-term stability, and cost-effectiveness of the biosensor [107]. There is sufficient scope to exploit the outstanding properties of CNT networks that promise to have a long-lasting impact on future technologies for biosensing.

### 3.5 Graphene-based Biosensors

Graphene is a one-atom-thick planar sheet of sp$^2$-bonded carbon atoms with a hexagonal lattice structure. It is known to be the thinnest material (thickness is 0.35 nm) known to date [109, 110]. Graphene
is the basic building block for several graphitic materials, such as fullerenes, CNT, and graphite. The unique structure of graphene provides remarkable electronic, optical, mechanical, thermal, and EC properties that make it a potential candidate for next-generation electronic materials. Some of the properties are listed below [111, 112]:

- It possesses a high surface area of >2,600 m²g⁻¹, which is greater than that of SWCNT (≈1,315 m² g⁻¹) and graphite (≈10 m² g⁻¹).
- The mechanical strength of graphene is very high (i.e., the breaking strength is ≈40 N m⁻¹ and the Young modulus is ≈1.0 TPa).
- It has very high heat conductivity (5,000 W m⁻¹K⁻¹), electronic conductivity (200,000 cm² V⁻¹ s⁻¹) and electrical conductivity (≈64 mS cm⁻¹) at room temperature (RT).
- Weakly scattered (λ scattering >300 nm) ballistic transport of charge carriers behaves as massless fermions at RT [113].
- The band gap of graphene can be chemically and geometrically tuned, and it shows a quantum Hall effect at RT.

Other properties, such as excellent conductivity (a good low-noise material electronically), exceptional biocompatibility, easy functionalisation, and mass production, make graphene a promising material for biosensors. In comparison with CNT, graphene shows potential advantages of being cost-effective as well as having a high surface area, ease of processing and being safe to use [112, 114]. Different methods have been proposed to synthesise graphene [115–117]. Geim and co-workers prepared graphene sheets by mechanical repeated peeling (‘exfoliation’) of highly oriented pyrolytic graphite (‘Scotch-tape method’), which is generally used for making proof-of-concept devices [118–120]. Other methods, such as mild exfoliation of graphite and thermal decomposition of silicon carbide wafers under ultra-high vacuum conditions, have also been proposed
The major issue with these approaches is the low yield of graphene. The most economical way for mass production of graphene is by chemical or thermal reduction of graphite oxide (GO) [115]. GO has a non-planar structure with distorted sp² carbon network because it contains significant amounts of oxygen-containing groups, which can be used for biosensor applications. Graphene derived from GO reduction is called ‘functionalised graphene sheets’ or ‘chemically reduced graphene oxide (rGO)’. This contains a partly restored sp² lattice that also provides some degree of oxygen-bearing groups [123]. rGO due to the presence of lattice defects displays a ‘wrinkled’ structure which is entirely different compared with the rippled structure observed for pristine graphene [124]. Comparison of the structure of graphene with that of CNT reveal that structural differences such as tubes versus sheets have a significant role in the design of biosensors. The presence of oxygen-containing groups at the edges/surface of graphene has been found to influence the EC performance of the heterogeneous electron transfer rate. These functional groups provide a favourable environment for the controlled functionalisation and immobilisation of biomolecules on the graphene surface [125, 126]. Graphene exhibits excellent promotion of electron transfer and has been used for the fabrication of biosensors for many enzyme-based biosensors. Several reports have been published on the application of ultrathin multi-layer graphene platelets as a transducing material for the biosensing of glucose [126]. Graphene can enhance direct electron transfer between enzymes and electrodes. Shan and co-workers and Kang and co-workers reported the direct electrochemistry of GOx on graphene. It has been reported that N-doped graphene provides enhanced oxidation currents for the enzymatic detection of glucose compared with conventional graphene materials [127]. Graphene has recently been used for the direct detection of DNA hybridisation using the oxidative signals of DNA bases [128]. Dong and co-workers showed that chemically reduced GO can provide well-resolved EC signals of all four bases (guanine, adenine, thymine and cytosine) with higher sensitivity compared with graphite. This increase in sensitivity is due to the high defect density of rGO, which
provides a superior EC performance [129]. Srivastava and co-workers reported rGO-based EC immunosensors for food toxin (aflatoxin B1 [AFB1]) detection (Figure 3.6) [130]. The EC-sensing studies of the immuno-electrode as a function of AFB1 concentration showed higher sensitivity, improved detection limit and long-term stability which could be attributed to the excellent EC properties, large surface area and rapid electron transfer kinetics of rGO. The superior sensing performance of the rGO-based immunosensor reveals its potential application for EC biosensing applications. Graphene has been used as a substrate in fluorescence quenching (FQ) for ultrasensitive detection of aptamers (thrombin). The specific aptamer was labelled with fluorescent dye and graphene was used as a substrate for the non-specific adsorption of the fluorescent dye-labelled aptamer. This approach resulted in the formation of quadruplex-thrombin complexes with weak affinity to graphene. In the fabricated system, graphene quenched the fluorescence signal due to a transfer of fluorescence resonance energy from the dye to graphene, and the change in conformation of the system led to a change in FQ [131, 132]. Fluorescence-based detection has been used for the detection of DNA viruses using graphene microarrays [133, 134].

The reported disadvantages of pristine graphene include aggregation, poor solubility, and processability, and these disadvantages are big obstacles in the fabrication of biosensors. It is, therefore, necessary to modify graphene with other nanomaterials (noble metals, transition metals, polymers, biomolecules) so that the as-prepared multi-functional hybrid materials can take full advantage of the superior properties of graphene [135]. These modifications will not only overcome the demerits of pristine graphene, but also result in the generation of new features. Further, the controlled synthesis of graphene (with a controlled number of layers and footprint area) remains a challenge [136, 137]. More research should be focused towards understanding the effects of oxygen moieties on the biosensing characteristics of biosensors. An in-depth understanding of the science at the interface of graphene and biomolecules may lead to the development of POC devices [138].
Figure 3.6 Application of GO in biosensor (A) fabrication of graphene flakes on ITO substrates by EC exfoliation of graphite sheets via $K^+$ intercalation and sodium dodecyl sulfate (SDS) stabilisation. (B) GO-based immunosensor for aflatoxin detection. (A) Reproduced with permission from M.D. Mukherjee, C. Dhand, N. Dwivedi, B.P. Singh, G. Sumana, V.V. Agarwal, J.S. Tawale and B.D. Malhotra, Sensors and Actuators B: Chemical, 2015, 210, 281. ©2015, Elsevier [128] (B) Reproduced with permission from S. Srivastava, V. Kumar, M.A. Ali, P.R. Solanki, A. Srivastava, G. Sumana, P.S. Saxena, A.G. Joshi and B.D. Malhotra, Nanoscale, 2013, 5, 3043. ©2013, Royal Society of Chemistry [130]
3.6 Quantum Dot-based Biosensors

Quantum dots (QD) are colloidal nanocrystalline semiconductors that possess unique properties due to quantum-confinement effects. In a bulk semiconductor, the electron-hole pair is dependent on the characteristic length called the ‘exciton Bohr radius’. Thus, if the size of a semiconductor crystal approaches the size of the exciton Bohr radius of the material, the electron energy levels are treated as being discrete, and this discrete energy level is called ‘quantum confinement’ [139, 140]. Depending on the particle size, QD have a broad continuous absorption spectrum that extends from the ultraviolet to the visible wavelength. Compared with traditional fluorophores, QD show broad excitation and narrow size-tunable emission spectra, negligible photobleaching, and high photochemical stability [139, 141, 142].

QD were first discovered in 1980 in glass crystals by the Russian physicist Alexei Ekimov [143]. Subsequently, Louis E. Brus observed a similar phenomenon in colloidal solutions [144]. The traditional core nanocrystals in QD are usually composed of elements from groups III–V, II–VI, or IV–VI of the periodic table. To improve the stability and photoluminescent quantum yield of the core nanocrystals, a layer of a few atoms with a higher band-gap semiconductor is usually introduced, which results in the formation of core-shell nanocrystals [145]. This coating not only improves the quantum yield, but also prevents the leaching of metal ions from the core [139, 146]. The synthesis of QD has been reported via top–down processing methods and bottom–up approach. In the top–down approach, molecular beam epitaxy, ion implantation, electron beam lithography, and X-ray lithography are used to reduce the bulk semiconductor to nano-size. Among the various methods, molecular beam epitaxy can be used to self-assemble QD from III–V semiconductors and II–VI semiconductors using the large lattice mismatch [147]. It is one of the most reported methods for the controlled shapes and sizes and desired packing geometries of QD. However, the major drawback with these methods is the structural imperfections obtained by patterning and the possibility of incorporation of impurities into the crystal during
processing [148]. The bottom–up approach is subdivided into two methods (wet-chemical and vapour-phase), which involve the colloidal self-assembly of particles in the solution followed by their chemical reduction [149–152].

The photoluminescence (PL) properties of QD depend mainly on the large surface area-to-volume ratios of QD, quality and properties of the nanocrystal surface, and the core quality [139]. Understanding the surface chemistry of QD is crucial in the fabrication of biosensors because it governs the change in the quantum yield, changes in PL decay, spectral shifts, and the appearance of undesirable band-gaps [153]. Moreover, it also allows bioconjugation, imparts aqueous solubility, and does not hinder the efficient use of Förster resonance energy transfer (FRET), bioluminescence resonance energy transfer, charge-transfer or electrochemiluminescence as a transduction method. Bioassays and bio-probes that use QD as donors in FRET are the most developed and widespread approach to integrating QD into biosensors [154, 155]. Several enzymes, antibodies, small-molecule binding proteins, and oligonucleotides are among the biorecognition agents that can be coupled to QD [156, 157]. In EC sensing, an ongoing trend is the application of QD due to their high surface-to-volume ratio, high reactivity and ultra-small size, and they have been used for the detection of various analytes (Figure 3.7). The small size results in: higher speeds (because electrons have a shorter distance to travel); lower voltage requirements for achieving the same field; higher component densities and chip functionality such as multi-analysis capability [158]. Considerable variation in particle valence, and electron transfer are due to minute changes in the external environment. In the light of these facts, QD have been used to fabricate a biosensor that has been found to exhibit rapid responses and high sensitivity and selectivity [159]. Several sensing modalities are expected to benefit from use of QD-based detection methods, the most common of which continues to be based on fluorescence [160, 161]. Recently, QD-based immunoassays with ultrahigh sensitivity have been developed using novel readout strategies, such as EC detection, barcodes and microfluidics (MF). Zou and co-workers integrated an immune-chromatographic test
Figure 3.7 Fabrication of (A) a SnO$_2$ QD-based xanthine biosensor ($XO_x$: xanthine oxidase). (B) Cadmium sulfide (CdS) QD-based cholesterol sensor (TGA: thioglycolic acid).

(C) Functionalised CdS QD-based biosensor platform for LDL detection (AAB: apolipoprotein B-100 antibodies; Cys: cysteine; and LDL: low-density lipoprotein). (D) CdTe-based biosensor for cancer detection [AS: (3-aminopropyl)-trimethoxysilane; MB: methylene blue; pDNA: plasmid deoxyribonucleic acid; SEM: scanning electron microscopy; and TEM: transmission electron microscopy].

strip assay using fluorescence. A simple method was developed for analyses of protein-kinase activity using unmodified cadmium telluride (CdTe) QD as fluorescent probes [162]. The use of QD in NA detection has recently garnered considerable attention whereby DNA or RNA segments, acting as recognition moieties, are conjugated onto QD surfaces to form fluorescent probes for genetic-target analyses. The fundamental theory for detection is the high specificity of hybridisation between multi-coloured QD-DNA probes and the target strand with a complementary sequence. It was observed that, after coupling with a DNA sequence probe, QD with different emission colours were applied for multiplexed detection of the complementary sequences that were immobilised on a microarray platform [163–165]. Using these QD barcodes, Giri and co-workers detected up to nine gene fragments (pathogens such as syphilis, human immunodeficiency virus, malaria, hepatitis B and C), with great accuracy [166]. Gerion and co-workers employed QD-conjugated DNA oligonucleotides as hybridisation targets for the identification of single nucleotide polymorphisms and single base deletions of the tumour suppressor gene P53 on a complementary DNA microarray [167]. Single-molecule DNA imaging was reported via QD [168] whereby the two extreme ends of a DNA primer were labelled with biotin and digoxigenin, which were then immobilised onto a glass surface. The outcome of this study provided information regarding DNA orientation but also allowed long-standing observation by fluorescence microscopy.

QD nanocrystals are made of a series of semiconductor NP that can be detected readily by highly sensitive EC techniques, thereby allowing the design of simple and inexpensive EC systems for detecting ultra-sensitive, multiplexed assays. QD may be used to improve the efficiency of photochemical reactions, and are coupled to bioreceptors to generate novel photoelectrochemical systems. Despite their numerous advantages, it is apparent that QD can only complement conventional methods using organic fluorophores. The major drawback associated with QD lies in their size, because FRET efficiency is highly dependent on the donor-pair acceptor distance, and is possible up to ≈10 nm [169–173]. Currently, few QD-based optical biomedical applications are available because
most of them suffer from instability. The major drawback is the: particle aggregation that results in colloidal instability; photooxidation in air (which results in shrinkage of the QD core and thus blueing and bleaching of its spectrum); photoblinking of a single QD because of photoionisation by an Auger process; and photo brightening on continuous excitation as a result of trap states within the nanocrystal [174]. If these problems could be solved, the performance of QD-based optical biosensors can be improved further.

3.7 Conclusions

Nanomaterial-based biosensors show excellent attractive prospects and hence can be used in food analyses, process control, clinical diagnoses, and environmental monitoring. Some noble MNP-based biosensors are under development and efforts are underway to understand the interaction between biomolecules and nanomaterials so that they can be integrated into future diagnostic platforms. Biological molecules possess unique structures and functions, so complete exploration of the structure and function of nanomaterials and biomolecules to fabricate biosensors remains a great challenge. The processing, characterisation, interface problems, availability of high-quality nanomaterials, and mechanisms governing the behaviour of these nanoscale composites remain a challenge for fabrication of a suitable device. Future work should concentrate on understanding the mechanism of interaction between nanomaterials and biomolecules on the surface of electrodes, and using the novel properties of nanomaterials to fabricate a new generation of biosensors. Significant research in the area of noble MNP-based biosensors will enable fabrication of biosensors that can be used in clinical and POC diagnostics. However, several other nanomaterials have not yet been studied for their potential application in the field of biosensors due to application diversity, and there is an urgent need for understanding the properties of these nanomaterials.
Efforts should be made towards the integration of these nanomaterial-based biosensors into processing systems such as MF or ‘lab-on-a-chip’ to automate the processing and analysis of samples completely. Until now, only a few nanomaterial-based biosensors have been embedded in MF devices. Thus, efforts should be directed towards the fabrication of MF devices by incorporating these nanomaterial-based sensors. Another problem with nanomaterial-based biosensors is their reproducibility, because control of the structure and arrangement of nanomaterials on the sensor surface is difficult. To overcome the poor reproducibility of nanomaterials, the controlled synthesis, ‘tailoring’ of nanomaterials, and their implementation in sensing platforms are among the most important research topics in nanomaterials. Attention should be paid towards the printing of these nanomaterials through different techniques (inkjet, gravure, and screen printing) on low-cost, flexible substrates such as polymers and paper to develop inexpensive and disposable nanomaterial-based sensors.

References


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Nanomaterial-based Biosensors


Nanomaterial-based Biosensors


Nanomaterial-based Biosensors


4 Conducting Polymer-based Biosensors

4.1 Introduction

In general, polymers have been used traditionally as inert, insulating and structural packaging materials, as well as electrical insulators and textiles, where their mechanical and electrical-insulating properties are paramount [1–4]. However, in the 1980s, many researchers showed that modification of a certain class of polymers resulted in semiconducting properties. Hence, scientists from many disciplines have been combining expertise to study organic solids that display remarkable conducting properties [5–7]. Organic compounds that efficiently transport charge are divided into three classes: (i) charge transfer complexes, (ii) organometallic compounds and (iii) conjugated organic polymers [8, 9].

A new type of polymer called intrinsically conducting polymers (ICP), or electroactive polymers, has recently been proposed [10]. These polymers have been found to exhibit interesting optical and electrical properties [11]. Moreover, compared with other inorganic crystalline semiconductors (silicon), electrically conducting polymers (CP) are molecular in nature, and they lack a long range order. The necessary condition for a polymer to behave as an ICP is that there should be an overlap of the molecular orbitals (MO), thus allowing formation of a delocalised molecular wave function [12]. For the free movement of an electron through a polymer lattice, the MO must be partially filled [13]. ICP are also referred to as ‘conjugated polymers’ having alternate single–double or single–triple bonds in their backbone that are capable of conducting electricity if doped [14–16]. Doping of a CP maintains electrical neutrality and it can be done chemically or electrochemically, which leads to oxidation or reduction of the π
electronic system, p-doping and n-doping [5, 17, 18]. The structural framework of a CP consists of alternate single–double carbon–carbon (carbon–nitrogen) bonds in the polymer backbone chain, which makes them distinct from other polymers. All CP have a backbone of σ-bonds between each sp² hybridised carbon atom and an unpaired electron (π electron) per carbon atom. This allows the formation of π-bands by overlapping of the remaining out-of-plane pₓ orbitals, leading to electron delocalisation along the polymer backbone [17]. This delocalised electronic state allows charge carriers to move along the backbone of the polymer chain, and is determined by the number and type of atoms within the repeat units. Thus, it can be assumed that the π-band plays a significant part in determining the semiconducting/metallic properties of CP [5, 13].

CP complexes in the form of tetracyano- and tetraoxalato-platinates have been known for some time [2, 19]. However, intense research on CP was started soon after polysulfur nitride [(SN)x] was discovered (1975) which, at lower temperatures, shows superconducting behaviour [20, 21]. The rediscovery of polyacetylene (PA) in 1977 (discovered initially by Shirakawa and co-workers using a Ziegler–Natta type polymerisation catalyst) by MacDiarmid and Heeger at the University of Pennsylvania marked the way for the development of CP [22, 23]. The electrical conductivity of PA can be increased by several orders (from 10⁻⁵ to 10⁻⁹ Scm⁻¹) by doping it with an oxidising agent [I₂, AsF₅, NOPF₆ (p-doping) or reducing agents (sodium napthalide; n-doping)] [23–25]. This helped towards the discovery of other new CP systems. Ivory and co-workers discovered polyparaphenylene (PPP) in 1979, which was known to form highly conducting charge transfer complexes with n- and p-type dopants [26]. Further, it was observed that doping with AsF₅ increased its conductivity from 10⁻⁵ to 500 cm⁻¹. The charge transport in PPP was attributed to the presence of polaron/bipolarons, which could be explained using theoretical models and measurements of electron spin resonance. Researchers are now focusing on studying the structure–property relationships of doped CP. The doping of CP is a unique and central theme that combines all CP and differentiates them from other polymers [27].
The doping process includes the conversion of an organic insulating or semi-CP to an electronic polymer that exhibits metallic conductivity ($1-10^5$ Scm$^{-1}$). Doping is basically a charge-transfer reaction that results in partial oxidation/reduction of the polymers which can be reversible upon de-doping if the polymer again retains its original backbone [28, 29]. Doping in conjugated polymers is interstitial, whereas in inorganic semiconductors it is substitutional. CP can be chemically or electrochemically doped (p-or n-doped) to obtain a metallic state. In some cases, doping can also be done without the introduction of a dopant ion (i.e., by using field-induced charging) [30, 31]. If a CP is doped by oxidation or reduction, the number of delocalised $\pi$ electrons in the backbone of the CP changes, and this is termed ‘redox doping’. To further maintain charge neutrality, counter ions are introduced into the CP. Redox doping can be classified further into three types: (i) p-doping, (ii) n-doping and (iii) charge injection doping and photo-doping. In p-doping, the electrons are removed from the polymer backbone, whereas the addition of electrons to the chain takes place in n-doping [17, 18]. Doping can be done using a chemical method in which the polymer is treated with an oxidising agent ($I_2$ vapours) or a reducing agent (alkali vapours). In electrochemical (EC) doping, a three-electrode system is used and the polymer is coated on a working electrode, which is placed in an electrolyte solution in which the polymer is insoluble [32]. The difference in potential between the electrodes results in crossing of the charges into the polymer by the addition or removal of electrons, and an appropriate counter ion enters into the polymer film to maintain charge neutrality. In photo-doping, the insertion of cations or anions into the polymer chain by irradiating the polymer with photons of energy that is higher than the band gap of the CP leads to the up-gradation of electrons to higher energy levels in the band gap [23]. In non-redox doping of CP, the number of electrons associated with the polymer chain is kept constant, resulting in the re-arrangement of energy levels in the CP. The conversion of the emeraldine base (EB) form of polyaniline (PANI) to protonated EB (polysemiquinone radical cation) after treatment with a protic acid is a good example of non-redox doping. Using this process, it was observed that the conductivity of PANI increased by approximately
ten orders of magnitude. Thus, the reversible interchange between redox states in a CP gives rise to changes in its properties such as polymer conformation, doping level, conductivity, and colour. These properties make CP suitable for applications in various electrochromic devices and biosensors.

CP have attracted much interest as suitable matrices for the immobilisation of biomolecules onto a transducer surface [33–35]. CP films have been found to be appropriate for the fabrication of multi-analyte biosensors because they allow for the immobilisation of biologically active molecules on electrodes of any size or geometry [36]. The electrochemically deposited CP have considerable flexibility in the available chemical structure, which can be modified by chemical modelling and synthesis, to modulate the required electronic and mechanical properties. Moreover, the EC synthesis of the CP facilitates direct deposition of the polymer on the electrode surface, which entraps the biomolecules simultaneously [36, 37]. Thus, it is easier to control the spatial distribution of the immobilised biomolecules, film thickness, and biological activity by manipulating the state of the polymer. CP are known to have a three-dimensional (3D) electrically conducting structure and, in neutral aqueous solution, they are known to be compatible with biological molecules. Further, the electronic conductivity of CP varies over several orders of magnitude in response to variation in the pH and redox potential of their environment [38]. The conducting behaviour of the polymers facilitates efficient transfer of electric charge produced by the biochemical reaction to the transducer. Also, CP exhibit exchange and size-exclusion properties due to which they are highly sensitive and specific towards substrates [39–41]. These materials have an important role in obtaining good limits of detection and fast responses because the redox reaction of the substrate, catalysed by an appropriate enzyme, takes place in the bulk of the polymer layer [42]. The structures of some CP used commonly in biosensors are shown in Figure 4.1.
Figure 4.1 Structures of some CP used commonly in biosensors (PPy: polypyrrole and PTh: polythiophene). Reproduced with permission from M. Gerard, A. Chaubey and B.D. Malhotra, Biosensors and Bioelectronics, 2002, 17, 345. ©2002, Elsevier [1]
In the next section, we discuss the application of different CP in biosensors with particular emphasis on PANI, polypyrrole (PPy) and polythiophene (PTh) because these CP can be prepared readily.

### 4.2 Application of Polyaniline in Biosensors

PANI belongs to a semi-flexible CP family. It was discovered in the nineteenth century, and was known formerly as ‘aniline black’. Later, it was reported that PANI had high conductivity, low cost, and several exciting features:

- It can be deposited on the sensor electrode readily and directly;
- Thickness can be controlled readily;
- Provides excellent redox conductivity and polyelectrolyte characteristics;
- High surface area for the immobilisation of biomolecules;
- High chemical specificity;
- PANI has long-term environmental stability; and
- The physical and chemical properties of PANI can be tuned readily.

The structure of PANI comprises of ‘reduced’ (benzenoid diamine) and ‘oxidised’ (quinoid diamine) repeating units in which the oxidation state can be defined by the value of $m$ (Figure 4.2) [43]. On the basis of different redox forms, PANI exits in three forms: (i) leucoemeraldine (LE), which is a fully reduced state; (ii) perigraniline (PG), which is a fully oxidised state with imine links instead of amine links; and (iii) EB, which is neutral or doped, with imine nitrogens protonated by an acid (Figure 4.3) [44]. Among these three forms of PANI, EB has been found to be the most useful because it is highly stable at room temperature. If doped (emeraldine salt) with an acid (e.g., HCl), it
becomes electrically conducting. The other two forms (LE and PG) have reduced electrical conductivity even if doped with an acid. These forms of PANI can be interconverted by chemical or EC oxidation or reduction [45].

Figure 4.2 (A) 3D and (B) 2D structures of PANI. Reproduced with permission from C. Dhand, M. Das, M. Datta and B.D. Malhotra, *Biosensors and Bioelectronics*, 2011, 26, 281. ©2011, Elsevier [43]

Figure 4.3 Chemical structures of emeraldine (i); before protonation (EB); (ii)–(iv) after 50% protonation [(ii) formation of bipolaron; (iii) formation of polaron; and (iv) separation of two polorons]. Reproduced with permission from C. Dhand, M. Das, M. Datta and B.D. Malhotra, *Biosensors and Bioelectronics*, 2011, 26, 2811. ©2011, Elsevier [43]
PANI is a p-type semiconductor, and the holes are the major charge carriers in PANI [46, 47]. PANI is semicrystalline, the heterogeneous system having a crystalline (ordered) region dispersed in an amorphous region [48]. The delocalised π-bonds in the PANI chain have been found to be responsible for its semiconducting properties. If PANI is doped with an acid, there is formation of bipositive species called ‘polarons’ and ‘bipolarons’, as shown in Figure 4.3. These polarons in the PANI structure are responsible for the conduction of electrical charge through a ‘hopping’ mechanism (intra-chain or inter-chain) in its crystalline region [49]. The structure of polarons consists of a cation radical of one nitrogen atom that acts as a ‘hole’ to carry the charge whereas the electron from the adjacent nitrogen (neutral) jumps to this hole and becomes electrically neutral, resulting in movement of the hole. However, this type of movement is forbidden in the bipolaron structure because the two holes are adjacent (Figure 4.4). In the LE/PG structure, the electronic environments of all nitrogen atoms along the polymer chain are similar, and the protons from a dopant can be attracted by the nitrogen atom, leading to protonation of a few nitrogen or free nitrogen atoms situated side by side across the chain. This causes an irregular arrangement in the polymer chain, resulting in the formation of only a few polarons [50].

For biosensing applications, PANI is an efficient mediator for speedy transfer of electrons in redox or enzymatic reactions [51]. PANI acts as a mediator, due to the delocalised redox charges present a series of conducting grains (polarons) in its crystalline emeraldine hydrochloride salts-I phase. It is considered to be an attractive polymer because it shows two redox couples in the correct potential range. This facilitates an enzyme–polymer charge transfer, thus acting as a self-contained electron-transfer mediator [52]. There is, thus, no need for additional diffusional mediators for the sensing system for electron transfer. For sensor application, PANI offers a broad range of opportunities to couple a desired bioanalyte, as well as non-specific interactions, into the surface of the transducer. It provides excellent electrical conductivity and rate of energy migration, which leads to enhanced sensitivity. Further, the chemical and structural flexibility that surrounds the amine nitrogen linkage can be used for efficient
binding and immobilisation of biomolecules. Many methods have been adopted for the immobilisation of biomolecules onto PANI. Among these, the most common methods are physical entrapment, physical adsorption, covalent binding and crosslinking using multifunctional reagents. Moreover, the physical and EC properties of a biosensor can be modulated by observing the change in the shape and dimension of PANI. Different structures (nanowires, nanospheres, nanorods and nanotubes) can be prepared by varying the synthesis or processing conditions, and these structures have a vital role in improving the sensing performance of the biosensor [53–56].

**Figure 4.4** Schematic of electron transfer from the biochemical reaction site to the electrode through a conducting PANI network in an amperometric biosensor. Reproduced with permission from C. Dhand, M. Das, M. Datta and B.D. Malhotra, *Biosensors and Bioelectronics*, 2011, 26, 2811. ©2011, Elsevier [43]
Several reports have suggested that nanostructured polyaniline (N-PANI) exhibits higher sensitivity and faster response time compared with the conventional bulk counterpart. N-PANI, due to a higher effective surface area and shorter penetration depth for target molecules, enhances the activity of the desired catalyst [57]. Similarly, polyaniline nanospheres (PANI-NS) were chemically fabricated, and it was revealed that PANI nanospheres, due to the large aspect ratio of these films, show a better detection range with faster response time and shelf-life (Figure 4.5) [58]. Given these interesting properties, several biosensors based on PANI have been reported. Tao and co-workers fabricated a glucose nanosensor based on PANI/enzyme nanojunctions by bridging two nanoelectrodes separated by a small gap (20–60 nm) with PANI/glucose oxidase (GOx) [59]. A fast EC response (<200 ms) was obtained, with a small size of nanojunction sensor, and the enzyme was regenerated naturally without the need for a redox mediator [59]. PANI has been used as a host polymer and redox indicator for GOx, thus providing a path for elimination of redox dyes during the optical detection of glucose [59].

Different composites of PANI with various conductive nanomaterials [carbon nanotubes (CNT), graphite oxide (GO), gold nanoparticles (AuNP), platinum (Pt) NP] can be utilised for the development of highly sensitive, durable, and broad-ranging biosensors. In this context, a novel glucose biosensor with high sensitivity and selectivity was fabricated by self-assembling GOx and Pt on nanofibrous PANI. The fabricated biosensor showed biocompatible performance and showed an excellent amperometric response to glucose [60]. PANI nanofibres were used for electrical contacting of AuNP with glassy carbon for the fabrication of nanocomposite structures. This matrix was used for electrocatalysis and enzyme (GOx) immobilisation [61].
Figure 4.5 Pictorial representations of the morphological transformation of the polyaniline nanotube(s) (PANI-NT) to PANI-NS, immobilisation of cholesterol oxidase (ChOx) and the biochemical reaction involved in cholesterol sensing [CSA: camphor sulfonic acid; EDC: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide; EG: ethylene glycol; ITO: indium-tin-oxide; LSV: linear sweep voltammetry; and NHS: N-hydroxysuccinimide]. Reproduced with permission from C. Dhand, M. Das, G. Sumana, A.K. Srivastava, M.K. Pandey, C.G. Kim, M. Datta and B.D. Malhotra, Nanoscale, 2010, 2, 747. ©2010, Royal Society of Chemistry [58]
A glucose biosensor was recently fabricated by *in situ* electropolymerisation of aniline onto a microporous PANI-coated Pt electrode in the presence of GOx. The prepared bioelectrode combined the properties of the microporous polymer material, and electropolymerisation resulted in good selectivity, sensitivity, and stability [62]. Self-assembly of a poly(diallyldimethylammonium chloride)/GOx multi-layer was accomplished in the whole wall of as-synthesised ordered porous PANI (inverse opal) for increasing the quantity of GOx in contact with the electrode directly. This resulted in direct electron transport between the enzyme and electrode, leading to higher sensitivity and short response time [63].

Recent reports have suggested that PANI can be used to fabricate reliable, accurate and efficient cholesterol biosensors. Cholesterol oxidase (ChOx) was directly immobilised onto an electrochemically deposited PANI film. The incorporation of Triton X-100 (surfactant) in PANI resulted in improved biocompatibility and efficient immobilisation of ChOx, leading to high sensitivity and a low Michaelis constant value [64]. Khan and co-workers fabricated a cholesterol biosensor via covalent immobilisation of ChOx on electrochemically prepared PANI film in the presence of TX-100 [65]. Dhand and co-workers reported the fabrication of N-PANI film using a novel electrophoretic technique for application to cholesterol biosensors. This N-PANI-derived bioelectrode was found to detect cholesterol with excellent sensitivity, selectivity and at very low values to reveal enhanced enzyme (ChOx)–substrate (cholesterol) interactions. Covalent immobilisation of ChOx onto PANI-NS prepared by morphological transformation of micelle polymerised camphor sulfonic acid-doped PANI-NT was reported by Dhand and co-workers for cholesterol detection. Thus, the prepared ChOx/PANI-NS/indium-tin-oxide (ITO) bioelectrode could be used to detect cholesterol at a wide concentration range with enhanced sensitivity. The response time for the PANI-NS-based bioelectrode is 10 s and the shelf-life of the sensor is 12 weeks (Figure 4.6) [66].
EC deoxyribonucleic acid (DNA) biosensors based on PANI are being studied widely. In this context, plasmid deoxyribonucleic acid (pDNA) is immobilised onto a deposited PANI film for the
fabrication of a biosensor [67]. Singh and co-workers fabricated a sexually transmitted disease (*Neisseria gonorrhoeae*) sensor based on a nucleic acid (NA)-functionalised N-PANI-coated probe onto an ITO-coated glass plate. The fabricated N-PANI-based bioelectrode was highly sensitive and could be used to distinguish *Neisseria gonorrhoeae* from other pathogens and spiked samples from the urethral swabs of patients (Figure 4.7) [68]. Arora and co-workers reported the application of PANI for ultrasensitive and specific detection of *Escherichia coli*. The pDNA specific to *Escherichia coli* was immobilised using biotin-avidin chemistry. Then, the fabricated bioelectrodes were utilised to electrochemically detect target DNA and differentiate between sub-femtomolar complementary and non-complementary target sequences present in the sample [69]. It was reported that transfer of a pDNA-modified PANI-intercalated GO nanocomposite onto a carbon-paste electrode could be used to monitor DNA hybridisation with complementary-deoxyribonucleic acid (C-DNA). Using square wave voltammetry (SWV), the genosensor was able to detect C-DNA in a linear range of 275–551 μg mL⁻¹ with detection limit of 29.34 μg mL⁻¹ and a response time of 30 min [70]. An EC DNA sensor was recently fabricated using PANI nanowires that were electrochemically deposited onto a glassy carbon electrode (GCE). Detection of EC DNA hybridisation using SWV showed limit of detection of $1.0 \times 10^{-12}$ mol L⁻¹ of cDNA with methylene blue as an indicator [71]. Peptide NA was used for the development of the NA sensor for specific detection of *Mycobacterium tuberculosis* on electrochemically-polymerised PANI-coated Au electrode. Soni and co-workers prepared the PANI–Au nanocomposite-based electrode using chronopotentiometry and electrophoretic techniques [72]. The electrodes provided an increased surface area due to their porous structure and were used further for the fabrication of genosensors specific for *Neisseria gonorrhoeae*. The wide detection range ($10^{-6}$ to $10^{-16}$ M), reduced response time (60 s) with high stability (4 weeks) and reusability (12 times) of the genosensor were demonstrated. The fabricated genosensor based on a PANI–Au nanocomposite was highly specific and hence could be used to distinguish clinical samples of *Neisseria gonorrhoeae* from other non-gonorrhoeae and Gram-negative bacteria species [73].
Efforts have been made to develop PANI-based label-less immunosensors. Acrylic acid was grafted onto a PANI surface for generation of carboxylic groups used for covalent binding of antigen by EDC-NHS chemistry [74]. Bandodkar and co-workers developed a reagentless capacitive immunosensor based on the capacitance change in a parallel plate capacitor by covalently immobilising anti-human immunoglobulin G (IgG) on electrophoretically deposited N-PANI film [75]. An electrochemical impedance spectroscopy-based immunosensor was reported for monitoring of the intermolecular interactions between anti-human α-fetoprotein (AFP) monoclonal IgG and AFP by electrochemically entrapping the antibody within
the PANI matrix. The resulting immunosensor had a linear dynamic range of 200–800 ng mL\(^{-1}\) of AFP [76]. A few reports are available on the use of PANI in the development of electroconductive hydrogels (ECH). An ECH is a hybrid network fabricated from conventional insulating polymers that have been combined with a CP. The insulating polymer provides the 3D aqueous gel, whereas the CP imparts electrical conductivity to the scaffold. Zhai and co-workers reported an ultrahigh-sensitive glucose biosensor based on PANI-platinum nanoparticle hydrogel heterostructures that have very low response times (3 s) [77].

The main advantage of using PANI for biosensor fabrication lies in its capability as an entrapment matrix for the desired biomolecule. PANI functionalisation with a biospecific agent provides a more efficient biosensor platform for immobilisation of a bioanalyte. Although it is one of the most explored CP for biosensor development, it has some drawbacks that have limited its commercialisation: an ageing effect; optical and EC instability; and non-availability of standard/optimised deposition techniques. The ageing of PANI with time slowly and spontaneously degrades its chemical structure, leading to its diminished electrical conductivity [42, 78]. Efforts should be made to customise the morphology by controlling the synthetic conditions that yield different nanostructures of PANI (nanotubes, nanorods and nanoparticles). The structural changes that are realisable with PANI are made possible by the use of dopants such as high-molecular weight sulfonic acids [79]. These PANI nanostructures are likely to overcome the processability issues associated with PANI. Moreover, the integration of PANI with nanomaterials may lead to new hybrid systems that couple the recognition or catalytic properties of biomaterials with attractive electronic and structural characteristics.

### 4.3 Conducting Polypyrrole-based Biosensors

PPy, also called ‘pyrrole black’, is formed due to the oxidation of pyrrole in air. It was reported first in 1960, and is an inherent CP with unusual electrical properties [80]. PPy has attracted particular interest because it can be prepared readily, has high conductivity,
flexibility, stability, biocompatibility, and excellent mechanical properties. It is usually fabricated in its oxidised conducting state via simultaneous polymerisation and oxidation of the \( \pi \) system of the final polymer. It can be electrochemically or chemically reduced to obtain the neutral polymer. In 1963, the research team of D.E. Weiss reported I\(_2\)-doped PPy, and the chemical structure, charge-transfer complex and electronic properties of PPy was studied thoroughly [81, 82]. PPy synthesised by a conventional chemical method was found to be insoluble in common solvents because of strong inter-chain interactions. PPy can be synthesised using:

(i) chemical initiation by oxidative agents;
(ii) photoinduced synthesis;
and (iii) EC activation by anodic current. Other chemicals and biochemical methods were used to prepare a controlled shape and size (several nanometres up to several micrometres) of PPy [83, 84]. In chemically-induced polymerisation, PPy was produced in bulk solution, and a small amount of the PPy was deposited onto the substrate [85]. The main problem of depositing the PPy film onto a suitable substrate is the insolubility of PPy in common solvents. However, doping with other agents increases the solubility of the PPy. The main obstacle in the use of this deposition method is the poor adherence of PPy on the transducer surface [86, 87]. To avoid poor adherence, the preferred way of depositing the PPy onto a suitable substrate is through electrochemical polymerisation (EP) using an EC cell. In the EP process, by applying a well-defined potential and known current passing through the EC cell, a thin PPy layer can be deposited using various solvents (acetonitrile, water) [88]. Moreover, the nanostructure of the PPy is paramount. Hence, the synthesis can be undertaken using water at neutral pH because it may help in entrapment/doping of PPy by various biomaterials (organic molecules, proteins, DNA) [34]. This electrochemically synthesised PPy has excellent conductivity. The high adherence of these films makes them stable substrates for the fabrication of a biosensor. Further, the EC properties of PPy strongly depend on the redox state of this polymer. At a positive potential, over-oxidation of PPy occurs, resulting in decreased conductivity of PPy and the possibility of leakage of anionic molecules which become incorporated into the polymeric backbone [87]. Conversely, over-oxidation of PPy
usually takes place at a lower positive potential in water and oxygen-containing environment due to partial destruction of the polymeric backbone, resulting in the generation of oxygen-containing groups such as carboxyl, carbonyl and hydroxyl [89, 90].

The necessity to detect an analyte at neutral pH range leads to the electrical inactivity of the deposited CP [91]. Compared with PANI, PPy can be deposited readily from neutral-pH aqueous solutions containing pyrrole monomers. PPy can be used for discrimination between cations and anions because the permeability and permselectivity of PPy are known to depend on the counter ion incorporated during polymerisation as well as on the ions present in the samples [92, 93]. It was observed that PPy protected the electrode from fouling by the biomolecular substances (proteins) present in samples of blood serum and urine. The stability of PPy-based biosensors is sufficient and determined mainly by PPy degradation in the surrounding water if a biosensor is used for continuous measurements. The following characteristics of PPy make it a suitable candidate for biosensing applications [94]:

- PPy can be synthesised electrochemically, and the analytical properties can be tuned readily.
- PPy protects the electrodes from fouling and other interfering materials.
- PPy has been found to be biocompatible, resulting in minimal and reversible disturbance to the working environment.
- PPy can be used as a redox mediator by directly transferring electrons from redox enzymes towards electrodes.

Many catalytic biosensors based on PPy were recently designed that allow precise detection of an analyte at very low concentrations. The application of PPy modified by enzymes in the development of catalytic biosensors initiated by entrapment of GOx within PPy for glucose sensing was reported. A stable and homogeneous hybrid film of PPy and copper hexacyanoferrate was prepared by the EC method.
for H₂O₂ catalytic reduction in the presence of Na⁺ or K⁺ [95]. The fabricated biosensor showed exceptional catalytic properties towards H₂O₂ detection, and the reported sensor performance was higher than that of Prussian Blue and other analogues, and the increased EC performance was attributed to the electronic conductivity of the PPy matrix [95]. PPy can be doped with several dopants to obtain the enhanced electrical conductivity of PPy, which results in the improved performance of the desired sensor. For instance, polymer films can be doped with anionic or cationic species (during polymerisation) or the insertion of a large dopant anion [polyvinyl sulfonate (PVS), p-toluenesulfonic acid, and dodecylbenzenesulfonic acid] into a PPy film during electropolymerisation. Integration of the dopant increased the porosity of the film, which helped facile immobilisation of the enzymes [96]. Using the EC method, a PPy–PVS nanocomposite film was fabricated onto an ITO electrode, followed by enzyme immobilisation by crosslinking via glutaraldehyde on the hybrid film. The biosensor showed a good response in terms of the dynamic range of detection, short response time, long lifetime and stability. Layer-by-layer (LbL) deposition of PPy has also been utilised for biosensor fabrication. Shirsat and co-workers deposited multi-layers of LbL-assembled PPy and CNT films on Pt-coated polyvinylidene fluoride membranes. EP was used for deposition of the PPy film, whereas the CNT layers were coated using a vacuum filtration technique [97]. This multi-layer structure comprised of the favourable features of PPy and CNT, and provided an excellent matrix for the immobilisation of GOx. Also, the fabricated sensor showed an enhanced linear range, response time and sensitivity [98]. Different nanostructures of PPy have been synthesised. For example, PPy nanotubes were synthesised and used for enhanced adsorption of GOx in glucose biosensors [98]. The nanotubes were synthesised using a solution of pyrrole and NaPF₆ at a fixed current density for 90 s. This PPy nanotube-based biosensor exhibited enhanced sensitivity, improved response time and a linear range [99].

PPy, due to its ease of synthesis at neutral pH, has been used extensively as a versatile immobilisation matrix in the design of immunosensors, DNA sensors and molecular imprinting technologies. It provides an efficient DNA-sensing platform if
electrodeposited onto an electrode surface, and acts as an interface for the linking of pDNA (Figure 4.8) [100]. Livache and co-workers reported the electrosynthesis of a PPy-DNA composite through copolymerisation for detection of DNA hybridisation on the surface of modified microelectrodes [101]. During the synthesis of the PPy-DNA composite, a mixture of pyrrole and a pyrrole bearing a specific DNA probe was electro-oxidised. The incorporation of DNA dopants into a PPy network using an EC quartz crystal microbalance exhibited strong affinity for the target DNA [4]. Youssoufi and co-workers developed a new type of EC DNA hybridisation sensor based on DNA-functionalised PPy in which the PPy precursor contained a loosely bound ester group that was used for covalent immobilisation of the amino-labelled DNA probe [102]. The EC response of this sensor showed a change in the voltammetric signal when hybridised with its complementary probe, whereas no change in signal was observed in the presence of a non-complementary target DNA sequence. This fabricated sensor could detect the target DNA, and the limit of detection of the biosensor obtained was $1 \times 10^{-2}$ nmol. Acid-functionalised PPy was used as an alternative for fabricating label-free sensors because it allowed the immobilisation of DNA by using various functional groups (–SH, –NH$_2$ and –COOH) [103, 104]. The development of DNA sensors based on PPy and PPy derivatives using a modified fluorine-doped tin-oxide electrode has also been reported. However, the selectivity, sensitivity, and reproducibility of this DNA sensor were found to be poor. To overcome these sensing characteristics researchers introduced other nanomaterials (CNT, metals, metal oxides) which resulted in the formation of PPy composites. An impedance-based DNA sensor was fabricated on a GCE modified with a PPy-MWCNT composite [105, 106]. The carboxyl functionalised-MWCNT and PPy were electrodeposited on the GCE for immobilisation of the amino-terminated pDNA. The PPy/MWCNT-COOH-modified electrode showed increased surface area, resulting in the high electron transport and improved sensitivity and selectivity of this NA sensor.
PPy has been used most extensively for the fabrication of different types of biosensors. It has been used as an attractive, versatile material that is suitable for preparation of various catalytic and affinity-based biosensors. The use of PPy in conjunction with bio-affinity reagents can act as a robust tool for a broad range of applications for EC detection and device fabrication. Further, the use of a wide variety of counter ions may result in a significant change in affinity at PPy ion-exchange sites. Further understanding of the association of the immobilised bioanalyte with the PPy matrix can lead to the design of smaller, more compact and portable biosensors.
4.4 Polythiophenes-based Biosensors

Among the various conjugated polymers, PTh display a unique combination of efficient electronic conjugation, chemical stability, and incredible synthetic versatility [107]. Further, the conducting organic materials allow tuning of the properties to be accessed through functionalisation of the thiophene monomer ring [108]. Many PTh derivatives have been proposed for electroanalysis, and the most widely used is 2,2'-bithiophene, which can be used to synthesise unsubstituted PTh films [109]. Dimers are used because a very high potential is required to oxidise the unsubstituted thiophene, which may concurrently induce over-oxidation of the polymer chains formed. In the event of over-oxidation, there may be formation of chemical functionalities on the thiophene ring that may interrupt conjugation of the π electron system, resulting in an irreversible decrease in the conductivity of the polymer [108, 110]. The properties of PTh-modified electrodes are known to depend on the oxidation potential of the monomer. They are related to the potential at which the relevant polymer film becomes electrically conductive, and can act as the redox mediator towards species in the solution [111]. Therefore, oxidation of the polymer at very low potential results in wider potential options at which different analytes are electroactive, and may lead to higher resolution of the resulting voltammogram.

Different chemical or EC oxidative methods have been used to synthesise PTh. However, the conducting nature of PTh is strongly favoured by a less positive potential whereby the growing oligomer chains oxidise with respect to the starting monomer. In brief, the process can be divided into four steps [107, 112]:

- Generation of a radical cation and coupling with the other monomer.
- Fast coupling of the monomer within a reaction layer thin enough with respect to the diffusion layer.
• The chain grows progressively by further radical–radical coupling and the reaction takes place in the vicinity to the electrode surface.

• Precipitation of the polymer onto the electrode surface when a certain length is achieved by the polymer or the polymer becomes insoluble.

The actual mechanism of the electro-polymerisation deposition is not understood fully and different alternatives have been proposed. However, this field is still open for discussion. For example, how does the p-doping of the polymer occur? It is an important parameter towards realisation of the specific characteristics of the polymer [113]. Several experimental factors selected for electro-generation of the polymer may affect the structural, morphological and chemical characteristics of the film. The chain length of the resulting polymer depends on various factors, such as the concentration of the monomer, nature of the solvent, and the supporting electrolyte. Thus, for the fabrication of a PTh-based sensor, the two key points that need to be optimised pertain to the film thickness and conductivity of the electrode-coating at different applied potentials [114, 115].

For sensing applications, two main classes of PTh derivatives have been investigated. The first class is represented by ‘neutral’ PTh (which is often insoluble in water) and the second class of PTh comprises of ionic side chains (which make these polymers soluble in water) [42]. In the first class of polymer, the specific receptors are covalently bound to the PTh backbone, whereas the second class of polymer can be particularly useful for the detection of biomolecules (DNA and proteins) [116]. For most functionalised PTh-based sensors, the detection of the target is related to modification of the optoelectrical properties of the polymeric transducer through conformational changes. For immobilisation of the enzyme on the PTh surface, two conventional procedures have been reported. In the first method, the positive charge on the growing polymer chains
during electro-generation can be used to electrostatically interact with enzymes that are negatively charged (pH should be above the isoelectric point). In this process, the biological entities are dissolved in the polymerisation solution (including monomers) in the presence of a suitable buffer [117, 118]. The procedure is simple and rapid, but it requires a higher concentration of the enzyme. Moreover, the monomer should be soluble in water. Further, after film formation, the thickness and homogeneity of the enzyme within the polymer are questionable. To overcome this limitation, organic composites were synthesised for increased loading of the enzyme. An example is the electro-generation of poly(3,4-ethylenedioxythiophene) (PEDOT) in the presence of polyethylene glycol (PEG) (Figure 4.9) [119]. The PEG derivative bears a carboxylic acid group that binds covalently with the enzyme through amine terminal groups. In the second method, the enzyme is allowed to react chemically with the polymer surface to form covalent bonds or electrostatic interactions. This helps to stabilise the enzyme on the polymeric surface. This process can be used for monomers that are soluble in organic media, but which bear functionalisation that interacts stably with the enzymes. In this category, the most extensively used thiophene derivative is thiophene-3-acetic acid, which contains external carboxylic groups that form amidic bonds with the amine groups present in enzymes.

Genosensors based on PTh and its derivatives have been studied widely. The formation of DNA–DNA duplexes during hybridisation on the PTh-modified electrode surface provides high rigidity to the polymeric structure, thus hindering swelling of the polymer induced by p-doping [120]. Upon hybridisation, the negative-charge densities (due to the phosphoric groups of the oligonucleotide chain) at the interface with the solution increase, which opposes access of the anions from the supporting electrolyte to the polymer film, hence opposing p-doping of the polymer [121, 122]. The high rigidity of the structure and lowering of conductivity has been attributed to this effect. This method of detection of DNA hybridisation was studied widely during the 1990s, and there are two main restrictions to this process [123]:
**Figure 4.9** Stratagem used for fabrication of disposable sensor strips using PEDOT:PSS (CEA: carcinoembryonic antigen and PSS: polystyrene sulfonate). Reproduced with permission from S. Kumar, S. Kumar, C.M. Pandey and B.D. Malhotra, *Journal of Physics: Conference Series*, 2016, **704**, 012010. ©2016, IOP Publishing [119]

- The polymerisation of thiophene derivatives having DNA sequences is restricted to very short nucleotide sequences.

- The system is limited to organic solvents only because most of the PTh derivatives in aqueous media cannot be used for p-doping.

To overcome the first drawback, the oligonucleotides can be linked covalently to PTh derivatives bearing functional groups after polymerisation. Recently, use of metal nanoparticles (Au, Pt) with PTh led to stabilisation of the DNA chains and further increased the performance of the biosensor [124].

In spite of these interesting developments, further research should be done to improve the stability and performance of the material. The biggest problem in using PTh is the complicated and time-consuming
procedure that limits the customised synthesis of the material. Further, combining PTh, with biological ligands, nanomaterials, and optical amplification tools ( Förster resonance energy transfer, fluorescence chain reaction), may result in the development of point-of-care devices.

4.5 Conclusions

CP have been used for the fabrication of highly stable, economic and easy-to-use biosensing devices. The improved sensitivity and selectivity of EC biosensors has been attributed to their excellent electrical conductivity. These organic conducting materials can be electrochemically grown on a very small electrode. CP are biocompatible with biological molecules in neutral aqueous solutions. Therefore, various biomolecules can be immobilised onto these CP readily without a loss in activity. Different morphologies of CP have been explored for the fabrication of EC sensors and biosensors. However, the sensing performance of the biosensor can be improved further through novel methods for surface modification and 3D structures with enhanced surface areas. Extensive efforts are required before these interesting conducting biomolecular electronic materials are utilised by biosensor industries.

References


Biosensors: Fundamentals and Applications


Conducting Polymer-based Biosensors


Conducting Polymer-based Biosensors


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Biosensors: Fundamentals and Applications


5 Applications of Biosensors

5.1 Introduction

With increases in the global population, several new, life-threatening diseases caused by various pathogens have come into existence. Some of the deaths from such diseases are primarily due to a delay in making the diagnosis, the side effects of drugs, and the material needed for rapid detection, prevention or cure [1]. Further, an increased number of pollutants arising due to excess use of chemicals have led to increases in mortality rates. There is, thus, an urgent need for the development of rapid sensing methods to monitor and control the release of contaminants and pathogens [2].

In this context, a novel electronic device for various applications has motivated many researchers to focus on the fabrication of biosensors [3, 4]. These bioelectronic devices offer real-time, on-site, simultaneous detection of multiple pathogenic agents by utilising the selectivity of biomolecules and the power of nanoelectronics [5]. The fabrication of desired biosensors requires the collective efforts of chemists, biologists, physicists, and engineers [2]. Biosensors represent a broad area of emerging technology for applications in various fields such as medical diagnoses, environmental monitoring, and food industries [6]. Biosensors combine a biochemical recognition component integrated with a physical transducer, which converts a biochemical event into a measurable signal, thus leading to the detection of biological molecules [7]. Several biosensors based on different metabolites used to monitor clinically relevant parameters have been reported [8, 9]. Most of the biosensors rely on the various transducers used for the detection of a particular analyte [10]. However, the use of silicon microfabrication for electrochemical (EC) and optical sensors is expanding because it can be used for the development of on-chip amplification of electronic
signals and data processing [11, 12]. Biosensor fabrication comprises of two broad categories of instrumentation [11, 13]:

- Sophisticated laboratory machines capable of providing a rapid, accurate and convenient measurement of complex biological interactions.

- User-friendly, portable devices that can be used by non-specialists for point-of-care (POC) diagnostics.

Only a few reports are available on the application of these methods in a clinical diagnosis. The only exception is the glucose biosensor, which represents ≈90% of the global biosensor market. The major problem is the application of the biosensor in real samples, whereby interference with undesired molecules results in low accuracy and selectivity during the measurement [14]. This is important because the treatment of an individual depends on the level of a clinical marker. In the next section, we discuss some of the recent applications of biosensors in clinical diagnostics, environmental monitoring, as well as the processing of food, water, and agricultural products (Figure 5.1).

**Figure 5.1** Scheme showing the various applications of biosensors

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*Biosensors: Fundamentals and Applications*
5.2 Biosensors for Food/Water Safety

The progressive increase in the quantity and number of items in the food market has led to the emergence of many biological, chemical and physical threats to food products [15]. The food industry integrates the processing and commercialisation of agricultural commodities for specific markets and consumer demands [16]. There are various steps in the agricultural and food-production chain that are susceptible to several threats. These food products are transferred to different parts of the world. There is, thus, a chance of loss of the quality and the transmission of pathogenic disease [17]. The threat may be biological or chemical, and it may arise from environmental contamination, or during the handling, processing, packing, and distribution of food [18, 19].

In recent years, the demand for the production and commercialisation of foods has increased, resulting in the search for innovative products and technologies. Such innovative products could be a partial solution to the continuing food crises by providing increased sanitary standards [20]. The World Health Organization (WHO) defines foodborne illnesses as diseases which are ‘Infectious or toxic in nature and are caused by agents that enter the body through the ingestion of contaminated food and water’ [20, 21]. According to a WHO report, every year millions of people around the world are affected by foodborne diseases [22]. Some of the causes of the diseases include infections due to bacteria, viruses, biotoxins, additives, food nutrients, pesticides, residues of veterinary drugs, and processing operations [23]. These pathogens/additives may generate polycyclic aromatic hydrocarbons derived from protein decomposition or mutagenic agents (e.g., tryptophan) in cooked foods, and which may result in risks to the health of consumers [23, 24].

Currently, key elements that influence consumer behaviour are food safety and food quality [25, 26]. Leading causes of contamination worldwide are microbial toxins and agrochemicals [27]. The presence of a pathogenic microorganism in food can lead to severe
health consequences in animals and humans. Among the various
foodborne pathogens, the most common are mycotoxins, exotoxins
and enterotoxins from *Escherichia coli* O157:H7 [28, 29]. These
mycotoxins mainly affect babies, young children, the elderly and
sick people [30, 31]. The identification and detection of foodborne
toxins/pathogens are essential for ensuring the quality of the product.
Moreover, detection of these pathogens relies on conventional
culturing techniques that are very elaborate, expensive, time-
consuming and which are not suitable for POC diagnostics [32].
Therefore, food industries require real-time, portable sensors for
pathogen detection with higher sensitivity and selectivity. In this
regard, biosensors can be an excellent option for agricultural and food
sectors to control the production processes and ensure the quality
and safety of food using reliable, fast and cost-effective procedures
[33]. In the next section, we discuss the role of biosensors for the
detection of these foodborne pathogens, and discussion is limited
to detection of the important foodborne toxicants *Escherichia coli*,
*Salmonella*, aflatoxin (AF) B and ochratoxin-A (OTA).

### 5.2.1 Biosensors for Detection of Foodborne/Waterborne Pathogens

#### 5.2.1.1 Biosensors for *Escherichia Coli* Detection

*Escherichia coli* is a Gram-negative, intestinal bacterium which
was discovered first in 1885 by the German bacteriologist Theodor
Escherich [34]. Most *Escherichia coli* strains are non-pathogenic,
and some of them are essential components of the healthy human
intestinal tract [35, 36] and resist the intestinal invasion of virulent
strains [37]. However, some virulent *Escherichia coli* strains may
result in severe infection to living beings, and emergency recall of
food products [38].

*Escherichia coli* comprises of a diverse group of bacteria. An ample
range of pathogenic *Escherichia coli* strains causes diarrhoea,
urinary-tract infection, inflammation of the lung and respiratory
system, and many other diseases, which leads to death in the aged and infants [39, 40]. Among the various pathogenic strains, the potent Shiga-like toxin secreted by *Escherichia coli* O157:H7 may cause severe haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans [41, 42]. The first incident recorded for *Escherichia coli* O157:H7 was in 1975 for a patient suffering from HC [43]. This incident was not taken seriously until 1982, when scores of people in Michigan and Oregon in the USA were infected with severe gastrointestinal disease due to consumption of beef patties contaminated with *Escherichia coli* O157:H7 [43]. Since then, diseases associated with *Escherichia coli* O157: H7 have been reported worldwide, including USA [44], Europe [45], Japan [46] and Australia [47]. Although *Escherichia coli* is regarded primarily as a cause of foodborne diarrhoea, the diversity, frequency, potential severity and the economic impact of *Escherichia coli* are as broad as any bacterial pathogen [48]. *Escherichia coli* O157:H7 is highly toxic to humans with an infectious dose as low as 1–10 colony-forming units (CFU) [49]. The pathogenesis of *Escherichia coli* O157:H7 in children can cause severe complications, leading to HUS and kidney failure [50]. Patients infected by *Escherichia coli* O157:H7 sometimes have no visible symptoms, making the diagnosis difficult and, often, they are misdiagnosed [50]. In many developing countries, *Escherichia coli* O157:H7 is the most common bacterial enteric pathogen, accounting for ≈20% of cases. In 2011, the diseases induced by a type of enterohaemorrhagic *Escherichia coli* O104:H4 caused massive outbreaks in Europe [51]. Many cases were reported in several other countries, including Poland, Switzerland, the Netherlands, Denmark, Sweden, the UK, Canada and the USA [52].

The main reservoir of *Escherichia coli* O157:H7 is cattle. The primary reports of *Escherichia coli* O157:H7 infections are associated with undercooked beef and raw milk [53]. *Escherichia coli* O157:H7 is carried more frequently in younger cattle than adult cattle, and the range of *Escherichia coli* O157:H7 in cattle manure is $10^2$–$10^5$ CFU g$^{-1}$ [54]. The other animal sources of *Escherichia coli* O157:H7
are chicken, deer, sheep and pigs [54]. Foods such as unpasteurised milk, cheese, juices, alfalfa, radish sprouts, lettuce, spinach and water are sometimes contaminated with *Escherichia coli* O157:H7. The infection is mainly due to cross-contamination (contact with the faeces of infected animals or people). Paths usually associated with the transmission of *Escherichia coli* O157:H7 and other enterohaemorrhagic *Escherichia coli* are person-to-person or animal-to-person [54–57].

Many microbial testing approaches (e.g., culture, enrichment, isolation, and phenotypic analysis) are being used in laboratories. These are followed by serological confirmation, which delays the detection process [58]. Conventional microbial methods show high sensitivity and reliability. The limitations include time and labour. In a well-controlled environment, it can take 18–24 h for a single microbial cell to grow into a colony consisting of 10^6 cells [59]. Moreover, most detection methods require assorted instruments, such as incubators, humidistsats, and signal-analysis equipment. The non-portability of these instruments is a major issue. Also, these techniques do not meet the requirements of POC clinical diagnostics.

The use of biosensors for *Escherichia coli* detection has recently emerged as an attractive and cost-effective technique for the development of POC devices. The availability of a biosensor for detection of *Escherichia coli* O157:H7 may help in the rapid prognosis of the disease, resulting in decreased morbidity and mortality in the result of an outbreak [60].

For the detection of *Escherichia coli* O157:H7 in food, two primary detection protocols have been used: biosensors based on deoxyribonucleic acid (DNA) hybridisation and immunosensors. In DNA-based biosensors, researchers have used thiolated single-stranded DNA probes specific to *Escherichia coli* O157:H7 (*eaeA* gene) that was self-assembled onto a quartz crystal microbalance.
sensor [61]. The fabricated bioelectrode was hybridised further with complementary-deoxyribonucleic acid (C-DNA) and amplification using an asymmetric polymerase chain reaction (PCR) with biotin-labelled primers. This sensor showed a linear working range \((2.67 \times 10^2 \text{ to } 2.67 \times 10^6 \text{ CFU ml}^{-1})\) with a limit of detection of \(2.67 \times 10^2 \text{ CFU ml}^{-1}\). Pandey and co-workers fabricated an EC genosensor based on self-assembly of 1-fluoro-2-nitro-4-azidobenzene-modified octadecanethiol for *Escherichia coli* detection. The biosensor showed high sensitivity (\(0.5 \times 10^{-18} \text{ M}\)) and linearity (\(0.5 \times 10^{-18} \text{ to } 1 \times 10^{-6} \text{ M}\)) with a response time of 60 s when methylene blue was used as a redox indicator. It was revealed that other pathogens, such as *Klebsiella* pneumonia, *Salmonella typhimurium* and other Gram-negative bacteria did not significantly affect the response of this sensor [60]. In another report, self-assembly of cysteine (Cys) hierarchical structures was used for the detection of an *Escherichia coli*-specific DNA probe. This nucleic acid (NA)-based sensor exhibited a linear response to C-DNA in the concentration range \(10^{-6} \text{ to } 10^{-14} \text{ M}\) with a response time of 30 min (Figure 5.2) [62]. Some of the disadvantages of the NA-based biosensor included extraction of DNA from culture samples and processing involving laborious pre-treatment steps [63]. Thus, efforts have been made to develop affinity-based biosensors (using antibody–antigen interactions) that may allow the detection of *Escherichia coli* O157:H7 cells directly from food samples. Ruan and co-workers developed an impedance-based immunosensor for the detection of *Escherichia coli* O157:H7 by immobilising the bacteria to electrodes functionalised with specific antibodies [64]. For amplification of the signal, a secondary antibody conjugated with alkaline phosphatase as the labelled enzyme was used. The fabricated biosensor was able to detect the target bacteria with a limit of detection of \(6 \times 10^3 \text{ cells/ml}\). In another work, researchers developed a label-free immunosensor for the detection of *Escherichia coli* using indium-tin-oxide (ITO)-interdigitated electrodes wherein the size between the electrode and bacterial cells was compatible [65]. Cystine microstructures (CM) with high uniformity having a size of 50 μm and 10 μm,
respectively, were used to fabricate a label-free high-performance EC immunosensor by immobilising monoclonal antibodies specific to *Escherichia coli* (Figure 5.3). The design of the fabricated biosensor demonstrated high performance with enhanced sensitivity (4.70 to $3 \times 10^9$ CFU ml$^{-1}$ in a linear sensing range), a long shelf-life (35 days) and high selectivity over other bacterial pathogens. The enhanced performance was attributed to a novel micro-structure in which the CM provided higher surface coverage for the immobilisation of antibodies, providing excellent electronic/ionic conductivity for the increase in sensitivity [66]. Further, an EC-immunosensing strategy based on copper(II)-assisted hierarchical cysteine (CuCys) structure was utilised to fabricate an immunosensor by covalently immobilising monoclonal antibodies specific for *Escherichia coli* O157:H7. Under optimal conditions, the fabricated immunosensor was found to be sensitive and specific for detection of *Escherichia coli* O157:H7 with a lower limit of detection of 10 CFU ml$^{-1}$ [67]. Sensing characteristics were further improved on addition of graphene oxide (GO) to the Cys hierarchical structures. Electrochemical impedance spectroscopy (EIS) investigations revealed that the self-assembly of reduced graphene oxide (rGO)-CysCu onto a gold (Au) electrode provided a relatively larger surface area and excellent charge-transfer rate. Under optimal conditions, the calibration plot obtained for *Escherichia coli* O157:H7 was approximately linear from 10 to $10^8$ CFU ml$^{-1}$, with a limit of detection of 3.8 CFU ml$^{-1}$, and the total assay time was <1 h. The proposed method was used to differentiate *Escherichia coli* O157:H7 from non-pathogenic *Escherichia coli* (DH5α) and other bacterial cells in synthetic samples [68]. Many other EC immunosensors have been developed for detection of *Escherichia coli* using nanomaterials such as gold nanoparticles (AuNP), metal nanoparticles (MNP), and carbon nanotube(s) (CNT) [64, 6, 68–77] (Table 5.1). These biosensors have shown potential for the development of POC devices for detection of *Escherichia coli* in food. Intensive efforts are required to commercialise these biosensors.
Table 5.1 Comparison of response characteristics of EC immunosensor for detection of *Escherichia coli* O157:H7

<table>
<thead>
<tr>
<th>Immobilisation matrices</th>
<th>Detection technique</th>
<th>Detection range (CFU/ml)</th>
<th>Limit of detection (CFU/ml)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNP-modified graphene paper</td>
<td>EIS</td>
<td>$1.5 \times 10^2$ to $1.5 \times 10^7$</td>
<td>$1.5 \times 10^2$</td>
<td>[65]</td>
</tr>
<tr>
<td>AuNP-coated SiO$_2$ assembled on the fullerene, ferrocene and thiolated chitosan composite</td>
<td>CV</td>
<td>$3.2 \times 10^1$ to $3.2 \times 10^6$</td>
<td>15</td>
<td>[69]</td>
</tr>
<tr>
<td>Inter-digitated array microelectrode</td>
<td>EIS</td>
<td>$4.36 \times 10^5$ to $4.36 \times 10^6$</td>
<td>$10^6$</td>
<td>[70]</td>
</tr>
<tr>
<td>ITO chip</td>
<td>EIS</td>
<td>$6 \times 10^4$ to $6 \times 10^7$</td>
<td>$6 \times 10^3$</td>
<td>[64]</td>
</tr>
<tr>
<td>Boron-doped diamond</td>
<td>EIS</td>
<td>$4 \times 10^4$ to $6 \times 10^6$</td>
<td>$4 \times 10^4$</td>
<td>[71]</td>
</tr>
<tr>
<td>Hyaluronan-modified nanoporous membranes</td>
<td>EIS</td>
<td>10 to $10^5$</td>
<td>$1.0 \times 10^1$</td>
<td>[72]</td>
</tr>
<tr>
<td>Core-shell Cu@Au NP on PS-modified ITO chip</td>
<td>Anodic stripping voltammetry</td>
<td>50 to $5 \times 10^4$</td>
<td>30</td>
<td>[73]</td>
</tr>
<tr>
<td>Ferrocene-functionalised ZnO nanorods</td>
<td>DPV</td>
<td>$1.0 \times 10^2$ to $1.0 \times 10^6$</td>
<td>50</td>
<td>[74]</td>
</tr>
<tr>
<td>GO–silver NP composites/Au electrode</td>
<td>Solid-state voltammetry</td>
<td>50 to $1 \times 10^6$</td>
<td>10</td>
<td>[75]</td>
</tr>
<tr>
<td>Chitosan–MWCNT–SiO$_2$/thionine nanocomposites and AuNP layer on amine-terminated alkanethiol 11-amino-1-undecanethiol hydrochloride/Au electrode</td>
<td>CV</td>
<td>$4.1 \times 10^2$ to $4.1 \times 10^5$</td>
<td>250</td>
<td>[76]</td>
</tr>
<tr>
<td>Interaction of lectins with carbohydrate components from bacterial cell surface was used as the recognition principle</td>
<td>Surface plasmon resonance</td>
<td>$3.0 \times 10^3 - 0.0 \times 10^8$</td>
<td>$3.0 \times 10^3$</td>
<td>[77]</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td>Cystine flower self-assembled on Au electrode</td>
<td>EIS</td>
<td>$10$ to $3 \times 10^9$</td>
<td>$4.3$</td>
<td>[68]</td>
</tr>
<tr>
<td>Cystine palm leaf structure self-assembled on Au electrode</td>
<td>EIS</td>
<td>$10^3$ to $1 \times 10^9$</td>
<td>$9.64 \times 10^2$</td>
<td></td>
</tr>
<tr>
<td>CuCysNf/Au electrode</td>
<td>EIS</td>
<td>$10$ to $1 \times 10^9$</td>
<td>$10$</td>
<td>[67]</td>
</tr>
<tr>
<td>rGO-Cys/Au</td>
<td>EIS</td>
<td>$50$ to $1 \times 10^9$</td>
<td>$32$</td>
<td>[60]</td>
</tr>
<tr>
<td>rGO-CysCu/Au</td>
<td>EIS</td>
<td>$10$ to $1 \times 10^9$</td>
<td>$3.8$</td>
<td></td>
</tr>
</tbody>
</table>

AgNP: Silver nanoparticle(s)
CV: Cyclic voltammetry
DPV: Differential pulse voltammetry
MWCNT: Multi-walled carbon nanotube(s)
Nf: Nafion
NP: Nanoparticle(s)
PS: Polystyrene

Figure 5.2 NA biosensor based on cystine flowers for *Escherichia coli* detection. Reproduced with permission from C.M. Pandey, I. Tiwari and G. Sumana, *RSC Advances*, 2014, 4, 31047. ©2014, Royal Society of Chemistry [78]

Figure 5.3 Different steps of the fabrication of immunosensor-based CM. Reproduced with permission from C. Mouli Pandey, G. Sumana and I. Tiwari, *Biosensors and Bioelectronics*, 2014, 61, 328. ©2014, Elsevier [66]
Salmonellosis is one of the most lethal diseases caused mainly by *Salmonella* species (*Salmonella enteritidis* and *Salmonella typhimurium*) [79]. According to a WHO report, every year ≈100,000 deaths occur due to infection by this species. The symptoms from *Salmonella* infection include pyrexia, abdominal pain, diarrhoea, nausea, and vomiting [80]. This foodborne pathogen (*Salmonella enteritidis*) can be electrochemically detected using streptavidin-modified nanoporous silicon-based biosensors immobilised with biotinylated DNA. It was observed that the DNA probe was specific to the target DNA, and that the porous silicon-based biosensor provided more active surface area with excellent sensitivity (1 ng ml$^{-1}$) than a planar silicon-based biosensor [81].

Microsensors have been fabricated for the detection of *Salmonella typhimurium* using EIS at concentrations in the order of 500 CFU ml$^{-1}$ which is lower than the level for this infectious pathogen [82]. Dungchai and co-workers immobilised monoclonal antibodies on PS for capturing bacteria followed by addition of polyclonal antibody conjugated with AuNP to bind bacteria in the presence of a copper-enhancer solution and ascorbic acid. It was revealed that the copper released upon reduction became deposited on AuNP. Also, by anodic stripping voltammetry, direct detection of *Salmonella typhimurium* was observed, with a limit of detection of 98.9 CFU ml$^{-1}$ [83]. The detection of *Salmonella* in commercial pork samples was also reported. A reusable capacitive immunosensor involving ethylenediamine and AuNP grafted on a glassy carbon electrode (GCE) electrode were used. EIS was employed to measure the interaction between monoclonal antibodies and AuNP conjugated with *Salmonella* species. A limit of detection of $1 \times 10^2$ CFU ml$^{-1}$ was obtained [84].

MNP (TiO$_2$) were used for facile and sensitive detection of *Salmonella* in milk [85]. In this report, the bacteria in milk were captured by
antibody-conjugated MNP, which was further separated using an external magnetic field. A limit of detection of 100 CFU ml\(^{-1}\) was obtained for *Salmonella* in milk.

5.2.2 Biosensors for Mycotoxin Detection

Mycotoxins are relatively small (molecular weight \(\approx 700\)) toxic chemical products formed as secondary metabolites by some fungi (*Fusarium*, *Aspergillus* and *Penicillium*). These mycotoxins are found mostly in crops or during storage of food, including cereals, groundnuts, coffee, cocoa, spices, oil seeds, dried peas, beans and various fruits. Mycotoxins enter the human food chain via consumption of meat or other animal products (milk, cheese and eggs) as the result of livestock eating contaminated feed. These ubiquitous toxic compounds are produced under certain climatic conditions in the field and during inappropriate storage, being found mainly in agricultural commodities and animal feedstuff [86]. Contamination by mycotoxins causes serious problems to the health of humans and animals, such as liver/kidney diseases, damage to the nervous system, immunosuppression and carcinogenicity [87]. Mycotoxins are becoming part of the food chain because it has been reported that 25% of the worldwide crops are affected by toxigenic fungi. Around 300 mycotoxins are known but only a few are considered to play a significant part in food safety. Some of the important mycotoxins are: AF (B1, B2, B3 and M1), OTA (B and C), fumonisins, trichothecenes, citreoviridin, patulin, citrinin and zearalenone.

5.2.2.1 Biosensors for Aflatoxin Detection

AF are a group of fungal secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* under certain conditions. They are the most widely spread group of toxins resulting
in contamination of food products [88]. The growth of *Aspergillus* mould occurs mostly on crops, such as grains and nuts, and can be detected occasionally in milk, cheese, corn, peanuts, cottonseed, almonds, figs, spices, and various other foods. Meat, eggs, and dairy products are sometimes contaminated because of the animal consumption of AF-contaminated feed [89]. Among the 18 types of AF known, the main members are B1, B2, G1 and G2. AF are known to be potent toxic, carcinogenic, mutagenic and immunosuppressive in the order of B1 > G1 > B2 > G2 [90, 91]. Among these, AFB1 is predominant and is known as a potent human carcinogen and primarily responsible for liver cancer in animals. AFB1 occurs in the low sub-nanogram per gram range in various food products and is carcinogenic. Low ppb concentrations are usually involved, so very sensitive analytical methods are needed for AFB1 [92, 93]. Hence, thin-layer chromatography and high-performance liquid chromatography with fluorescence or mass detection are considered the official methods for AFB1 monitoring [94, 95]. Routine screening of AFB1 is commonly done by enzyme-linked immunosorbent assay (ELISA), which can process many samples in one assay. Though chromatographic techniques provide a low limit of detection, they are expensive, require many samples and skilled personnel, and are time-consuming. Moreover, these methods involve pre-concentration and clean-up procedures for the removal of matrices and interference from samples. On the other hand, ELISA is a long, laborious and tedious process with a possibility of cross-reactivity that may give rise to false-positive results for a single mycotoxin determination [96, 97]. There is, therefore, an urgent need for the development of miniaturised, multiplexing and portable devices for *in situ* analyses of several compounds simultaneously.

The development of an EC biosensor for AFB1 detection has recently aroused much interest due to its high sensitivity, fast detection, high signal-to-noise ratio, and simplicity [92, 98]. An EC immunosensor based on a screen-printed electrode was reported for AF detection in barley and milk [99]. Many nanostructured materials, including platinum (Pt), gold (Au) and nickel (Ni) were recently used for AFB1 detection. Wang and co-workers immobilised an
anti-AFB$_1$-functionalised magnetic core-shell Fe$_3$O$_4$/SiO$_2$ composite nanoparticles (NP) on a QCM surface with an external magnet for AFB$_1$ detection [100]. The detection range obtained was 0.3–7.0 ng/ml. Piermarini and co-workers demonstrated an EC immunosensor array using a 96-well screen-printed microplate for AFB$_1$ detection with a detection range of 30 pg/ml with a working range of 0.05–2 ng/ml [101]. Sharma and co-workers reported an EC sensor for AFB$_1$ detection via the immobilisation of antibody molecules onto cysteamine functionalised-AuNP. A linear detection range of 10–100 ng dl$^{-1}$, sensitivity of 0.45 μA ng$^{-1}$ dl, limit of detection of 17.90 ng dl$^{-1}$ and response time of 60 s was observed [102]. Srivastava and co-workers used a thin film of carboxylated-multi-walled carbon nanotubes (c-MWCNT) for EC detection of AFB$_1$. The thin film of c-MWCNT was deposited onto an ITO-coated glass electrode by an electrophoretic deposition method [92]. Further, the surface of the c-MWCNT/ITO electrode was functionalised with monoclonal AFB$_1$ antibodies (anti-AFB$_1$). The developed immunosensor had higher sensitivity (95.2 μA ng$^{-1}$ ml cm$^{-2}$), with a limit of detection of 0.08 ng ml$^{-1}$ and a linear detection range of 0.25–1.375 ng ml$^{-1}$. It was observed that a low value for the association constant (0.0915 ng ml$^{-1}$) resulted in the high affinity of immunoelectrodes towards AF [103]. These researchers utilised a chemically active rGO-based EC biosensing platform for AFB$_1$ detection. The observed EC-sensing results of the anti-AFB$_1$/rGO/ITO-based immunoelectrode obtained as a function of AFB$_1$ concentration showed a high sensitivity of 68 μA ng$^{-1}$ml cm$^{-2}$, limit of detection of 0.12 ng ml$^{-1}$, linear detection range of 0.125–1.0 ng mL$^{-1}$ and long-term stability (45 days) [104]. The EC property of rGO was found to be improved further with the incorporation of AuNP, as indicated by the higher CV current of a rGO-Au hybrid electrode that was almost double that compared with that of rGO alone. The resulting rGO-Au nanohybrid-based biosensor showed excellent sensitivity (182.4 μA ng$^{-1}$ml cm$^{-2}$), wide linear detection range (0.1–12 ng ml$^{-1}$) and high stability (56 days) towards the detected AFB$_1$. The sensing performance of rGO-Au composite material was found to be superior to that of MWCNT, rGO and other reported biosensors for AFB$_1$ detection (Figure 5.4) [103–105].
5.2.2.2 Biosensors for Ochratoxin Detection

OT, especially OTA (Aspergillus and Penicillium fungi), were isolated first in 1965. OT occur in a large variety of commodities, mainly in cereals [106]. In cereals, OT are produced mainly by Penicillium verrucosum whereas, on grapes, coffee, and cocoa it is formed by Aspergillus carbonarius. OTA is allowed in very small concentrations (0.5–10 μg kg\(^{-1}\)) and depends on the nature of the food. OTA has been found to be responsible for the contamination of several agricultural products, and has been detected in cereals (e.g., wheat, barley, rice and sorghum), cereal products, species, dried fruits, beer and wine [107].

OTA is the most detected mycotoxin in human blood worldwide due to its binding with plasma protein; half-life of \(\approx35\) days in serum; enterohepatic circulation; reabsorption from urine [108]. Its detection clearly demonstrates OTA exposure in the blood, urine of humans and

Figure 5.4 Fabrication of a bovine serum albumin (BSA)-anti AFB\(_1\)/rGO-Ni NP/ITO immunosensor for AFB\(_1\) detection [EDC: N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide and NHS: N-hydroxysuccinimide]. Reproduced with permission from S. Srivastava, V. Kumar, K. Arora, C. Singh, M.A. Ali, N.K. Puri and B.D. Malhotra, RSC Advances, 2016, 6, 56518. ©2016, Royal Society of Chemistry [105]
in food eaten by humans [109]. Moreover, OTA has many adverse effects in animal species: teratogenicity, immunotoxicity, genotoxicity and mutagenicity [110–112]. Several biosensors have been reported in the recent past for OTA detection in food products. Tsai and co-workers fabricated a piezoelectric immunosensor based on a self-assembled monolayer of 16-mercaptophexadecanoic acid using QCM via immobilisation of anti-OTA antibodies [113]. The detection range of the fabricated immunosensor was 50–1,000 ng/ml with a limit of detection of ≈16.1 ng/ml with negligible interference. Ngundi and co-workers reported a rapid and highly sensitive competitive immunoassay for the detection and quantification of OTA [114]. In other cereals, the limit of detection for OTA was 3.8–100 ng/g, whereas a limit of detection of 7 and 38 ng/g was observed in coffee and wine, respectively. Khan and Dhayal prepared a film of chitosan (CS) and TiO₂ NP to immobilise antibodies for OTA detection using EIS. The CS-TiO₂-based immunosensor exhibited linearity of 1–10 ng/dl with the limit of detection of 1 ng/dL [115]. Khan and co-workers fabricated an impedance-based immunosensor for OTA detection using a composite film of polyaniline (PANI) and CS to immobilise rabbit-immunoglobulin G (r-IgG) [116]. OTA interaction with IgG resulted in increased charge-transfer resistance values and showed a linear response up to 1–10 ng/ml with a sensitivity of 53 ± 8 ml/ng [116]. An impedometric biosensor was fabricated by Ansari and co-workers in which zinc oxide (ZnO) NP were used for OTA detection. The immunosensor exhibited wide linearity (0.006–0.01 nM/dm³), a limit of detection of 0.006 nM/dm³, a response time of 25 s and sensitivity of 189 Ω/nM/dm³cm⁻² [117]. It was revealed that the surface-charged CS film could be used to detect OTA via immobilisation of r-IgG and bovine serum albumin (BSA) to block non-specific binding sites. The presence of functional (NH₂/OH) groups in CS provided a favourable microenvironment for efficient immobilisation of IgG, thus leading to enhanced electron transfer to the electrode. This CS-based immunosensor showed an improved linear range (1–6 ng dL⁻¹), low limit of detection (1 ng dl⁻¹), fast response time (25 s), high sensitivity (4.8 × 10⁻⁸ A dl⁻¹), reproducibility (>10 times) and long-term stability (30 days) compared with that of the CS/ITO-based immunosensor [118].
another work, r-IgG and BSA were immobilised onto sol–gel-derived nanostructured cerium(IV) oxide (CeO$_2$) film fabricated onto an ITO substrate for OTA detection. EC studies revealed that nano-CeO$_2$ provided a favourable environment with increased electroactive surface area for r-IgG loading with the desired orientation, resulting in enhanced electron communication between r-IgG and the electrode. This BSA/r-IgG/nano-CeO$_2$/ITO immunoelectrode exhibited improved characteristics such as linear range (0.5–6 ng dl$^{-1}$), low limit of detection (0.25 ng dl$^{-1}$), fast response time (30 s) and high sensitivity (1.27 μA/ng dl$^{-1}$ cm$^{-2}$) [119]. Further, the researchers incorporated the nano-CeO$_2$ onto a CS matrix to prepare a CS-nano-CeO$_2$ nanocomposite film onto the ITO substrate for immobilisation of r-IgG and BSA. The results of EC studies indicated that the presence of nano-CeO$_2$ in the CS-nano-CeO$_2$/ITO nanobiocomposite resulted in an increased effective surface area of CS, which led to improved loading of r-IgG. The EC response of the BSA/r-IgG/CS-nano-CeO$_2$/ITO immunoelectrode obtained as a function of OTA concentration exhibited a linearity of 0.25–6.0 ng dl$^{-1}$, a limit of detection of 0.25 ng/dl, and a response time of 25 s. It was observed that the BSA/r-IgG/CS-nano-CeO$_2$/ITO immunosensor exhibited improved sensing characteristics compared with sol–gel-derived nano-CeO$_2$ and CS-based immunoelectrodes (Figure 5.5) [119, 120]. Similarly, a nano-SiO$_2$ and CS-based nanobiocomposite film was deposited onto an ITO substrate for co-immobilisation of r-IgG and BSA for OTA detection. The observed three-dimensional arrangement of nano-SiO$_2$ in the CS matrix showed excellent film-forming ability of CS. This resulted in an enhanced effective surface area of CS-nano-SiO$_2$ nanocomposite for r-IgG immobilisation and enhanced electron transport between r-IgG and the electrode. It was observed that the BSA/r-IgG/CS-nano-SiO$_2$/ITO immunoelectrode could be used for OTA detection with improved sensing characteristics, such as linearity (0.5–6 ng dl$^{-1}$), limit of detection (0.3 ng dl$^{-1}$), response time (25 s) and sensitivity (18 μA ng/dl cm$^{-2}$) [121]. Surface-charged Fe$_3$O$_4$ NP were self-assembled in a CS biopolymeric matrix to prepare nanobiocomposites for co-immobilisation of r-IgG and BSA. It was observed that nano-Fe$_3$O$_4$ resulted in an increased electroactive surface area wherein the surface charge of nano-Fe$_3$O$_4$ for affinity to
oxygen supported r-IgG immobilisation. The results of DPV studies indicated that Fe₃O₄ NP provided an increased electroactive surface area for r-IgG immobilisation and facilitated improved electron transport between IgG and electrode. The BSA/r-IgG/CS-nano-Fe₃O₄/ITO immunoelectrode exhibited improved sensing characteristics such as a low limit of detection (0.5 ng dl⁻¹), fast response time (18 s) and high sensitivity (36 μA/ng dl⁻¹ cm⁻²) with respect to other CS-based nanobiocomposite immunoelectrodes [118].

In spite of the interesting research mentioned above on the detection of food toxicants using various novel nanocomposites, efforts should be made to utilise matrices for analyses of real samples. Further, work must be made to integrate these platforms with a micro-fluidic

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**Figure 5.5** Schematic for the fabrication of a self-assembled monolayer-based impedimetric platform for OTA detection. Reproduced with permission from P.R. Solanki, A. Kaushik, T. Manaka, M.K. Pandey, M. Iwamoto, V.V. Agrawal and B.D. Malhotra, *Nanoscale*, 2010, 2, 2811. ©2010, Royal Society of Chemistry [122]
system for development of POC devices for detection of other food toxins, such as fumonisins, citreoviridin, patulin, citrinin and zearalenone [122].

5.3 Biosensors for the Defence Industries

A wide range of synthetic chemicals, toxins (plant or animal origin) and biological materials, including various disease-causing microorganisms as well as some bacterial exotoxins, have been used as warfare agents [123–125]. It has been predicted that the threat from the production of these chemical and biological warfare agents will increase significantly. These chemical and biological warfare agents not only cause disease in humans and animals, but also damage foods and plants [126, 127]. Therefore, for protection against such toxic agents, early detection and identification of these toxins is needed. Further, information about the type of microorganism or agent used during an attack before causing the disease or exposure will allow an early diagnosis of the possible disease, and may lead to its appropriate treatment [128]. Therefore, there is an urgent need for rapid and cost-effective commercial devices that can detect the microbial contamination present in food, industrial water wastes, and clinical samples. To date, only superficial research has been done regarding the development of biosensors for detection of chemical warfare agents [128].

Efforts have been made to utilise biosensor technology to ensure early detection of chemical warfare agents, especially nerve gasses [129]. The latter have the same structure as organophosphorus insecticides. It is possible to use a biosensor for detecting organophosphates (OP) and carbamate pesticides. Several potentiometric biosensors have been fabricated for detection of OP compounds, whereby the change in pH accompanying the hydrolysis of acetylcholinesterase (AchE) or butyrylcholinesterase (BuCh) has been utilised [130]. Tran-Minh and co-workers (1990) used pH electrodes coated with acetylcholinesterase and an acrylamide/methacrylamide polymer
membrane which was incubated for 1 h in the presence of paraoxon or malathion [131]. For malathion, the lower limit of detection was $10^{-1}$ M and for paraoxon it was $10^{-9}$ M. The biosensor could be used repeatedly by regenerating with 1 mM 2-pyridine aldoxime. The catalysis reaction of the acylcholinesterase-based sensors could be given as [132] Equations 5.1 and 5.2:

$$R\text{-choline} + H_2O \rightarrow \text{choline} + R\text{-COOH} \quad (5.1)$$

$$R\text{-thiocholine} + H_2O \rightarrow \text{thiocholine} + R\text{-COOH} \quad (5.2)$$

where R is usually an acetyl or butyryl moiety. OP pesticides in soil extracts were measured using butyrylcholinesterase immobilised on pre-activated nylon-polyamide membranes held in situ on the surface of a pH electrode by a nylon mesh [133]. The response to BuCh was measured before and after inhibition by OP compounds, and the limit of detection for ethyl- parathion was found to be 3.9 ppm of soil ($\approx 5 \times 10^{-7}$ M in the extract). This fabricated biosensor was inexpensive and could be prepared readily, and there was no need to reactivate or reuse the membranes after enzyme inhibition. The sensor could be disposed after each use. The only problem with this sensor was the activity, which was reduced to 65–85% of its original activity after 3–7 days in the buffer.

Researchers have reported use of amperometric sensors for OP detection based on sequential enzymatic reactions catalysed by ACh and choline oxidase. For this purpose, two enzymes were co-immobilised on a cellulose nitrate membrane. It was observed that the ACh activity was proportional to the production of hydrogen peroxide ($H_2O_2$), which was measured at a platinum (Pt) electrode [86]. A decrease in current due to inhibition of ACh activity by $\leq 10$ nM paraoxon could be determined. A similar sensor for paraoxon was fabricated by Palleschi and co-workers by immobilising choline oxidase, and the pesticide and ACh were incubated together in solution. In both cases, incubation times of 0.5–2 h were required for maximum sensitivity [87].
Apart from OP-based biosensors, there has been a report for detection of hydrogen cyanide, which is a byproduct of industrial processes and organic synthesis. An amperometric biosensor was fabricated using membrane-enclosed cytochrome oxidase (CyO) wherein oxygen was reduced through successive electron transfers from a modified Au electrode to cytochrome c and then to CyO. It was observed that, at ambient oxygen concentration, the current was proportional to the CyO activity, and inhibition of the current occurred in the presence of cyanide, H₂S or azide ions, which were bound at the metal centres of the enzyme. The sensor reached a steady response to a given concentration of inhibitor in ≈1 min, and could measure concentrations of cyanide down to 0.01 ppm in the solution phase [134]. A CyO-based cyanide sensor with improved stability was reported for a flow-injection system. In this case, a phospholipid was incorporated with CyO and cytochrome c in a carbon paste electrode covered with a dialysis membrane. The limit of detection was 0.5 μM and the response time of the sensor was <2 min [135].

Although a number of reports are available on biosensor technologies that are capable of detecting certain toxic organic materials at low concentrations, commercialisation remains a problem. The primary factor limiting marketing of a biosensor for detection of toxic chemicals is the availability of suitable biomolecules, enzymes, or receptors [136]. There is a considerable need for biosensors that could be utilised for detection of OP and cyanide at low concentrations.

### 5.4 Biosensors for Clinical Diagnostics

The diagnosis and monitoring of various diseases require intensive routine examination of blood samples and other associated tests. Appropriate times, trained manpower and sophisticated analytical techniques are required for undertaking clinical trials. Several of the detected analytes are specific for a given disease, and can be helpful to monitor its progress [137]. Further, the importance of these clinical tests is determined by their sensitivity, specificity and response time. In this context, biosensors may be a boon for
monitoring clinically important parameters such as blood glucose, urea, lactate, cholesterol and uric acid. These biosensors offer an advantage to additional laboratory analyses of relevant substances for clinical analyses [2]. In the next section, we discuss some recent examples of biosensors being used for the detection of several clinically relevant metabolites.

### 5.4.1 Biosensors for Glucose Detection

Diabetes mellitus (DM) is a leading healthcare issue in most countries. It is the most common endocrine disorder of carbohydrate metabolism, and one of the leading causes of morbidity and mortality worldwide. According to a WHO report, nearly 171 million persons worldwide were reported to have DM in 2000, and this figure is expected to increase to 366 million by 2030 [138]. It is estimated that DM prevalence among adults (20–79 years of age) would be 6.4%, and by 2030 it will increase to 7.7%, thereby affecting 439 million adults [139]. Several laboratory tests have been used for the diagnosis and management of DM, and the most useful tool for DM is monitoring of the haemoglobin (Hb)\(_\text{A1c}\) level in blood [140].

For the maintenance of normal blood glucose levels, many glucose-measuring devices have been reported. The first *ex vivo* continuous glucose monitoring system (CGMS) of blood glucose was proposed in 1974 whereas, in 1982, an *in vivo* glucose monitoring was demonstrated [141]. CGMS thus provides real-time data of an internal insulin-release system that helps in improved control of DM. Two types of CGMS are being used: (i) continuous subcutaneous glucose monitors and (ii) continuous blood glucose monitors. However, these CGMS cannot be used directly for measuring blood glucose because the electrode surface may be contaminated by proteins and other coagulation factors. Hence, a subcutaneously implantable needle-type electrode was developed by Shichiri and co-workers to monitor the blood glucose level in interstitial fluid [142]. Some of the needle-type CGMS devices approved by the US Food and Drug Administration (FDA) which are being used widely are: the
Minimed Guardian REAL-Time system by Medtronic (Minneapolis, MN, USA); SEVEN by Dexcom (San Diego, CA, USA); Freestyle Navigator by Abbott (Abbott Park, IL, USA) [141]. These fabricated devices display updated real-time glucose concentrations every 1–5 min, and the disposable sensor can be used for ≥7 days. However, the accuracy of this CGMS device is lower than that of the traditional glucose biosensors in terms of biocompatibility, calibration, long-term stability, specificity, linearity, and miniaturisation. Therefore, the clinical usefulness of CGMS has not yet been established [141].

Most POC glucose biosensors used nowadays are based on disposable, screen-printed enzyme electrode test strips that have EC cells. These strips consist of glucose dehydrogenase with a cofactor (pyrroloquinolinequinone, nicotinamide adenine dinucleotide and flavin adenine dinucleotide), or glucose oxidase along with a redox mediator [141, 143, 144]. The test strip is inserted first into a meter. Then, a small volume of capillary blood is obtained from the fingertip (using a lancing device) and placed onto the test strip. Finally, a conversion factor is used and the measurement results are displayed as plasma-glucose equivalents according to the recommendation from the International Federation of Clinical Chemistry and Laboratory Medicine. NPL Glucosense was developed at the Council of Scientific & Industrial Research-National Physical Laboratory in India. It is based on a screen-printed graphite electrode having a mediator incorporated in the working electrode (WE) (Figure 5.6). The sensors are fabricated using thin film microfabrication technology on a disposable cartridge. The biosensor is available in the Indian market for consumers.

Efforts are in progress for the development of non-invasive glucose analyses using optical (polarimetry, Raman spectroscopy, infrared absorption spectroscopy, photoacoustics, and optical coherence tomography) or transdermal approaches [145–148]. Optical glucose sensors detect changes in the physical properties of light in the anterior chamber of the eye or interstitial fluid. GlucoWatch Biographer, manufactured by Cygnus (Redwood City, CA, USA), was the first transdermal glucose sensor approved by the US FDA. This watch-like device uses the transdermal extraction of interstitial fluid
by reverse iontophoresis. However, the major issues with this device are the long warm-up time, false alarms, inaccuracy, skin irritation, and sweating. Efforts should be made for the development of reliable non-invasive glucose devices which can be used for POC diagnostics.

Figure 5.6 NPL Glucosense developed at the National Physical Laboratory

5.4.2 Biosensors for Cholesterol Detection

The determination of cholesterol level is clinically imperative because the abnormal concentration of cholesterol in the body results in hypertension, hyperthyroidism, anaemia and coronary artery diseases [149]. A correct protocol for the determination of blood cholesterol concentration is based on the inherent specificity of an enzymatic reaction. Many reports are available for cholesterol-based biosensors. Cholesterol oxidase (ChOx) has been used for
the development of cholesterol biosensors. It is a FAD-containing flavoenzyme that catalyses the dehydrogenation of C(3)-OH of the cholestan system. Utilising FAD, ChOx catalyses the oxidation and isomerisation of 3-hydroxysteroids having a \textit{trans} double-bond 5–6 in the steroid ring yielding the corresponding 4-3-ketosteroid and \( \text{H}_2\text{O}_2 \). The oxidised FAD is a primary acceptor of hydrides from alcohol. The reduced FAD then transfers the redox equivalents to oxygen as the final acceptor (Figure 5.7).

![Figure 5.7 Pathway of the ChOx enzyme reaction. Reproduced with permission from S.K. Arya, M. Datta and B.D. Malhotra, \textit{Biosensors and Bioelectronics}, 2008, 23, 1083. ©2008, Elsevier [149](#) Conducting polymers (CP) can be used as matrices for the fabrication of cholesterol biosensors. Among the various CP, the most commonly used polymers are polypyrrole (PPy) [150–155], PANI [156–158], diaminonapthalene [159] and polyvinylferrocenium [160]. Over-oxidised PPy film was used for the entrapment of ChOx within PPy film. These PPy films provided an anion-exclusion property so that interference from electroactive species was minimised [161]. These researchers used the polymer bilayer formed by an inner PPy layer and outer poly(\( \alpha \)-phenylenediamine) layer. The inner PPy layer was used for the entrapment of ChOx, whereas the outer layer was used to reduce interference and provided selectivity to the biosensors. Further, to improve the sensitivity of the biosensor, a Pt layer was deposited.
underneath [162]. Solanki and co-workers used poly(aniline-co-
pyrrole) film as a matrix in a cholesterol biosensor fabrication which
showed a sensitivity of 93.35 μA mM⁻¹ [163].

Several nanomaterials have been utilised for the detection of
cholesterol because they provide a high surface area for higher
enzyme loading and a compatible microenvironment that helps
the enzyme retain its bioactivity. In this context, various NP [164]
such as AuNP [165–167], Pt [168] and Ag [169, 170] were used for
the development of a cholesterol biosensor. AuNP were used as an
interfacial layer for the immobilisation of CHOx on an Au electrode
surface. It was proposed that AuNP provides an environment for
the increased electrocatalytic activity of ChOx and thus improves
the analytical performance of the biosensor in terms of stability
[171]. Matharu and co-workers used Langmuir–Blodgett (LB) films
of AuNP for cholesterol detection at low potential (0.3 V) without
the need for an artificial mediator [171]. The use of AuNP resulted
in an improved analytical performance of the fabricated cholesterol
biosensor. Several other researchers used nanomaterials in the form
of composite materials for developing cholesterol biosensors. For
example, CNT and NP of Au, Ag, Pt, and TiO₂ were used in the form
of nanocomposites for the development of cholesterol biosensors
[170, 172–174]. Most of the reported biosensors used CS as the
dispersing medium for CNT because CS provided biocompatibility
to the CNT matrices [175]. Similar to CNT, doping of ZnO with
metals such as Au, Ag, Cu and Pt was shown to result in the
enhanced properties of ZnO nanostructures that were beneficial for
the performance of a cholesterol biosensor [176, 177].

Microfluidic (MF) platforms have been reported for cholesterol
biosensing applications [178]. Ali and co-workers illustrated fabrication
and integration of a novel MF biochip using a MWCNT and NiO
NP. The nanocomposite was integrated with a polydimethylsiloxane
(PDMS) microchannel using a photolithographic technique, and the
surface of nanocomposite-microchannels was functionalised with
ChOx and cholesterol esterase (ChEt) (Figure 5.8). The fabricated MF
chip was utilised to measure the chronoamperometric change with
variation in concentrations of cholesterol oleate (0.25–12.93 mM).
A linear relationship was obtained between the chronoamperometric change and concentration of cholesterol oleate. The novel integrated system provided high reproducibility, selectivity and excellent sensitivity of 2.2 mA/mM/cm² [179].

Figure 5.8 (i) Schematic of a MF biochip used for detection of total cholesterol (an ordered arrangement of this microsystem is assumed); (ii) Photograph of a real MF biochip for cholesterol detection; and (iii) an enlarged view of an optical microscopic image of the MF biochip. Reproduced with permission from M.A. Ali, S. Srivastava, P.R. Solanki, V. Reddy, V.V. Agrawal, C. Kim, R. John and B.D. Malhotra, *Scientific Reports*, 2013, 3, 2661. ©2016, Macmillan Publishers Ltd [179]
The combination of several new techniques and expertise in the fields of physical chemistry, biochemistry, physics of film thickness, materials science, and electronics has revealed promise for the development of viable cholesterol biosensor [180]. Contrary to expectations expressed in market surveys that the biosensor market has not expanded as predicted, the development of accurate and low-cost commercial cholesterol biosensors and capturing of market akin to glucose will surely boost the market of biosensors for healthcare.

5.4.3 Biosensors for Cancer Detection

Cancer is a leading cause of death worldwide and its global burden continues to increase primarily because of the growth of the world population alongside increasing adoption of cancer-causing behaviours. According to GLOBOCAN estimates for 2012, 32.6 million people worldwide were living with cancer, and there were 14.1 million new cancer cases. Among these, 57% (8 million) of new cancer cases, 65% (5.3 million) of cancer deaths, and 48% (15.6 million) of cancer cases were reported in less developed countries such as India. The high mortality rates of cancer patients in developing countries is most likely a result of the delay in diagnosis and limited access to timely and standard treatment.

Many biosensors have been reported in the recent past based on DNA, antibody, cell and aptamers for cancer detection [181–183]. Sharma and co-workers fabricated a sensitive EC biosensor employing cadmium telluride (CdTe) quantum dots (QD), and its composite with CS. The QD self-assembly was sought to provide improved fundamental characteristics to the electrode interface in terms of high electron kinetics, electroactive surface area, and diffusion coefficient. This QD-modified electrode was used as a transducer surface for covalent immobilisation of a chronic myelogenous leukemia (CML)-specific probe oligonucleotide. The sensing characteristics of this biosensor showed potential for detection of the target oligonucleotide at ≤1.0 pM. Furthermore, the PCR-amplified CML-positive patient samples with various breakpoint cluster region–Abelson murine
leukemia viral oncogene homolog 1 transcript ratios could be electrochemically distinguished from healthy specimens, indicating the promising application of this QD-based biosensor for clinical investigations [181]. The same research team electrophoretically deposited a nanostructured composite of CS–CdTe–QD onto an ITO-coated glass substrate. This novel composite platform was explored for detection of CML by an immobilising amine-terminated oligonucleotide probe sequence. EC results carried out using DPV showed that this NA sensor could be used to detect ≤2.56 pM of the complementary target [184]. Further, to improve the limit of detection of the QD-based biosensor, the phase behaviour of a trin-octylphosphine oxide-capped cadmium selenide quantum dots (QCDte) monolayer and binary mixed monolayer with stearic acid (SA) were studied. Subsequently, deposition of the QCDTe–SA monolayer onto pre-hydrophobised ITO substrate was carried out using the LB technique to obtain a nanopatterned array of QCDTe. Analyses of EC results revealed that this biosensor could detect target DNA at 10^{-6} to 10^{-14} M within 120 s, had a shelf-life of 2 months and could be used about eight times [185]. Similarly, Pandey and co-workers reported controlled deposition of amino-functionalised (Am)-Si@ZnO nanoassemblies using the LB technique onto an ITO-coated glass substrate. The monolayers were deposited by transferring the spread solution of Am-Si@ZnO, in chloroform with SA at the air–water interface, at optimised pressure (16 mN/m), concentration (10 mg/ml) and temperature (23 °C). These electrodes were covalently immobilised with the amino-terminated oligonucleotide probe sequence of CML via glutaraldehyde as a crosslinker. The results of sensing studies carried out using EIS revealed that this Am-Si@ZnO LB film-based NA sensor showed a linear response to complementary DNA (10^{-6}–10^{-16} M) and a limit of detection of 1 × 10^{-16} M. This fabricated platform was validated with samples from CML-positive patients and the results demonstrated its great potential for clinical diagnostics (Figure 5.9) [186]. The different fabricated NA sensors for CML detection are summarised in Table 5.2 [181, 184–191].

Apart from the DNA sensor for the detection of cancer, various methods were proposed for detection of the oral cancer. Kumar and co-workers reported application of a silanised nanostructured
zirconia which was electrophoretically deposited onto ITO-coated glass for covalent immobilisation of CYFRA-21-1 antibodies. This biosensing platform was utilised for simple, efficient, non-invasive, and label-free detection of oral cancer. The biosensor was used for detection of the concentration of an oral-cancer biomarker in saliva samples, with high sensitivity (2.2 mA ml ng⁻¹) and stability of 6 weeks. The results of these studies were validated via ELISA [192] (Figure 5.10). In another work, the authors uniformly decorated nanostructured zirconium dioxide (ZrO₂) (average particle size, 13 nm) on rGO to avoid coagulation of ZrO₂ NP and to obtain enhanced EC performance of a ZrO₂–rGO nanocomposite-based biosensor. This immunosensor exhibited a wider linear detection range (2–22 ng ml⁻¹), excellent sensitivity (0.756 μA ml ng⁻¹) and lower detection of limit (0.122 ng ml⁻¹) [193]. Numerous reports have been published on the detection of various cancer biomarkers. However, efforts should be made to execute on-chip EC immunoassays in a single platform.

**Figure 5.9** Schematic illustration for the (A) synthesis of Am-Si@ZnO and (B) LB deposition Am-Si@ZnO on an ITO electrode and subsequent DNA sensor fabrication. Reproduced with permission from C.M. Pandey, S. Dewan, S. Chawla, B.K. Yadav, G. Sumana and B.D. Malhotra, *Analytica Chimica Acta*, 2016, 937, 29. ©2016, Elsevier [186]
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Immobilisation matrix</th>
<th>Technique used</th>
<th>Response time (s)</th>
<th>Detection limit (M)</th>
<th>Reusability</th>
<th>Shelf-life</th>
<th>Range (M)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amine-terminated DNA/GCE</td>
<td>DPV</td>
<td>300</td>
<td>$5.9 \times 10^{-8}$</td>
<td>_</td>
<td>_</td>
<td>1.25 x 10^{-7} to 6.75 x 10^{-7}</td>
<td>[194]</td>
</tr>
<tr>
<td>2</td>
<td>Hairpin DNA/GCE</td>
<td>DPV</td>
<td>_</td>
<td>$5.3 \times 10^{-9}$</td>
<td>_</td>
<td>_</td>
<td>6.2 x 10^{-8} to 3.1 x 10^{-7}</td>
<td>[195]</td>
</tr>
<tr>
<td>3</td>
<td>Amine terminated DNA/GCE</td>
<td>DPV</td>
<td>_</td>
<td>$6.7 \times 10^{-9}$</td>
<td>_</td>
<td>_</td>
<td>1.8 x 10^{-8} to 9.1 x 10^{-8}</td>
<td>[196]</td>
</tr>
<tr>
<td>4</td>
<td>pDNA/CS-CdTe/ITO</td>
<td>DPV</td>
<td>60</td>
<td>$2.56 \times 10^{-12}$</td>
<td>5–6 times</td>
<td>6 weeks</td>
<td>1 x 10^{-11} to 1 x 10^{-6}</td>
<td>[188]</td>
</tr>
<tr>
<td>5</td>
<td>QCdTe-SA-LB/ITO</td>
<td>DPV</td>
<td>120</td>
<td>$1 \times 10^{-14}$</td>
<td>8 times</td>
<td>2 months</td>
<td>1 x 10^{-14} to 1 x 10^{-8}</td>
<td>[192]</td>
</tr>
<tr>
<td>6</td>
<td>AuNP/PANI/CS-graphene sheets</td>
<td>EIS</td>
<td>_</td>
<td>$2.1 \times 10^{-12}$</td>
<td>_</td>
<td>_</td>
<td>1 x 10^{-11} to 1 x 10^{-9}</td>
<td>[198]</td>
</tr>
<tr>
<td>7</td>
<td>CdTe/(3-aminopropyl)trimethoxysilane / ITO</td>
<td>DPV</td>
<td>120</td>
<td>$1 \times 10^{-12}$</td>
<td>_</td>
<td>_</td>
<td>1 x 10^{-12} to 1 x 10^{-6}</td>
<td>[191]</td>
</tr>
<tr>
<td>8</td>
<td>Amino-functionalised ZnO@SiO_{2}/ITO</td>
<td>EIS</td>
<td>60</td>
<td>$1 \times 10^{-16}$</td>
<td>8 times</td>
<td>2 months</td>
<td>1 x 10^{-16} to 1 x 10^{-6}</td>
<td>[193]</td>
</tr>
</tbody>
</table>

pDNA: Plasmid deoxyribonucleic acid

Figure 5.10 Schematic of the fabrication of a BSA/anti-CYFRA-21-1/3-aminopropyl triethoxy silane/ZrO$_2$/ITO immunoelectrode for the detection of oral cancer (AAPS: 3-aminopropyl triethoxy silane and TEOS: tetraethoxysilane). Reproduced with permission from S. Kumar, S. Kumar, S. Tiwari, S. Srivastava, M. Srivastava, B.K. Yadav, S. Kumar, T.T. Tran, A.K. Dewan, A. Mulchandani, J.G. Sharma, S. Maji and B.D. Malhotra, Advanced Science, 2015, 2, 1500048. ©2015, John Wiley & Sons [192]
5.5 Biosensors for Environmental Monitoring

With increases in the number of potentially harmful pollutants in the environment, there is a need for fast and cost-effective analytical techniques that can be used for the detection of these contaminants. For environmental screening and monitoring, there is a pressing requirement for a rapid, portable, and cost-effective method because the conventional techniques used are expensive and slow turnaround times. Various analytical methods have been reported [194, 195].

A biosensor can be a useful tool for the assessment of biological quality or chemical monitoring of inorganic and organic pollutants as well as environmental and quality control. Compared with conventional analytical techniques, biosensors provide the possibility for portability, on-site follow-up to measure pollutants in complex matrices with simple sample preparation [196, 197]. Although the accuracy of the biosensor cannot be compared with that of conventional analytical techniques, it can provide enough information for screening and routine testing of samples. Several technical and commercial obstacles need to be addressed [198]:

- Presence of various potential pollutants with a wide range of chemical classes
- Complexity and a broad range of environmental matrices
- Wide dynamic range of pollutant concentrations
- Presence of co-contaminants with pollutants
- Lack of well-established data quality
- Lack of a sufficient market for a particular application
- Other requirements for regulatory acceptance
Most of the commercially available sensors have been used for the detection of leaks in petroleum products. However, the lower limit of detection of these products remains a challenge, and there is a need for the development of sensitive and specific immunochemical reagents that can be employed for the development of *in situ* biosensor methods. Moreover, these biosensors must undergo extensive field testing to avoid false-positive and false-negative results.

### 5.6 Conclusions

There is a broad range of applications of biosensor technology in different areas, and biosensors are likely to have significant roles in the analysis and control of biological systems. For successful fabrication of biosensors, there should be better combination of biosensing and biofabrication with synthetic-biology approaches using EC, optic or bioelectronic principles or a combination of all these protocols. Biosensors for measurement of different blood metabolites (glucose, lactate, and urea) using EC and optical transducers are being commercially developed. Some of them are being used in the laboratory, and in POC diagnostics. On the other hand, further research is needed to develop immunosensors that can compete with traditional immunoassays [199]. These immunosensors are easier to use and hold promise for POC application, especially for cancer diagnostics. Further, the development of different molecular tools (genomic and proteomic) to identify tumours and produce molecular signatures has provided new opportunities for the application of biosensors in cancer testing. The use of biosensors for cancer detection may increase assay speed and flexibility, thus enabling multi-target analyses, automation and reduced costs of diagnostic testing [200]. There is a need for cost-effective wearable, integrated and less-invasive sensors amenable to mass production to bear the rising healthcare costs together with consumer demand.
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Applications of Biosensors


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Applications of Biosensors


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Biosensors: Fundamentals and Applications


6 Challenges and Prospects

6.1 Introduction

Biosensors have been projected to play a significant role in applications in various fields: agriculture, food safety, environmental and industrial monitoring and security [1, 2]. In spite of these exciting applications and a plethora of research publications and patents, a lot must be done for the commercialisation of biosensor technologies [3, 4]. The key issue underlying such commercialisation is the slow and limited technology transfer [5, 6]. However with recent developments in analytical chemistry, there is a possibility for speedy automation, miniaturisation and integration of a system with high throughput for multiple tasks [7]. For successful fabrication of a biosensor, the system must be versatile, support interchangeable biorecognition elements, and be miniaturised to allow automation for parallel sensing with ease of operation at a competitive cost [8]. Thus, significant investment must be made in research and development for biosensor commercialisation [7, 9].

The most important part of biosensing is selection of the target analyte and interactions with the biorecognition element [10]. Traditional analytical sensing systems provide large biorecognition elements that can detect the presence of a specific analyte [11, 12]. In this context, affinity-based biosensors based on various transducer elements for detection of antibodies and deoxyribonucleic acid (DNA) have major roles [13, 14]. Further, increased efforts in this research area may lead to the introduction of labels, label-free, nanoparticles, multi-functional matrices, carbon nanotube(s) (CNT) and other methods to meet the desired sensing characteristics [15–17]. However, for the commercialisation of such biosensors,
more work is needed to improve the stability, specificity, selectivity, sensitivity, rapid detection, in vivo measurement, calibration, and ease of production [18, 19]. Different biorecognition elements used in biosensors are based on various principles and specific conditions. There is a possibility to obtain improved biosensing characteristics using labels and label-free methods, switching and displacement strategies, signal turn-on and turn-off moieties, signal processing, and enhancement/amplification [10, 20]. For the commercialisation of biosensors, the challenges noted below must be addressed.

6.2 Challenges in Biosensing

6.2.1 Preparation of Samples

Recent advances in biosensor technology and signal amplification have led to the highly sensitive detection of desired analytes. For field application, sample preparation is a major step in translating biosensors from the laboratory to the clinic [7, 21]. Preparation of the sample involves basic steps such as enrichment of the target analyte, removal of matrix inhibitors and volume reduction [22, 23]. Also, the type of biological sample, sample size, and target analyte concentration may affect sample preparation. Samples can be collected using a blood draw (serum analytes), a buccal swab (somatic cells), a lumbar puncture (cerebrospinal fluid), sputum, urine, or stool samples. These collected samples need further chemical/physical treatment before being loaded on the sensing device for the analysis. Preparation is easy for aqueous samples (blood, urine, saliva) but, for viscous solid samples (stool and sputum), additional steps such as digestion and homogenisation are necessary. Similarly, with nucleic acid-based biosensors, DNA extraction from culture samples and processing involves laborious pre-treatment steps [24]. There is, thus, a need for a device that uses a minuscule amount of the sample, and for all processes to be carried out on a single platform.
6.2.2 Separation of the Sample

The separation of the different biological components of a sample is a major step in biosensing because it may lead to a false result, especially if dealing with blood samples which contain plasma, white blood cell-rich buffy coats and red blood cells [25]. The conventional techniques used for the separation of these interferences in clinical laboratories are centrifugation and filtration [26]. Both of these methods require dedicated instruments and may result in ‘membrane clogging’ and hemolysis under high pressure. Recently, microfluidic (MF)-based alternatives have been proposed that facilitate integration with biosensors. One such MF technique relies on the Zweifach–Fung bifurcation (ZFB)-effect that rapidly separates plasma from blood samples [7, 27, 28]. MF-based alternatives for separation are under active investigation to facilitate integration with advanced biosensors [29].

In the ZFB method, blood cells travel into MF channels with a higher flow rate whereas plasma flows in the lower-flow-rate channel [30]. In one such application, researchers integrated a ZFB-based MF component with a DNA-encoded antibody array for rapid on-chip blood separation and measurement of a panel of plasma proteins using fluorescence detection with an assay time of 10 min. Other MF-based approaches have been reported that can separate plasma from whole blood but, as a result of the physical restriction of the flow rates, these techniques are limited to a small volume (≤100 ml) [30]. There is, thus, a need for MF chips that can be used for higher flow rates as well as for estimation of low concentrations of target analytes.

6.2.3 Immobilisation of Biomolecules on Suitable Matrices

The various features of the biosensor (sensitivity, specificity, selectivity, stability) rely on the biochemical recognition elements that are fixed closely to the transducer system [12]. The choice of the immobilisation process must be according to the selection of the
biorecognition element. The different immobilisation techniques such as adsorption, entrapment, crosslinking, and covalent binding have been discussed in Chapter 2. As with modification of the analyte, many different (and sometimes unusual) immobilisation techniques have been reported. This leads to a discrepancy when comparing the results obtained using different techniques. It can, therefore, be presumed that no immobilisation method can be suitable for addressing all the issues related to the immobilisation of various enzymes onto different transducer surfaces. Efforts should, therefore, be made to obtain improved methods of immobilisation. Hence, it is important that new protocols for biomolecule immobilisation are appropriately validated [31, 32]. Further, selection of the immobilisation process must ensure that the activity of the biomolecules is maintained and there is no hindrance to the active sites of bound biomolecules.

6.2.4 System Miniaturisation

For the commercialisation of a biosensor, the fabricated platforms should allow the user to add the sample, click ‘start’ and then display the results. However, it is a challenge to bring together all the biosensing components of sample preparation and analyte detection onto a single platform. Considerable efforts are required for the transfer of technologies from laboratories to the clinical market [33]. Recent developments in MF-based strategies are likely to facilitate system integration by using multi-layer soft lithography, multi-phase MF, electrowetting-on-dielectric, electrokinetics and centrifugal MF [33]. Different steps such as sample preparation, mixing, pumping and separation can be achieved using MF techniques.

Another challenge is the availability of a detection module for the integrated system. As in the case of optical detection, a bulky microscope and laser source are needed. However, it is not feasible practically in the clinical setup. Research should be more focused on the development of a portable detection system that is based on optical, electrochemical (EC) and magnetic transduction systems.
6.3 Future Prospects

The increasing demand for highly sensitive and selective biosensors requires a desired analyte to be monitored in real-time at minimal cost. There is, thus, a pressing need for the development of small, easy-to-use, smart and fast diagnostic systems. The future lies in the fabrication of ‘lab-on-a-chip’ (LOC) biosensors that can accommodate all the essential components microfabricated onto a single chip [34, 35]. This LOC can be used to simplify the biosensing process and can be a reliable monitoring tool for the detection of analytes in clinical laboratories. The combined efforts of scientists from different fields (chemistry, biology, physics, and engineering) may lead to the development of a reliable biosensing platform that can be used for different biomedical applications. Efforts are needed to improve the transduction, amplification and processing of biochemical signals. Increased efforts are required towards the development of implantable sensors (glucose, urea, cholesterol) for continuous monitoring of the target analyte. However, the major challenge in fabricating an implantable biosensor is biofouling, tissue destruction and infection around implanted sensors [36]. Given the technical feasibilities and difficulties, there is a possibility of greater success in developing hand-held biosensors than implantable devices. Some progress in biosensor techniques has been made in the recent past, and some of these biosensors necessitate further research for commercial application in point-of-care (POC) devices.

6.3.1 Paper-based Biosensors

For the fabrication of biosensors, matrices, such as gold (Au), indium-tin-oxide, and carbon electrodes are being widely used [37]. Some of the limitations in using these electrodes are high cost, hardness, brittleness, difficulty in functionalisation and disposabilty [38]. Another major problem with these conventional electrodes is the requirement of very high temperatures for their processing and the expertise to process them [39]. To overcome these limitations, efforts are being made towards the development of paper-based electrodes.
These cellulose fibre-based electrodes are lightweight, flexible, cost-effective, biocompatible and can be transported and stored readily. Mass production of these electrodes is easy and cheap and, due to their porous structures, the surface can be functionalised readily [39, 40]. To make the paper conduct, various organic and inorganic materials can be utilised. Modifying the paper with inorganic material may result in enhanced electrical performance. The major drawback in using inorganic material is the cost, processing difficulties and cracks in the film during bending/sintering. However, these limitations can be overcome by modifying the paper substrate with organic materials (CNT, conducting polymers) [41]. Kumar and co-workers used composites of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PSS) and CNT for fabrication of the conducting paper using a dip-coating method. It was observed that the conductivity of the paper increased to two orders of magnitude when it was treated with formic acid [42]. This was attributed to the removal of non-conducting PSS molecules from the surface of the conducting paper, leading to their rearrangement in the matrix. The fabricated conducting paper-based EC sensor was found to be flexible, conducting, and disposable. In another work, CNT were replaced with reduced graphene oxide, and resulted in improved EC activity and signal stability [43]. Both of these modified conducting paper-based sensors were used for the detection of cancer biomarkers, and enhanced sensitivity, lower limit of detection, and wider linear detection range were observed. Compared with the conventional electrode, it was noted that conducting paper-based EC biosensors were flexible, cost-effective, lightweight and could be easily disposed of [44].

With the initiation of MF technologies, there is a considerable scope for the development of smaller, faster, less expensive, and easier biosensing devices as an alternative to traditional analytical instrumentation. Paper-based MF technologies have garnered much interest because they use a common and inexpensive substrate material, and are capable of carrying out various measurements, and can be utilised as POC devices [45]. Martinez co-workers reported the first MF micro-paper-based analytical device (μPAD) for chemical analyses.
For the construction of μPAD hydrophobic patterning, the reagent can be used to define hydrophilic flow channels for directing the sample from an inlet to the designated location for subsequent analyses [47]. Nowadays, different techniques are being used for the fabrication of μPAD that are chemically modified further and sealed. This μPAD could perhaps be utilised for the detection of various analytes using different transducers. However, there is scope for the improvement of μPAD for clinical diagnostics.

### 6.3.2 Wearable Sensors

With the urgent need to obtain miniaturised, mobile and ubiquitous computing devices, there is an increased interest towards wearable technology [48]. It is an emerging field and is expected to create a revolution in clinical diagnostics for on-field monitoring. Wearable sensors comprise of different types of small physiological sensors, and transmission modules with processing modules. These sensors are low cost, wearable and can be used for continuous day-to-day monitoring of health, mental and activity [49]. These sensors can be in the form of ‘smart shirts’ or a thin bandage that allows continuous monitoring of blood pressure, blood glucose levels, and other biometric data [50]. The applications of these sensors are not limited to the biomedical field, but can also be integrated with other surfaces, such as buildings or vehicles [51, 52].

### 6.3.3 Biosensors for Detection of Cancer Biomarkers

Cancer can be defined as ‘abnormal and uncontrolled cell growth due to the accumulation of specific genetic and epigenetic defects that are environmental and hereditary in origin’. Unregulated cell growth leads to the formation of a tumour mass that, over time, becomes independent of normal homeostatic checks and balances [53, 54]. Tumour cells become resistant to apoptosis and other anti-growth defences within the body. During the development of
cancer, the tumour begins to spread beyond the site of origin and metastasises to other body organs and systems, at which point, the cancer is virtually incurable. Development of technologies to recognise and understand the hallmark of healthy cells and how these become cancerous can provide insights into the aetiology of cancer that can be useful for early detection, diagnosis, and treatment [55]. Previously, metastatic spread was thought to be a slow process in malignant progression, but recent work has shown that circulating tumour cells (CTC) can be found in the blood at an early stage. CTC are cells that are detached from a primary tumour and circulate in the bloodstream. They may constitute the ‘seeds’ for the subsequent growth of additional tumours (metastasis) in different tissues [56]. It has been hypothesised that these CTC may represent ‘cancer stem cells’ or be a population with high metastatic potential. The critical technical challenge for detection of CTC is to enrich and identify rare CTC in blood. Even for patients at an advanced stage of cancer, only a few CTC counts available per millilitre of blood. In recent years, significant efforts have been made into developing technologies that achieve specific and sensitive detection and capture of CTC [57].

### 6.4 Conclusions

The critical step in the fabrication of a miniaturised EC biosensor is selection of a suitable method for probe immobilisation. For example, the electrosynthesis of conducting paper allows precise control for probe immobilisation on surfaces regardless of their size and geometry [58]. Moreover, polymerisation occurs at the electrode surface, and probes can be easily entrapped in proximity to the electrode surface. Thus, the amount of the immobilised probe can be controlled readily by changing the concentration and thickness of the polymers [18]. Nanomaterials such as graphene combined with other nanoparticles (Au, platinum, palladium) can significantly facilitate biomolecule immobilisation and enhance
Challenges and Prospects

detection sensitivity [59]. The combined effect of both materials may result in the promotion of electron transfer between the active sites of the biomolecule and the detecting electrode [12].

The combination of various types of nanofabrication and chemical functionalisation, particularly at the nanoelectrode assembly interfaced with biomolecules, may result in the fabrication of advanced biosensors for the detection of DNA, protein, and enzymes [60]. Moreover, for the generation of a detectable signal, the desired analyte requires various pre-cleaning steps, redox species, the addition of excess reagent, and other chemical modifications [61].

With recent developments in fluorescent nanocrystals [quantum dots (QD)], research should be conducted towards the development of optical-based biosensors using QD [62]. QD are brighter than the molecular dyes used to ‘tag’ biomolecules, are resistant to photobleaching and can be utilised for multiplexed detection by controlling the size of fluorescent nanocrystals [63].

For the real-time monitoring and detection of infectious disease, optical-based biosensors can be powerful tools with a great future [64, 65]. Most pharmaceutical and biotechnology companies use high-end instruments in which surface plasmon resonance (SPR) arrays are equipped with autosamplers and robust acquisition of data. The recent technical achievements in SPR microarrays will compete against immunoassays, and may be helpful in the determination of various clinical parameters [66].

Commercialisation of a biosensor can be accomplished only if the biosensing platform works efficiently in a real-sample environment with high selectivity, sensitivity, and detection limits. Cross-reactivity with complex clinical samples often results in inaccuracy, low sensitivity, and a decrease in sensor performance. It is a challenge to see if using novel biorecognition elements can result in breakthroughs towards the successful commercialisation of biosensors.
References


Challenges and Prospects


Challenges and Prospects


Abbreviations

1D One-dimensional
2D Two-dimensional
3D Three-dimensional
AA Ascorbic acid
aAFB$_1$ Antibody of aflatoxin B1
AC Alternating current
AChE Acetylcholinesterase
AF Aflatoxins
AFB$_1$ Aflatoxin B1
AFP $\alpha$-Fetoprotein
AgNP Silver nanoparticles
AS (3-Aminopropyl)-trimethoxysilane
AS Amperometric sensor(s)
AuNP Gold nanoparticle(s)
BdNG 5′-Biotin end-labelled probe (20-mer) specifically targeting Opa gene of Neisseria gonorrhoeae
BSA Bovine serum albumin
$C_d$ Double-layer capacitor
C-DNA Complementary-deoxyribonucleic acid
CdS Cadmium sulfide
CdTe  Cadmium telluride
CE    Counter electrode
CEA   Carcinoembryonic antigen
CeO₂  Cerium(IV) oxide
CGMS  Continuous glucose monitoring system
ChEt  Cholesterol esterase
ChOx  Cholesterol oxidase
CM    Cysteine microstructures
CML   Chronic myelogenous leukemia
c-MWCNT Carboxylated multi-walled carbon nanotubes
CNT   Carbon nanotube(s)
CP    Conducting polymers
CS    Chitosan
CSA   Camphor sulfonic acid
CTC   Circulating tumour cells
CuCys Copper(II)-assisted hierarchical cysteine
CV    Cyclic voltammetry
CyO   Cytochrome oxidase
Cys   Cysteine
DNA   Deoxyribonucleic acid
dNG   Complementary target sequence of Neisseria gonorrhoeae
DPV   Differential pulse voltammetry
EB    Emeraldine base
EC    Electrochemical
ECH   Electroconductive hydrogels
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<td>EDC</td>
<td>N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene glycol</td>
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<tr>
<td>EIC</td>
<td>Electrochemical impedance circuit</td>
</tr>
<tr>
<td>EIS</td>
<td>Electrochemical impedance spectroscopy</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EP</td>
<td>Electrochemical polymerisation</td>
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<tr>
<td>$E_{PA}$</td>
<td>Potential of the anodic peak current</td>
</tr>
<tr>
<td>$E_{PC}$</td>
<td>Potential of the cathodic peak current</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
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<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
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<tr>
<td>FET</td>
<td>Field-effect transistors</td>
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<tr>
<td>FQ</td>
<td>Fluorescence quenching</td>
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<tr>
<td>FRET</td>
<td>Förster resonance energy transfer</td>
</tr>
<tr>
<td>GCE</td>
<td>Glassy carbon electrode</td>
</tr>
<tr>
<td>GO</td>
<td>Graphite oxide/graphene oxide</td>
</tr>
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<td>GOx</td>
<td>Glucose oxidase</td>
</tr>
<tr>
<td>HC</td>
<td>Haemorrhagic colitis</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>HUS</td>
<td>Haemolytic uraemic syndrome</td>
</tr>
<tr>
<td>ICP</td>
<td>Intrinsically conducting polymers</td>
</tr>
<tr>
<td>IEP</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>$I_{PA}$</td>
<td>Anodic peak current</td>
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<tr>
<td>$I_{PC}$</td>
<td>Cathodic peak current</td>
</tr>
<tr>
<td>ISE</td>
<td>Ion-selective electrode</td>
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ISFET | Ion-selective field-effective transistor  
---|---  
ITO | Indium-tin-oxide  
LB | Langmuir–Blodgett  
LbL | Layer-by-layer  
LDL | Low-density lipoprotein  
LE | Leucoemeraldine  
LOC | Lab-on-a-chip  
LSV | Linear sweep voltammetry  
MBA | 4-Mercaptobenzoic acid  
MF | Microfluidic  
MI | Molecular imprinting  
MIP | Molecularly imprinted polymer  
MNP | Metal nanoparticles  
MO | Molecular orbitals  
MOSFET | Metal oxide semiconductor field-effect transistor  
MR | Molecular recognition  
MWCNT | Multi-walled carbon nanotube(s)  
NA | Nucleic acid(s)  
NHS | N-hydroxysuccinimide  
NMO | Nanostructured metal oxide  
NP | Nanoparticle(s)  
N-PANI | Nanostructured polyaniline  
OP | Organophosphates  
OTA | Ochratoxin-A  
PA | Polyacetylene
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<tr>
<td>PANI</td>
<td>Polyaniline</td>
</tr>
<tr>
<td>PANI-NS</td>
<td>Polyaniline nanosphere(s)</td>
</tr>
<tr>
<td>PANI-NT</td>
<td>Polyaniline nanotube(s)</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>pDNA</td>
<td>Plasmid deoxyribonucleic acid</td>
</tr>
<tr>
<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PG</td>
<td>Pernigraniline</td>
</tr>
<tr>
<td>PL</td>
<td>Photoluminescence</td>
</tr>
<tr>
<td>POC</td>
<td>Point-of-care</td>
</tr>
<tr>
<td>PPP</td>
<td>Poly(paraphenylene)</td>
</tr>
<tr>
<td>PPy</td>
<td>Polypyrrole</td>
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<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>PSS</td>
<td>Polystyrene sulfonate</td>
</tr>
<tr>
<td>PTh</td>
<td>Polythiophene</td>
</tr>
<tr>
<td>PVS</td>
<td>Polyvinyl sulfonate</td>
</tr>
<tr>
<td>QCdTe</td>
<td>Cadmium selenide quantum dots</td>
</tr>
<tr>
<td>QCM</td>
<td>Quartz crystal microbalance</td>
</tr>
<tr>
<td>QD</td>
<td>Quantum dots</td>
</tr>
<tr>
<td>$R_{ct}$</td>
<td>Charge-transfer resistance</td>
</tr>
<tr>
<td>RE</td>
<td>Reference electrode</td>
</tr>
<tr>
<td>rGO</td>
<td>Reduced graphene oxide</td>
</tr>
<tr>
<td>r-IgG</td>
<td>Rabbit-immunoglobulin</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>Description</td>
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<tr>
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</tr>
<tr>
<td>$R_p$</td>
<td>Polarisation resistance</td>
</tr>
<tr>
<td>RP</td>
<td>Reporter probe</td>
</tr>
<tr>
<td>$R_s$</td>
<td>Solution resistance</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SA</td>
<td>Stearic acid</td>
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<tr>
<td>SAW</td>
<td>Surface acoustic wave(s)</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
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<tr>
<td>SELEX</td>
<td>Systematic evolution of ligands by exponential enrichment</td>
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<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
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<tr>
<td>SPW</td>
<td>Surface plasmon wave(s)</td>
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<tr>
<td>ssDNA</td>
<td>Single-stranded deoxyribonucleic acid</td>
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<tr>
<td>SWCNT</td>
<td>Single-walled carbon nanotube(s)</td>
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<tr>
<td>TGA</td>
<td>Thioglycolic acid</td>
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<tr>
<td>WE</td>
<td>Working electrode</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>XO$_x$</td>
<td>Xanthine oxidase</td>
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<td>ZFB</td>
<td>Zweifach–Fung bifurcation</td>
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<td>ZnO</td>
<td>Zinc oxide</td>
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<td>ZrO$_2$</td>
<td>Zirconium dioxide</td>
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<td>$Z_w$</td>
<td>Warburg impedance</td>
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<td>$\mu$PAD</td>
<td>Micro-paper-based analytical device</td>
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Biosensors have emerged recently as an interesting field of research owing to a plethora of applications in our daily lives, including food and process control, environmental monitoring, defence, and clinical diagnostics.

This update on *Biosensors: Fundamentals and Applications* focuses on the state-of-the-art of biosensor research and development for specialists and non-specialists. It introduces the fundamentals of the subject with relevant characteristics of transducer elements, as well as biochemical recognition molecules.

Different techniques for biomolecule immobilisation, the interaction of biomolecules with the sensor surface, and the interfacial properties are treated comprehensively with respect to their impact on the performance of a biosensor.

This book presents the current trends and developments in different nanomaterials and polymers involved in biosensor fabrication. The relative advantages and challenges of biosensor fabrication, along with the emerging paradigms and techniques that facilitate molecular-level detection in nanosensor devices, are discussed in depth.