Biosensors Components and Performance Factors

2.0   BIOSENSOR

Biosensor is a device used for the detection of an analyte, which combines a biological component with a physicochemical detector component (Fig. 2.1). Biosensors are incorporated with a biological material (*Eg.* tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids etc.) or a biological derived material associated with or within a physiochemical transducer or transducer micro-system, which may be optical, electrochemical, thermometric, piezoelectric or magnetic transducer.
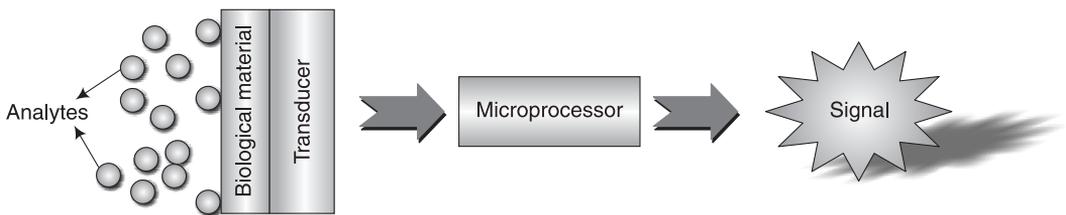

Fig. 2.1   *General layout of a biosensor.*

Biosensor functions by coupling a biological sensing element with a transducer usually yield a digital electronic signal which is proportional to the concentration of a specific analyte or group of analytes.

2.0.1   Principle

Biosensors consist of (i) a biological component for sensing the presence and concentration of a substance and (ii) a transducer device, which works according to the principle shown in Fig. 2.2. The sample is allowed to pass through a membrane so that selection may be exercised and the interfering molecules are retained outside the membrane. The sample then interacts with the biological sensor and forms a product, which may be an electric current/charge, heat, gas or a suitable chemical. The product then passes through another membrane and reaches the transducer,

The required components for a biosensor are bioreceptor molecules, biological material (biocatalyst), transducer, amplifier, processor, recorder and display device (Fig. 2.4).
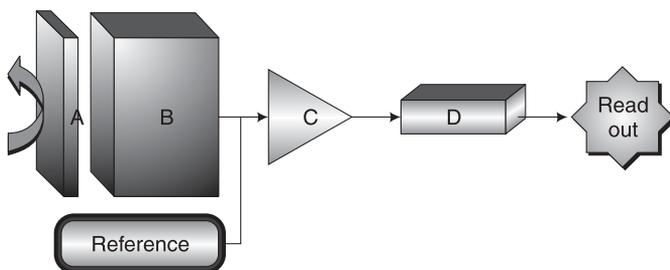

Fig. 2.4   *The main components of a biosensor. (A) biocatalyst (B) transducer (C) amplifier (D) processer.*

2.1.1   Bioreceptor Molecules

A receptor is used to find a target. In biosensors, the target is an analyte and the receptor is some substance which is stuck to the surface and binds to the target to allow it to be detected. Any biomolecule or molecular assembly that has the capability of recognizing a target substrate (i.e. an analyte) can be used as a bio-receptor.

Enzymes have been the most widely used bio-receptor molecules. Recently, antibodies and proteins are also used as bio-receptor molecules in biosensors. The specificity of a biosensor comes from the specificity of the bioreceptor molecule used. An enzyme is a good example. It has a three-dimensional structure that fits only a particular substrate. Antibodies represent one of the major classes of protein; they constitute about 20% of the total plasma protein and are collectively called immunoglobulins (Ig). The simplest antibodies are usually described as Y-shaped molecules with two identical binding sites for antigen. An antigen can be almost any macromolecule that is capable of inducing an immune response. The antibody has a basic structural unit consisting of four polypeptide chains - two light chains and two heavy chains. The antibody binds reversibly with a specific antigen. Unlike the enzymes and proteins, the antibodies do not act as catalysts. Their purpose is to bind foreign substances and antigens. Protein molecules show specific affinity for hormones, antibodies, enzymes and other biologically active compounds. These proteins are mostly bound to a membrane. Some examples are olfactory receptors for smelling, photoreceptors for eyes, etc.

2.1.2   Biological Elements

A biological element is a material related to biomolecules. The biological material may be a protein such as an enzyme, antibody; a nucleic acid (DNA or RNA), antibody fragment, a whole microbial cell, even a plant or an animal tissue and microbial products.

2.1.3   Transducer or Optode

A biosensor is a sensing device that consists of a biological component coupled to a transducer that converts biochemical activity into the most commonly electrical signal. The physico-chemical

transducer can be electrochemical (e.g., pH, polarographic, potentiometric or conductometric probes), thermal (*Eg.* thermistors), optical (fibre optic) or piezoelectric crystals.

Transducer is a device that converts energy from one form to another form, for example, telephone companies use transducers to convert sound energy into electrical energy to be carried by long distance through telephone lines and then another transducer at the receiving end to convert the electrical energy back into sound. In biosensors transducers convert the biochemical activity into electrical energy. The transducer converts a biorecognition event into a measurable signal that correlates with the quantity or presence of the chemical or biological target. The key part of a biosensor is the transducer (Fig. 2.5), which makes the use of a physical change accompanying the reaction.
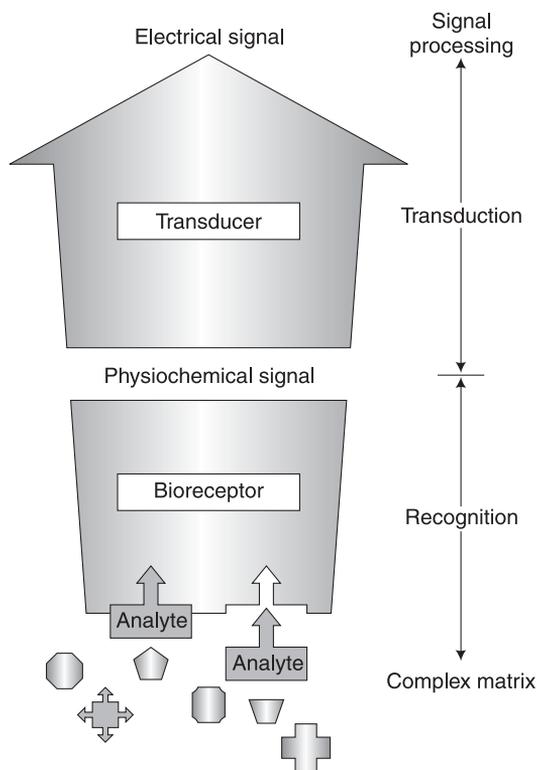

Fig. 2.5   *Transducer signaling process in biosensors.*

Depending on the analyte and bioreceptor, the transducer of a biosensor could utilize one of several mechanisms.

The transducer could rely on amperometric technology, which involves that detects changes in current. They measure currents generated when electrons are exchanged between a biological system and an electrode. Alternately, it may use a potentiometric mechanism which involves reactions that cause a change in voltage (i.e., potential at constant current) between electrodes and this change can be detected or measured.

A transducer that relies on resistive or conductive mechanisms will detect changes in conductivity between two electrodes. It can also utilize a piezoelectric mechanism. In a piezoelectric material, there is a coupling between its mechanical and electrical properties. The coupling can be used to create an electrical oscillator, the frequency of which can be varied and measured by varying a mass applied to its surface. In the case of a biosensor, that mass can change due to the reaction taking place on the surface. Thermal transducers measure changes in temperature. Optical transducers can measure light output during the reaction or a light absorbance difference between the reactants and products.

2.2 EVOLUTION

Biosensors can be classified into three generations according to the degree of integration of the separate components, i.e. the method of attachment of the biorecognition molecule or bioreceptor to the transducer. In the first generation, the bioreceptor is retained in the vicinity of the base sensor behind a dialysis membrane, is achieved by immobilization process (via cross-linking method) at a transducer interface or by incorporation into a polymer matrix at the transduction surface. In the second generation, the individual components remain essentially distinct (*Eg.* control electronics-electrode-biomolecule), while in the third generation the bioreceptor molecule becomes an integral part of the base sensing element (Fig. 2.6).
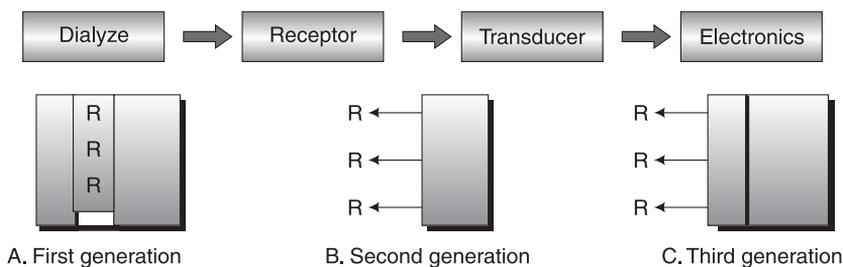

Fig. 2.6 *Three generations of biosensor.*

2.2.1 First Generation

In first generation biosensors (Fig. 2.6A), the normal product of the reaction diffuses into the transducer and causes the electrical response. Example for first generation biosensors are glucose biosensors, which were based on measuring the hydrogen peroxide concentration once it has diffused to an electrode. The electrode acts as the transducer where electron flow would produce a measurable current and was usually made out of either metal or carbon. This approach however had several problems. Firstly, the monitoring of hydrogen peroxide in this way requires a high operating potential. At this potential other unwanted electro-active substances that may be present in biological environments such as uric and other acids can react causing interference. Secondly oxygen has a limited solubility in many biological fluids which in turn limits the enzymatic reaction and thus the response of the biosensor.

Enhanced versions of the first-generation biosensor (Fig. 2.6A) have been developed which counteract these effects. One such design uses carbon electrodes coated in a graphite paste into

immobilising it directly onto an oxidised boron-doped diamond electrode. Carboxyl groups on the surface of the electrode covalently link with the glucose oxidase.
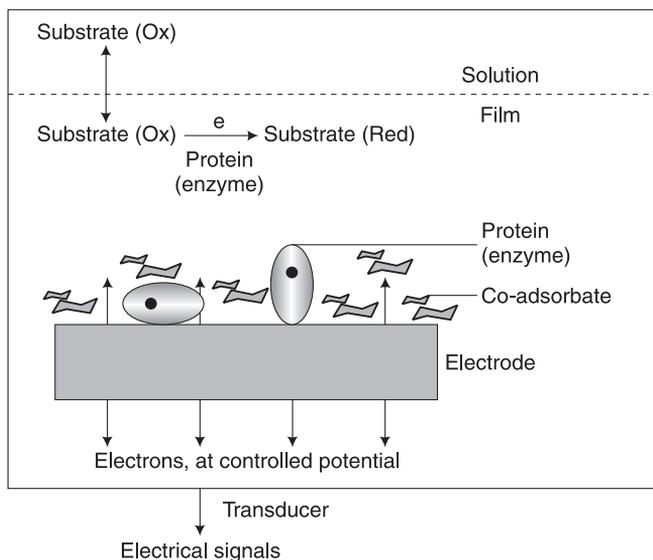

Fig. 2.7   *Biosensor electrode based on Protein-Film Voltammetry (PFV). (Source: http://www.jstage.jst.go.jp/ article/analsci/20/4/603/_pdf)*

2.3   LIMITATIONS

1. The first is the instability of the biological sensing component (enzyme, antibody, tissue, etc.), which can lose its activity in hours or days depending on the nature of the molecule and exposure to environmental stresses, such as pH, temperature or ionic strength.
2. The second limitation is on the size of the physico-chemical transducers being used in biosensors.

2.4   ADVANTAGES

1. They can measure nonpolar molecules that do not respond to most measurement devices.
2. They allow rapid continuous control.
3. Speed of response (typically less than a minute) and ease of use is the main advantages offered by biosensors.
4. Typically the smaller the device, the faster and more sensitive is the response.
5. Biosensors can easily detect analytes in the micromolar to nanomolar range.
6. Biosensors can serve exceptionally well in emergency.
7. Situations or for on-site field applications.

2.5   DISADVANTAGES

1. Heat sterilization is not possible as this would denature the biological part of the biosensor.
2. The membrane that separates the reactor media from the immobilized cells of the sensor can become fouled by deposits.
3. The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane.
4. Changes in the reactor broth (i.e., pH) can put chemical and mechanical stress on the biosensor that might eventually impair it.

2.6   BENEFICIAL FEATURES

A successful biosensor must possess the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses be stable under normal storage conditions except in the case of calorimetric enzyme strips and show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as independent of physical parameters as like stirring, pH and temperature. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should preferably be co-immobilized with the enzyme.
3. The response should be accurate, precise, reproducible and linear over the useful analytical range without dilution or concentration. It should also be free from electrical noise.
4. If the biosensor is to be used for invasive monitoring in clinical situations the probe must be tiny and biocompatible having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case the biosensor should not be prone to fouling or proteolysis.
5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
6. The biosensors have been considered to be superior and more sensitive, in comparison to physical instruments due to the following reasons:
   a. In a biosensor the immobilized biological material is present in intimate contact of a suitable transducer so that the biochemical signal is quickly converted into an electrical signal.
   b. The immobilization of biomolecules permits reuse of these molecules (which are expensive) and allows simplification of the entire apparatus.
   c. The biological sensing element is present in a small area and is very sensitive, thus facilitating analysis of substances in small quantities.
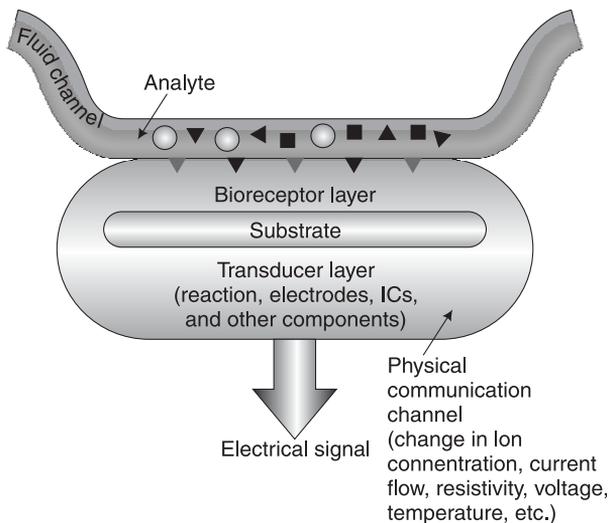   d. Biosensors may be developed according to specific needs and can be highly specific or show broad spectrum.

Fig. 2.8   *Representation of a generic biosensor.*

For fast detection with a readable electrical output to develop biosensors that combine various processes, such as pathogen cell lysis, debris removal, DNA cleanup, polymerase chain reaction (PCR) and fluorescence detection. To combine these and similar processes with semiconductor devices to create compact portable units for field must be used. The surface plasmon resonance (SPR) is used to detect biological molecules such as protein and DNA. In addition living cells that can react functionally to the presence of both biological and chemical threat agents are being placed on chips. Liquid-crystal detectors can be used for identification of pathogens.

Depending on the analyte, bioreceptor and transducer portion of a biosensor could utilize one of several mechanisms. The transducer could rely on amperometric technology, which involves devices that detect changes in current. They measure currents generated when electrons are exchanged between a biological system and an electrode (Fig. 2.8). Alternately, it may use a potentiometric mechanism which involves reactions that cause a change in voltage (i.e., potential at constant current) between electrodes and this change can be detected or measured.

A transducer that relies on resistive or conductive mechanisms will detect changes in conductivity or resistivity between two electrodes. A capacitive transducer relies on a biorecognition reaction, which causes a change in the dielectric constant of the medium in the vicinity of the bioreceptor. Such a capacitance measurement method can be used as a transducer.

A transducer can also utilize a piezoelectric mechanism. In a piezoelectric material there is a coupling between its mechanical and electrical properties. The coupling can be used to create an electrical oscillator, the frequency of which can be varied and measured by varying a mass applied to its surface. In the case of a biosensor the mass can change due to the reaction taking place on the surface. Thermal transducers measure changes in temperature.

Biosensors with optical mechanisms correlate changes in concentration, mass, or number of molecules to direct changes in the characteristics of light. One of the reactants or products of the

biorecognition reaction has to be linked to calorimetric, fluorescent, or luminescent indicators. An optical fibre might perform this linkage by guiding light signals from the source to the detector.

2.9.1 Device Characterization

Biosensor development programs generally aim to overcome the design limitations in current biosensor systems. For example, one of the challenges involved in biosensor design is achieving a stable, reproducible interface between the biological affinity elements and an inorganic transducer element in the sensor.

The desire to miniaturize biosensors for handheld portability, still achieve adequate sensitivity, imposes significant technical challenges in the coupling of biomolecules to transducer surfaces. Therefore, fast and accurate electrical characterization of biosensor devices and mechanisms in the development lab is essential for qualifying new designs. Because of the complexity in extracting cell and tissue signatures of agent activity and response, it is often desirable to conduct direct current-voltage (I-V) characterization on key components of the biosensor. I-V characterization requires only a small fraction of the time needed for most types of functional testing, but is a powerful predictor of full fledged operation. For example, I-V data can be used to study anomalies, locate maximum or minimum curve slopes, and perform reliable analyses. Depending on design specifications, I-V characterization is often suitable for sensors based on amperometric, potentiometric, conductive, resistive and thermal principles. Usually I-V testing applies a voltage or current to the device under test (DUT) and measures its response to that stimulus (Fig. 2.9). The test procedures may involve probing of integrated circuits to apply the stimulus to certain connection pads and measure the response on others.
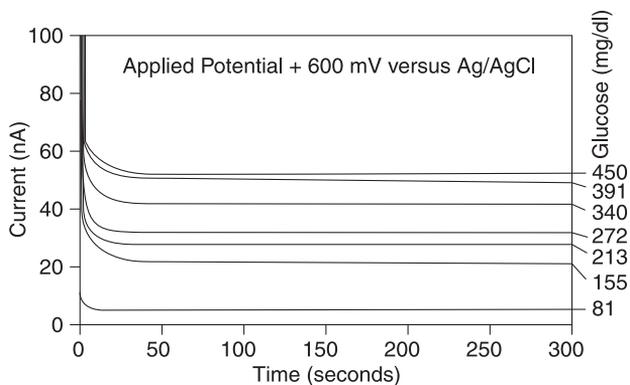
Fig. 2.9 *Electrical characteristics of a prototype electrochemical transdermal sensor based on a glucose oxidase reaction with blood plasma from capillaries.*

Depending on the Device Under Test (DUT) signal the glucose oxidase levels may be estimated. In this case, highly sensitive source, measurement instruments and test techniques that minimize external sources of error are necessary. When an optical mechanism is involved, I-V characterization may also involve simultaneous measurements of the wavelength or intensity of a light output with a photodetector. This is called L-I-V testing.

2.9.2   Device Performance

The biosensors used by medical practitioners, military personnel and public safety forces will be part of a portable system. This places restrictions on the operational power requirements of the sensors and may dictate the level of voltage or current output that can be provided to the measurement circuitry. In battery operated systems sensor output current can range from nanoamps to milliamps and voltage from nanovolts to volts. Different measurement techniques and tools are required for signal levels at the opposite ends of such wide ranges.

2.9.3   Voltage

Characterizing biosensor devices at voltage levels greater than 100 µV should be relatively easy. A sampling data acquisition system based on a PC plug in board may provide adequate resolution as would many programmable digital multimeters (DMMs) and self contained data loggers. For example, most laboratory grade DMMs provide enough range and resolution to make voltage measurements from 1 µV to 1000 V. For the PC based data-acquisition board solution measure over a variety of voltage levels depending on the resolution of the analog-to-digital (A/D) converter and its gain (Table 2.2).

Table 2.2   Voltage measurement resolution and maximum ranges for different A/D converter resolutions. *(Source: Data Acquisition and Control Handbook.)*

Resolution (volts input)	8^a 256^b	10^a 1024^b	12^a 4096^b	16^a $65{,}536^b$
0-10	39.06 mV	9.765 mV	2.441 mV	152.59 µV
0-5	19.53 mV	4.883 mV	1.221 mV	76.29 µV
±10	78.12 mV	19.530 mV	4.883 mV	305.20 mV
±5	39.06 mV	9.765 mV	2.441 mV	152.59 µV
±2.5	19.53 mV	4.883 mV	1.221 mV	76.29 µV
±1.25	9.76 mV	2.441 mV	610.350 µV	38.15 µV
Maximum input, 1-10[c]	9.960 V	9.990 V	9.9976 V	9.99985 V

[a]= Converter bits (n); [b]=Output states (2^n); [c]= Maximum input=resolution ¥ 2^n-1.

For measurements below 1 µV a nano-voltmeter should be considered instead of a data acquisition board or DMM solution. For example, the model 2182 by Keithley Instruments Inc. (Cleveland) is a low-noise digital nano-voltmeter with an A/D resolution in the 24-bit range. This type of instrument is optimized for accurate low level voltage measurements, even when the signal is approaching the theoretical (lower) limit associated with sensors that have low output impedance. Sensors with low output impedance require a voltmeter with a high input impedance to avoid measurement errors. Although the input impedance of a nano-voltmeter is similar to a DMM, it has much lower voltage noise and drift. This gives it much better voltage sensitivity, and it may be able to read down to 1 nV.

2.9.4   Noise

Noise is often a problem for tools used to measure voltage. Significant errors can be generated by noise that originates in the sensor, the measuring instrument, and sources external to the test circuit. External sources include electromagnetic fields (EMFs), measuring circuit ground loops

and thermal EMFs. Johnson noise is another form of thermal noise that occurs in every electrical component. Johnson noise establishes the ultimate limitation of the measurable signal level. For accurate measurements, the noise sources should be minimized as much as possible.

In some test environments, electrical noise is difficult to avoid. When that happens the measurement compensation techniques are needed. These are included in most bench top DMMs and nanovoltmeters to minimize electrical noise from a.c. lines and from random noise sources. These techniques are less likely to be available in PC-based data-acquisition systems but knowledgeable users can program a system for signal averaging to help reduce random noise and use longer A/D integration periods to minimize a.c. line noise.

2.9.5 Current Measurements

Different types of amperometric devices require different characterization approaches. Electrical currents can be measured with data-acquisition systems but the method of acquisition selected will depend on the current level and number of required measurement channels. Otherwise, I-V characterization of a device using a current loop is uncomplicated as long as the current source output voltage is high enough to overcome any test lead resistance. The corollary is that current loops are ideal when there is an appreciable distance between the signal source and the instrumentation (Fig. 2.10).

It may be desirable to qualify the output of a biosensor assembly in terms of engineering units such as concentration of the target analyte in milligrams per deciliter. In the case of an amperometric output the current is an indicator of the phenomenon actually being measured. Therefore it is desirable to have instrumentation that makes it easy to do the conversion. This requires that the instrument be able to perform internal calculation and scaling features that convert current readings to appropriate engineering units. For example, a transducer utilizing a 4–20 mA current loop might be calibrated for a concentration of zero mg/dl at 4 mA and a full scale concentration of 100 mg/dl at 20 mA.
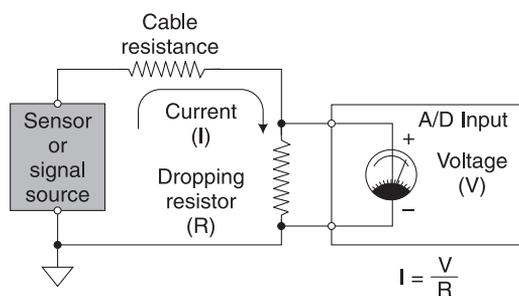

Fig. 2.10   *Current measurement using a dropping resistor and voltmeter.*

In the early stages of product development, it may not be necessary to do this conversion when the significance of the transducers output current level is already understood. In this case, the current reading is the parameter of interest and a voltage stimulus may serve as a proxy for the biological event during I-V characterization. Under these circumstances the current signal can be relatively high but may require care in selecting a dropping resistor for the measurement. The

current passes though the dropping resistor at the input stage of a data acquisition system and voltage is measured across the dropping resistor to determine the current level. This type of scaling is a common feature of data-acquisition systems.

However unlike voltage measurements current measurements may be subjected to "voltage burden" errors. Voltage burden is defined as the voltage drop across the input of an ammeter when it is inserted into a circuit. The dropping resistor (R) and A/D voltage input constitute an ammeter and the current flow can be calculated from the voltage drop across the resistor. Noise effects, similar to those for voltage measurements, must also be considered and are exacerbated by voltage burden.

The resistance value will normally be selected to provide a voltage drop corresponding to the full input range of the A/D board when the maximum anticipated current flows. For example, a 20-mA current produces a 10-V drop across a 500-ž resistor. Note that the sensor (i.e., current source) must be capable of developing a minimum output potential of around 10–11 V in order to achieve the full voltage drop across the resistor. This may not be a problem in the lab, but if a portable biosensor circuit is powered by only 6 V, it cannot drive more than 12 mA through the resistor. Furthermore, the resistor must have an adequate power rating (I2R) for the current and resistance values.

Issues such as these can be avoided by performing current measurements with bench top instruments that use either a small dropping resistance or a feedback ammeter circuit. In the former case, highly sensitive voltage measurements allow a small dropping resistance value. A feedback ammeter avoids this problem because its input circuitry consists of an operational amplifier, which has very low input resistance and a voltage burden that typically ranges from about 10 μV to 1 mV. This type of current-measurement circuit is typically used in a complete biosensor instrument.

DMMs typically use dropping-resistor ammeter circuits, whereas pico-ammeters and electrometers use feedback ammeter circuits. In either case, making connections to the signal source is straightforward, and they are suitable for measuring currents up to several amperes. At the other end of the scale, DMMs can measure currents down to about 10 nA, and picoammeters can measure currents as low as 10 fA. Another advantage of these instruments is their built-in signal-conditioning circuitry. Some DMMs can provide voltage, current, resistance, and temperature (thermocouple or thermistor) measurements up to 200 channels.

2.9.6   Source-Measure Instruments

In I-V characterization, the integration of a DC source and measuring instrument can be problematic because of intricate triggering issues. Such issues can often be avoided by using a tightly integrated source-measure unit. These high-precision instruments can act as either a voltage or current source with sweep, pulse, and compliance-limit capabilities, and simultaneously measure I and V parameters. Typical resolutions are in the range of micro volts and picoamperes.

The bipolar voltage and current sources of these instruments are controlled by a microprocessor, which makes I-V characterization much more efficient and simplifies instrumentation setup. Many different test sequences can be stored in its program memory and executed with a simple trigger signal. Test data can be stored in a buffer memory until an I-V sweep is completed and then downloaded to a PC for processing and analysis.

2.9.7   Cabling, Conductors and Capacitance

The connections between the instrumentation and the DUT are important parts of a measurement system. Understanding and managing the limitations of these connections is crucial for accurate measurements. Noise sources, cable length, and cable capacitance can affect the quality of any measurement, but the lower the signal level the more important these issues become. To minimize problems, the measurement circuit, its cables, and its connectors should be matched to the test signals. In addition, cables and test leads should be carefully routed and mounted.

1. Cabling

When evaluating a cable for the measurement application, several issues should be considered. The electrical noise present in the test environment should be quantified. Noise can be defined as any undesirable signal that is impressed upon a signal of interest.

Sources of electromagnetic noise include a.c. power lines, motors and generators, transformers, fluorescent lights, cathode-ray tube displays, computers, radio transmitters, etc. Depending on the nature of the signal and the noise, it may not be possible to separate them once the signal has been acquired at the instrumentation input terminals.

To the extent possible, test leads and cables should be routed to minimize their exposure to noise. The leads should then be mounted rigidly in place so that they cannot move and cause the generation of spurious voltage in the presence of electromagnetic fields.

In addition, the distance between the signal source and measurement system terminals should be measured. Wire has an electrical resistance that is dependent on its composition, length, and diameter. Resistance increases with increasing length and decreasing wire diameter. This resistance is a component of the total cable effects that become part of the analog input of a measurement circuit (Fig. 2.11). High cable resistance in conjunction with low A/D input resistance can result in a significant voltage drop through the interconnect wiring, resulting in measurement errors.

Lastly, it should be determined whether the data-acquisition channel has a single-ended or differential input. Single-ended signals (i.e., those referenced to ground) can be transmitted with two wires or with a shielded cable where the shield is tied to ground. For differential signals, at least two wires are needed to transmit the signal, which consists of a signal high and a signal low, neither of which is referenced to ground. Two individual conductors are sufficient to transmit the signal, but a twisted pair or shielded twisted pair provides greater noise immunity.

2. Conductors

The conductors used in shielded or unshielded cable can be made of solid or stranded wire. Solid wire results in minimum signal attenuation, but stranded conductors provide more flexibility and may be easier to route and mount. Conductors may consist of bare copper that is either plated with silver or tinned with solder. Connector and conductor materials should match to minimize resistance and thermally generated EMFs.

For the highest signal integrity, cables with shielded conductors should be used. Shielding reduces electromagnetic noise picked up by signal leads. It is also helpful in reducing electromagnetic radiation from conductors carrying high frequency signals. Shielding is constructed with different

types of wire braid or a combination of wire braid and foil. Multilayer or multibraid shields are more effective than a single layer in attenuating signal pickup and radiation. However, this tends to make cables more stiff and difficult to route and mount.

Several points should be considered when selecting shielded cable. For instance, higher-frequency noise is difficult to attenuate and requires more-elaborate shielding.

Simple spiral wire-wrap foil is the least effective type of shielding. Tight braiding, double braiding, or braiding plus foil offer more effective shielding. Lastly, caustic atmospheres, moisture, etc., can reduce the effectiveness of shielding. In some cases, these contaminants can leach into a cable and degrade the shielding far beneath the outer insulating jacket. Testing in such environments should be avoided, if possible.

3. Capacitance

For many DUTs, the output signal can be modeled as a voltage source in series with a resistance. Similarly, an analog instrument input can be modeled as a meter in parallel with an input resistance. During a measurement, the instrument input absorbs a small bias current that the source must be able to supply. The interconnecting cable is an essential part of this circuit and can introduce resistance, capacitance, and inductive effects that depend on length, gauge, composition, routing, and the physical environment.
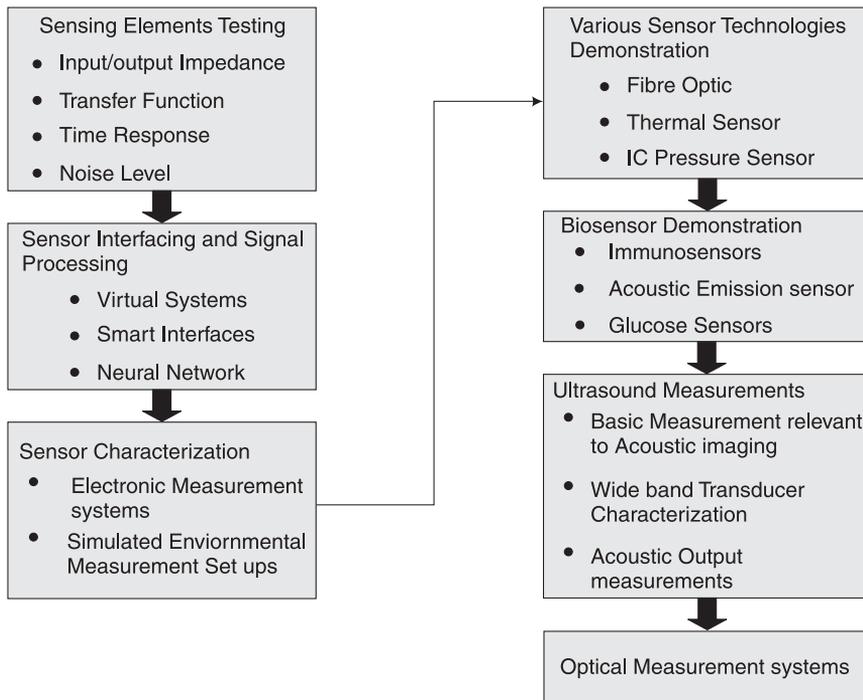

Fig. 2.11 *Testing process of biosensors.*

2.10.2   Outer Membrane

The outer membrane has to be compatible with the medium into which it will be placed. Therefore, the requirement will be different depending on the nature of the measurement medium. For example, the outer membrane for biosensor used in liquid samples should be different from that intended for implantation application. For the latter application, bio-compatibility becomes an issue (the rejection of the sensor by body may occur).

The outer membrane should offer low diffusional resistance to analytes while the resistance should be high for macromolecules.

For long term continuous use applications, the fouling of the membrane must be minimal. The fouling causes an increase in the diffusional resistance of the analyte and thus the signal of the sensor changes as the fouling progresses. If microorganism grows on the surface of the outer membrane, the passage of oxygen to the enzyme layer is hindered which makes the sensor to behave erroneously.

Table 2.3   Comparison of four enzyme immobilization methods.

	Adsorption	Entrapment	Covalent coupling	Crosslinking
Matrix material	ion exchange resins, active - charcoal, silica - gel, clay, aluminum oxide, porous glass.	alginate, carageenan, collagen, polyacrylamide, gelatine, silicon-rubber, polyurethens	agarose, cellulose, PVC, ion-exchange resins, porous glass	Cross-linking agents: glutaraldehyde, bisisocyanate,
Nature of bonding	reversible; changes in pH, ionic strength may detach the enzyme	physical entrapment	chemical bonding	entrapment; functionally inert proteins are often used together (BSA, gelatin)
Enzyme loading	low	low	high	high
Enzyme leakage	some	some	very low	low
Loss of activity	negligible	negligible	significant	small
Cost	inexpensive	inexpensive	expensive	inexpensive

2.10.3   Optimization of Biosensor

Variations of the diffusion resistance of the semipermeable membrane are being used to optimize the sensor performance and semipermeable membrane influences the response time and sensitivity. In contrast, thicker membranes such as polyurethane or charged material, significantly increase the measuring time, but may also lead to an extension of the linear measuring range. Table 2.4 lists some of the commercially available membranes that can be used as the outer membrane.

Table 2.4 Available pre-cast membranes.

	Collagen	Polycarbonate (Nucleopore)	Cellulose acetate
Characteristics Derivatizability	A hydroxylic natural protein easy	Uniform pore size	Slightly negative due to $-COO^-$ Easy
Temperature stability	O.K. at room T unstable at 37^oC	Stable	Stable
Selectivity	Exclude protein	Exclude protein	Exclude protein, retard transport of anionic species
Source	Sigma	Nucleopore	Amicon

2.10.4   Inner Membrane

The inner membrane should be permeable and selective to target species (for example H_2O_2 only for the current glucose sensor). Also, it should be as thin as possible and stable for long-term use. Some of the solution castable membranes and their characteristics are compared in Table 2.5.

Table 2.5 Solution castable membranes.

	Cellulose acetate	Nafion	Polyurethane
Characteristics	–	Perfluorosulphonic acid ionomer, Negatively charged	Widely varying MW dep. on source
Solvent	Acetone, Cyclohexanone	Low MW alcohols	Dissolves in 98% tetrahydrofuran, 2%Dimethylform-amide
Coating method	Dip coating, in-situ formation	Dip coating as thin as 1000A (with 5% solution)	Dip coating
Other characteristics	–	Tend to adsorb proteins & Cations, Not useful as an outer membrane	Biocompatible, retard glucose access to enzyme layer, passes O_2 well

2.10.5   Effect of Enzyme Loading

2.10.5.1   Internal diffusion

Usually in the operation of biosensors, the flow conditions are adjusted to provide a mass transfer rate from the solution to the membrane system faster than that of in the enzyme layer (the internal mass transfer). In the immobilized enzyme layer, reaction and diffusion occur simultaneously. Therefore, rigorous modeling is required to fully characterize the behavior of a biosensor. The key question in designing a biosensor is: (1) how thick should the enzyme layer be? and (2) how much enzyme has to be placed in the layer? Although rigorous modeling is required to fully characterize the behavior of biosensors, the design can be carried out by considering limiting cases.
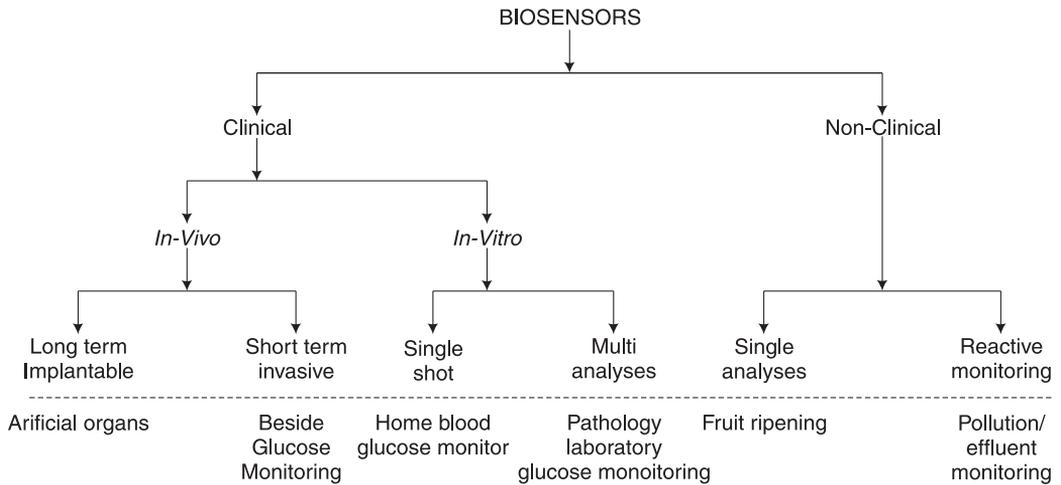
Fig. 2.12 *Potential applications of biosensors.*

Modern smart sensors, based on field-effect transistors (FETs), may combine several measurements in one sensor unit. This particularly applies in the case of ion sensors for sodium, potassium, calcium and pH. Attempts are also being made to make combination biosensors, e.g. for glucose, lactate and urea. Table 2.7 shows a list of common assays that are routinely needed for diagnostic work with patients.

Table 2.7 Common assays that is required in diagnostic medicine.

S.No.	Analyte	Method of Assay
1	Glucose, Lactate, Cholesterol	Amperometric biosensor
2	Urea, Penicillins	Ptoentiometric biosensor
3	Hepatitis B	Chemiluminescent immunoassay
4	Sodium, pH	Glass ion selective electrode

A potential dream application is to have an implanted sensor for continuous monitoring of a metabolite. This might then be linked via a microprocessor to a controlled drug-delivery system (e.g. an iontophoretic system) through the skin. Such a device would be particularly attractive for chronic conditions such as diabetes.

The blood glucose sensor would be monitored continuously and, as the glucose level reached a certain value, insulin would be released into the patient's blood stream automatically. This type of system is sometimes referred to as an artificial pancreas. The latter would be far more beneficial for the patient than the present system of discrete blood glucose analyses which involve pricking a thumb every time, followed by injection of large doses of insulin every few hours.

2.11.2   Control of Industrial Process

Sensors are used in various aspects of fermentation processes in three different ways, i.e.

1. Off-line in a laboratory

line if found unsatisfactory. Such an analysis would facilitate real-time control of products during manufacture.

2.11.5   Military and Defense Industry

In military and defense organizations, portable biosensors can be very useful for detection of toxic gases and the agents of chemical warfare, such as mustard and nerve gas.

'Bacteria' are our invisible friends and enemies. Some bacteria aid our digestion, others destroy our poisons. Still other "bugs" make us sick. Natural bacteria are a fact of life, living inside and outside bodies of living organisms. We have learned to live with them and they live with us. Soldiers and sailors in action worry that even more dangerous bacteria are lurking in the environment, these are named as killer bacteria, used as weapons. Such biological warfare agents are viewed by many to be as threatening to human life as nuclear weapons. The key to protecting a military unit or community from dangerous bacteria is to detect them before they reach their intended victims. People can then be warned to leave the area or wear protective gears. Bacteria can be detected using biosensors.