Electrochemical Biosensors

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Abstract

The technology supporting the development of electrochemical biosensors is a high interest area of current research programs. Broad interdisciplinary skills are required for the design and construction of these devices. To provide a brief overview of these biosensors, it is helpful to (1) define what is meant by a biosensor (2) explain the attributes and properties of an effective sensor. The detection approach is electrochemical in origin. This is a technical discipline that constitutes an area of specialization in its own right. The applicable biomaterials and how they produce detection signals brings together biochemical sciences with electrochemical phenomena. Finally a discussion of the immobilization technologies is presented. This yields a useful biosensor which is a significant materials science development effort. Also, list of references is presented that can lead the reader to current texts and major review articles addressing the technology in greater detail.

Keywords: biosensors, bioprobes, electrochemistry, sensors, detection

Introduction

The whole area of sensor development continues to be an extremely dynamic and growing area for scientific research. [1] It becomes immediately apparent that physical, chemical and biological sensor development is necessarily a multidisciplinary activity. It ranges within a number of complementary technical disciplines. The development of modern sensors has a close connection with developments in the semiconductor and materials industries. The developing technologies serve to provide microminiaturization on semiconductor substrates. The commercial potential of these sensor devices has been driven by the need for hydrocarbon detectors in the automotive industry, clinical assays and environmental monitoring. A very large purview of sensor technologies exists covering physical, chemical and biological measurements. The scope of this review shall attempt to restrict itself to a summary of the essential issues connected with electrochemical sensors as applied to bio-systems.

A problem arises in the attempt to draw hard and fast lines so as to appear to make clear and distinct classifications for the purposes of presentation. The dividing line could emphasize the nature of the operative recognition mechanism when generating the detection and measurement signal. For example, a biosensor can be viewed as a sensing device that employs a biological entity (enzyme, antibody, bacteria, etc.) as an intrinsic

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part of the sensing process. The difficulty quickly becomes evident that the monitoring parameters in bio-systems require combining materials components with making physical measurements. As a consequence the term bio-probe has found some acceptance. By applying such a term, one attempts to distinguish the sensing mechanism of species of biological origin from the general methodology used in sensing the parameters originating within biological systems.

The discussion that follows provides an overview of the discrete processes, materials and measurement methodologies.

Definition

Making progress in any technological area eventually requires a clear definition to guide further discussion. The following definition is a descriptive statement that alludes to a mechanistic interpretation. A committee of the International Union of Pure and Applied Chemistry has proposed the following as a recommended definition. [2]

An electrochemical biosensor is a self-contained integrated device capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element.

To illustrate this concept, Figure 1 shows a general block diagram for an electrochemical biosensor. The diagram illustrates the essential configuration of the biosensor together with a data collection device suitable for measuring, recording and reporting the analytical information developed by the sensor.

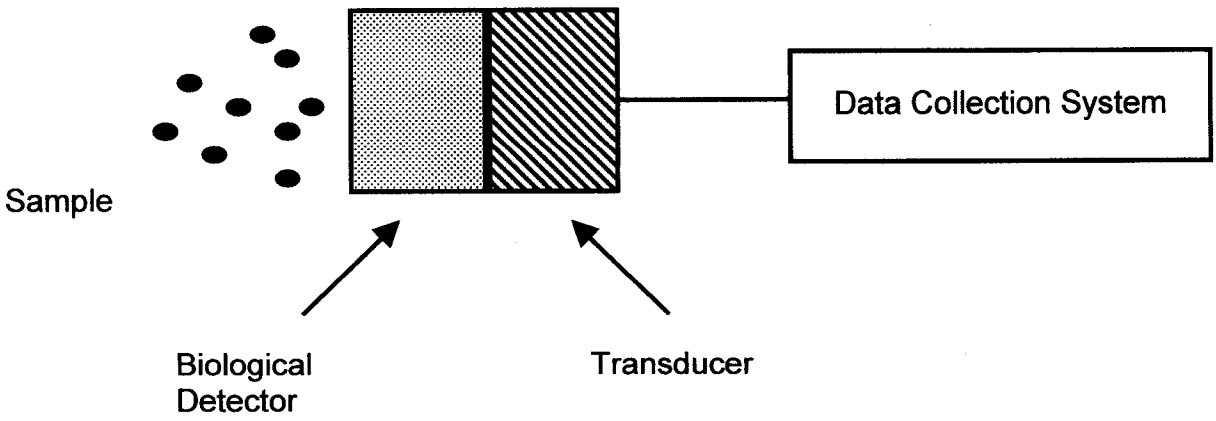

Fig.1. A general arrangement of the essential components of a biosensor system is shown. To be an electrochemical biosensor, the transduction element involves an electrochemical process.

Ideality as applied to sensors is more a hope than a reality. As a consequence, certain response specifications can be given that serve as a means for comparing the relative performance of sensors in any particular application.

Functional dependence of the amount of the sample to the response signal – Although a proportional signal would be the most desirable case, a well-defined mathematical relationship is also satisfactory. The reason for this is the availability of on-device signal processing that can do the data processing and reporting in real time. Of course, the relationship should be single valued or if not, the sought after value can be easily identified.

Absence of Hysteresis in the Sensor response – The signal should return to original baseline in any calibration step.

Fast response time – Basically, the signal should respond quickly to changes in the sensor environment. This is an important consideration when making real time measurements based on sensor readings. There are times when slow kinetic steps require stabilization times for achieving a stable and reliable measurement. The response time is an important figure of merit in designing sensor applications.

The Signal to Noise ratio (S/N) – The signal-to-noise ratio establishes the detection limit of the device. Ordinarily, the sensor determines the noise limit but this is not always the case. Some improvement is possible using noise filtering techniques.

Selectivity – The object of the measurement is to determine the existence and estimate the amount of the target species. The response of the sensor to any other species then constitutes an interference. Ideally, the sensor should respond only to the species being measured. If interferences exist, awareness of their presence and a means for correcting for the interfering component is helpful. Otherwise, the effect of the interference appears as a component in the error inherent in the measurement.

Sensitivity – Sensitivity is the rate of change of the detection signal with respect to the change in concentration of the detected species. The sensitivity changes as one approaches the noise threshold.

Electrochemical Detection

The electrochemical biosensor is characterized by its use of electrochemical methods as the means for transduction. [3] The simplest presentation currently in vogue for classifying these methods is Potentiometric, Amperometric and Conductometric.

1. Potentiometric sensors use the potential on an indicating electrode in an electrochemical cell measured at virtually zero current. This potential is determined from the measured voltage between an indicator electrode and a reference electrode. Since the potential of the reference electrode is an established constant, the potential of the indicator electrode is determined.

The potential of the indicator electrode is proportional to the logarithm of the concentration of the species being determined.

2. Amperometric sensors monitor the current measured at the indicating electrode as the consequence of a current passing through the cell. The magnitude of the current is correlated to the species being determined.
3. Conductometric sensors monitor the conductivity of an electrochemical cell and then use a correlation between the conductance and the concentration of the ionic species causing the conductivity.

This classification serves to generate distinct group classifications at the expense of a basic understanding of the processes themselves. Perhaps a few additional words of clarification would be helpful.

Potentiometric Measurements

It goes without saying that in order to make a voltage measurement across a cell, some finite current must pass however small that current might be. Because that finite current must flow, the kinetics of the electron transfer processes at the indicating electrode becomes an important consideration. The user would like that all of the reactions (chemical and electrochemical) occur at rates sufficiently fast that the overall reaction approaches a condition of thermodynamic reversibility. Alternatively, slow kinetics can make the potentiometric determination useless or associated with the slow step of the related kinetic processes. The proximity to thermodynamic reversibility means the body of thermodynamic data that is already in existence is available for interpretation of potentiometric measurements. For example, the electrode response follows the Nernst Equation which gives a first principles description of the electrochemical response of the interface. Thus the electrode response as a function of the logarithm of concentration is established. In this perspective, the potentiometric measurements have fundamental significance.

A special case and a significant application of potentiometry is the development of the ion selective electrodes. The original concept was the well-known glass electrode that monitors the pH of an electrolyte solution. The potential measurement of interest is the voltage drop that developed across a glass membrane that separates the analyte solution (the solution under test). The actual electrode within the glass electrode system is constructed as a fixed potential reference electrode. By doing that the potential drop across the membrane is what is being monitored. An important variant of this concept was the development of ion selective electrodes. In these electrodes species other than hydrogen ion are detected and measured. Also, pH can be determined using metal electrodes such as palladium hydride electrodes.

ISFETs are basically field effect transistors (FET) that incorporate an ion-sensitive surface. The surface electrical potential is dependent on the ions interacting with the semiconductor surface. This potential change can be monitored. The Ion Sensitive Field Effect Transistor (ISFET) is assembled by coating the sensor electrode with an

appropriately designed polymer layer. When the polymer layer is selectively permeable to analyte ions, these ions diffuse through the polymer layer. The consequence of this is a change in the FET surface potential. In a similar manner, an enzyme sensitive FET (IEFET) can be assembled.

Amperometric Measurements

Amperometry appears to be the name given to the set of electrochemical measurements where the electrochemical cell response occurs under a condition resulting in the passage of a current. A point of confusion can occur here because the set and a member of that set have the same name.

This particular logical problem is occasionally seen in other situations and has the tendency to result in some unintended circular arguments. This usually results in diminished clarity in expository presentations. Here we have a good example of that situation. When the term, amperometry, is used to identify the entire set of techniques, the amperometric designation focuses particular attention on the dynamic (non-equilibrium) aspect of the measurement. It that way, amperometry, as a name given to a collection of methods, stands separate from the potentiometric (or equilibrium) methods. However, the name, amperometry, also applies to a specific analytical technique. That is, amperometry is a technique wherein the measured current passing at an electrode that is held at a constant potential is monitored as a function of time. So, amperometry is the name of one specific technique belonging to a collection (or class) of techniques where the collection has the same name, amperometry. Clearly, this is not good form consistent with logical exposition. However, if one keeps that distinction in mind there should be no confusion.

The most commonly employed techniques in this amperometric grouping are voltammetry, amperometry (the specific application) and chronopotentiometry. In addition, there exists a rich assortment of other well-studied electroanalytical techniques that can also be applied. However, the data analysis is a little more sophisticated. These include: chronocoulometry as well as square wave and differential pulse voltammetric methods.

As a point of clarification, a naming methodology has evolved over time for electrochemical techniques used in analytical determinations. The techniques are distinguished by those experimentally measured parameters shown in a two dimensional graphical display of the data. For example, voltammetry displays the current and voltage response of the electrode system being examined. However, there are a number of variations of the voltammetric technique. Among them are cyclic, rapid scan, anodic stripping, stair case, hydrodynamic, rotating electrode, alternating current, etc. The differences relate to the unique mass transfer and charge transfer conditions existing at the immediate electrode interface applicable to that particular technique. A discussion of each of these methodologies can be very extensive and is the subject of a number of excellent monographs. In similar manner, chronopotentiometry and chronocoulometry

display electrode potential and charge passed as a function of time. Note that these methodologies have a number of well-studied variants as in the case of voltammetry

Conductometric Measurements

The conductance measurement uses the application of a low amplitude ac signal to an electrochemical cell. This is actually a special case of an impedance measurement. Technically, the conductance is the real part of the complex admittance of an electric circuit. The electrochemical cell can be represented in terms of an equivalent circuit. This circuit explains the measured impedance and phase angle over a finite frequency domain. By fitting the values of the circuit components and then correlating them with the changes taking place in the electrochemical cell, an excellent analytical method becomes available. This technique appears under different names including Electrochemical Impedance Spectroscopy (EIS).

A brief summary of the electrochemical transducers is presented in Table 1.

Table 1. Types of Electrochemical Transducers for Various Types of Measurements with Corresponding Analytes [4]

Measurement type	Transducer	Transducer analyte
1. Potentiometric	Ion-selective electrode (ISE);	K^+, Cl^-, Ca^{2+}, F^-
	Glass electrode;	H^+, Na^+ . . . ;
	Gas electrode;	CO_2, NH_3;
	Metal	redox species
	Metal hydride electrodes	H^+
	Ion-sensitive Field Effect Transistor (ISFET); Enzyme FET (ENFET)	H^+, K^+ . . .
2. Amperometric	Metal or carbon electrode;	O_2, sugars, alcohols . . alcohols . . . ;
	Chemically modified electrodes (CME)	sugars, alcohols, phenols, oligonucleotides . . .
3. Conductometric, Impedance	metal electrodes;	Urea, charged species, oligonucleotides . . .

Biomaterials for Biosensors

Recognition is the essential step in the detection process occurring in biological systems. An example of this is the use of an enzyme acting specifically to convert a reactant molecule into a product. Another example is that existing in immune systems where antigens interact with antibodies. The antigen is recognized as a foreign body. A specific antibody is generated to act against it by binding to it and operating to remove the antigen. Each type of cell has within it a unique signature in its DNA. All of the information contained in the DNA appears encoded in a series of amino acids and, as such, forms the identifying backbone of that structure. The recognition of these

sequences is of fundamental importance to the control, reading and detection of these molecular structures.

Enzymes

An enzyme is a biocatalyst. It acts to significantly increase the reaction rates of processes that would not normally take place under biologically ambient conditions of temperature, pressure and pH. Some enzymes show a specific sensitivity to a particular molecule (or substrate). Others might react with another substrate where a common amino acid linkage appears. It is also to be noted that many enzymatic reactions involve cofactors. These cofactors are other molecules or ions that assist in the reaction. During the catalysis, the cofactors may be chemically changed and, as a consequence, the resulting physicochemical effects can be used to monitor or detect the enzymatic process. A general list of enzyme properties that are advantageous in sensor applications are shown in Table 2.

Table 2. Enzyme Properties that are of Value in Sensor Development

1. High specificity for substrate or analyte
2. Reusability
3. Well understood mechanism of operation
4. Alternative methods for monitoring catalyzed reactions
5. Established methodologies for immobilization
6. Synthetic substrates available that provide products as a consequence of enzymatic conversion, that are detectable: direct determination of enzymatic activity is easily monitored
7. Many analytical formats available
8. Stable forms of enzymes available: may be produced from thermophilic organisms or generated by chemical or genetic mutation; a very active area for research and development
9. Commercial enzyme systems are well established (e.g., for determination of glucose, cholesterol and other biomolecules)
10. Relatively inexpensive to produce and purify
11. Many cell and tissue based sensors are enzyme based

Antibodies

An animal's immune system has two distinct parts: the innate and the adaptive immune systems. The innate system is mediated by cells having a nonspecific response to foreign molecules. Included among these cells are phagocytes that engulf foreign particles and natural-killer cells that bind to the foreign particles. The adaptive immune system produces molecules that have a specific response to foreign molecules based on their unique structure. The cells that mediate the adaptive immune system and produce antibodies are B-Lymphocytes. These antibodies are specific binding proteins that

selectively bind to the foreign molecules (the antigens) selectively. Each B-Lymphocyte is capable of producing an antibody. This antibody appears on the surface of the B-Lymphocyte as an antigen receptor. The antibody reacts specifically with the invading antigen.

By this specific recognition and interaction performed on the molecular level, antibodies and antigens can be exploited as a means for diagnostic testing. Antibodies can be raised in vitro so as to be able to detect specific molecules. In this way, antibodies may be used as the basis for an electrochemical detection system.

Immobilization of Biomolecules for Biosensors

Biomolecule Matrices for Biosensors

It is essential to create a biosensing surface where the sensing mechanism is immobilized. The biosensing surface may contain enzymes, antibodies, antigens, microorganisms, mammalian cells, tissues or receptors.

A short review of the immobilization procedures follows.

Physical adsorption on a solid surface

This method involves a physical attraction or adsorption onto a film of plastic, glass or cellulose. It is not considered a reliable or reproduceable method for building a biosensing matrix. Problems appear associated with leaching during storage. The absorptive forces can be disruptive by the effects of pH, temperature and ionic strength. The advantage of this method is its simplicity and chemically gentle environment.

Use of cross linking reagents

Bifunctional cross linking reagents can be used to stabilize proteins. The proteins can be cross linked to one another or other inert proteins. Greater sensor stability can be achieved by using an immobilized protein. However, some loss of activity occurs. The cross linking chemical could interact with the active sites as in the case of enzymes. In the case of immunosensors, antibody binding sites can be incorrectly oriented or blocked. In practice, membranes can be cast directly upon electrode surfaces.

Entrapment using a gel or polymer

A convenient and gentle method for immobilization is the physical entrapment of biomolecules in gel matrices. This method can permit the porosity or degree of cross linking to be controlled. The cross linking serves to constrain the biomolecules so

that leaching is minimized. This technique can be used to immobilize microorganisms.

Use of membranes to retain the biomolecule close to the electrode surface

By controlling membrane porosities, biomolecules can be retained near the transducer surface without the need for actual immobilization. Ion selective membranes can be used in conjunction with the contained biomolecular species.

Covalent attachment

The biosensing surface can be established by the chemical coupling of the biomolecules. Such matrices show a resistance to pH, temperature and ionic strength. The supports commonly used are inorganics, natural polymers and synthetic polymers.

Exploitation of biomolecular interactions

This is a miscellaneous category that relies on specific biochemical reactions whose properties can create a detectable response. The reviews of Guilbrault [5] and Taylor [6] cover enzyme, antibody and receptor immobilizations for biosensors.

Concluding Remarks

The exploitation of biospecific interactions has a key role to in the development of highly sensitive biosensors with clinical, pharmacological, and biotechnical applications. Recently published articles identify this area as one of the fastest growing technologies with diverse applications in many scientific fields. [7]

The development of highly sensitive biosensors is built on the biospecific interactions as the mechanism of detection and measurement. These biosensors have already found application in clinical, pharmacological and biotechnical analysis.

The preceding is a brief and constrained presentation of an interdisciplinary technology that has great depth and breadth. For those readers who would like to pursue a more detailed examination of this technology or to initiate experimental programs to develop the technology further, a list of general references and review articles are appended below and may be found to be useful. Another information source is the Internet. By using the search engines available, a search can be performed using the key words, "electrochemical biosensors." A useful search engine can be found having the URL address, http://www.google.com and can provide an interesting list of internet addresses where technical documents, manufacturer's information and presentation graphics. This information can be downloaded and structured as needed.

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