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Review Article

A Review on Electrochemical Biosensors: Principles and Applications

Mohammed Asef Iqbal[1*] , S.G. Gupta[2] and Hussaini S.S.[3]
1* Dept. of Microbiology, Milliya Arts, Science & Mgmt. Sci. College, Beed, MS, India
2 Government Institute of Forensic Science, Aurangabad, MS, India,
3 Dept. of Physics, Milliya Arts, Science & Mgmt. Sci. College, Beed, MS, India
*Email: e_bareed@yahoo.co.in

ABSTRACT
Electrochemical sensors for multiple analytes were the first scitifically proposed as well as commersialised biosensors. Electrochemical biosensors have been studied for a long time. Presently, typical platform of transducers based on semiconductors and screen printed electrodes is in practice for construction of biosensors. Among all biocomponents enzymes or enzyme labeled antibodies are presently the most common biorecognition tools used in biosensor. This review highlights the principle and some typical applications for the electrochemical biosensors. These have been categorized in three categories based on their operation and application as: potentiometric, amperometric and impedimetric transducers. The review also highlights some of the most typical assays used.
Keywords*: Biorecognition tools, enzyme electrode, multiple analytes, immunosensor, potentiometric, labeled antibodies, amperometric, impedimetric transducer*

INTRODUCTION

Past five decades has seen many advances in the field of basic and applied research concerning the electrochemical biosensors. The first enzyme electrode with immobilized glucose oxidase was introduced by Leland C. Clark at the New York Academy of Sciences Symposium in 1962 [1]. Springs Instruments (Yellow Springs, OH, USA) had placed the first commercially produced biosensor in the market in 1975. This was used for blood glucose detection in the diabetes patients. Nowadays various biosensor devices have been developed and commersialised with a wide range of applications such aa pathogen and toxin detection and even some are based on multichannel configuration [2,3].

A suitable enzyme in the bio-recognition layer is the most crucial part of an electrochemical biosensor, which provides electro-active substances for detection by physicochemical transducer as a measurable signal. The analyte being detected could serve either as the substrate for the enzyme or may function as its inhibitor, when a native enzyme is used as the biorecognition component. In addition, enzymes can be used as *Pohanka and Skládal: Electrochemical biosensors – principles and applications* labels bound to antibodies, antigens and oligonucleotides with a specific sequence, thus providing affinity-based sensors [4]. Among all the enzymes processed in biotechnology a limited number of enzymes are used for monitoring of clinical metabolites, these enzymes include glucose oxidase [5] and glucose dehydrogenase [6] for glucose assays, alcohol oxidase for ethanol [7], NADH dependent lactate dehydrogenase [8] and lactate:cytochrome c oxidoreductase for lactate [9,10,11, urease for urea [12] and cholesterol oxidase co-immobilized with cholesterol esterase for the cholesterol assay [13] from the group of oxidoreductases. Peroxidase and alkaline phosphatase are the most common enzyme labels for electrochemical affinity biosensors [14].

The electrochemical biosensors may use potentiometric, amperometric and impedimetric transducers for converting the chemical signals into a measurable amperometric signal.

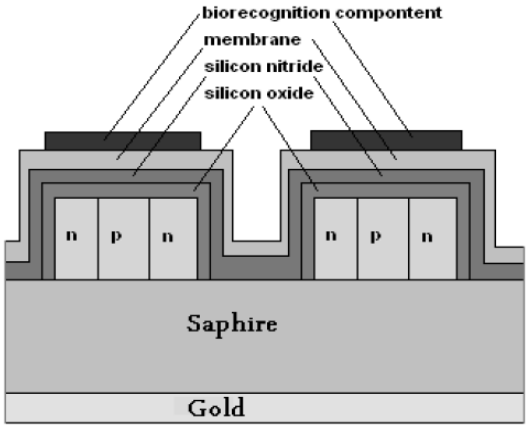

Fig. 1. Schematic drawing of a field effect transistor (n-p-n type) based biosensor**.**

POTENTIOMETRIC BIOSENSORS

These biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET). The primary output signal is possibly generated due to ions accumulated at the interface of ion-selective membrane. Current flow through the electrode is almost zero. The ion resulting from the enzyme catalysed reaction is followed up by the electrode [15]. For example, glucose oxidase can be immobilized on a surface of the pH electrode. Glucose does not have significant effect on the pH of the working media; however, gluconate resulting from the enzymic reaction causes acidification. A biorecognition element is immobilized on the outer surface or captured inside the membrane. In the past the pH glass electrode was used as a physicochemical transducer [16]. The Nernst potential of the pH glass electrode is described by the Nicolsky-Eisenman equation, of which the generalized form for ISE is as follows [17]:

$$E = E^0 + \frac{RT}{z_a F} \ln\left[a_a + \sum_{i=1}^{n} K_{a,i}(a_i)^{z_a/z_i}\right]$$

(*E* potential, *R* the universal gas constant, *T* temperature, *F* Faraday constant, *za* followed and *z*i interfering ion valence, *aa* activity of measured and *ai* activity of interfering ion and *Ka,i* represents the selectivity coefficient).

Semi conductor based physico-chemical transducers, are nowadays more common. ISFETs and LAPS (light addressable potentiometric sensor) based systems are more convenient for biosensor construction. The ISFET principle [18] is based on a local potential generated by surface ions from a solution. This potential modulates the current flow across a silicon semiconductor. The transistor gate surface is covered by a selective membrane in ISFET; this could be made from compounds such as silicon nitride (Si3N4), alumina (Al2O3), zirconium oxide (ZrO2) and tantalum oxide (Ta2O5) for pH detection.

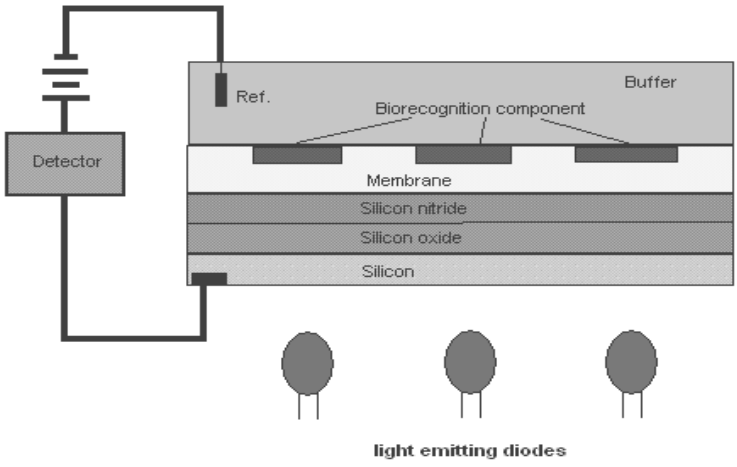

Fig. 2. Block diagram of the light addressable potentiometric sensor with biorecognition component bound intomembrane and with buffered reaction cell.

The LAPS principle [19] is based on semiconductor activation by a light-emitting diode (LED). The sensor is made from an n-type silicon typically coated with 30 nm of silicon oxide, 100 nm of silicon nitride, and indium-tin oxide. Voltage change as a function of medium pH in the LED activated zone is measured by the LAPS. This provides an opportunity for multiposition sensing and construction of an array of biorecognition zones.

A potentiometric biosensor with a molecularly imprinted polymer constructed for the herbicide atrazine assay allows detecting from 3×10-5 to 1×10-3 M [20]; for tracking the level of neurotransmitter serotonin, molecularly imprinted polymer was also used [21]. For creatine analysis, a potentiometric biosensor with co-immobilized urease and creatinase on the poly(vinylchloride) ammonium membrane was used [22]. ISFET with immobilized butyrylcholinesterase was employed for the glycoalkaloids assay [23]. For the detection of organophosphate pesticides A simple pH electrode modified with acetylcholinesterase (AChE) was used [24]. The LAPS biosensor was used for the *Escherichia coli* assay allowing detection as low as 10 cells/ml when the specific primary capture antibody was immobilized on the LAPS flow-through cell, and the secondary antibody labeled by urease for sandwich complex formation was used [25]. A commercial device Bio-Detector (Smiths Detection, Warrington, UK) based on the LAPS type biosensor is found in mobile laboratories for automated 8-channel analysis of biological agents.

AMPEROMETRIC BIOSENSORS

Amperometric biosensors are quite sensitive and more suited for mass production than the potentiometric ones [26]. The working electrode of the amperometric biosensor is usually either a noble metal or a screen-printed layer covered by the biorecognition component. Another economic option is Carbon paste with an embedded enzyme [27]. At the applied potential, conversion of electroactive species generated in the enzyme layer occurs at the electrode and the resulting current (typically nA to μA range) is measured [28]. The principle of the previously mentioned YSI 23A [29] can serve as an example:

glucose + GOD(FAD) 6 gluconolactone + GOD(FADH2) (1)

GOD(FADH2) + O2 6 GOD(FAD) + H2O2 (2)

H2O2 6 O2 + 2H+ + 2e- (3)

The reactions (1) and (2) are catalyzed by glucose oxidase (GOD) containing FAD as a cofactor. The last reaction is the electrochemical oxidation of hydrogen peroxide at the potential of around +600 mV.

Two- or three-electrode configurations are possible for working amperometric biosensors. The former case consists of reference and working (containing immobilized biorecognition component) electrodes. Limited control of the potential on the working electrode surface with higher currents is one of the main disadvantages of the two electrode configuration, and because of this, the linear range could be shortened. This problem could be solved by employing a third auxiliary. Now voltage is applied between the reference and the working electrodes, and current flows between the working and the auxiliary electrodes. A common screen-printed three electrode sensor is shown in Fig. 3.

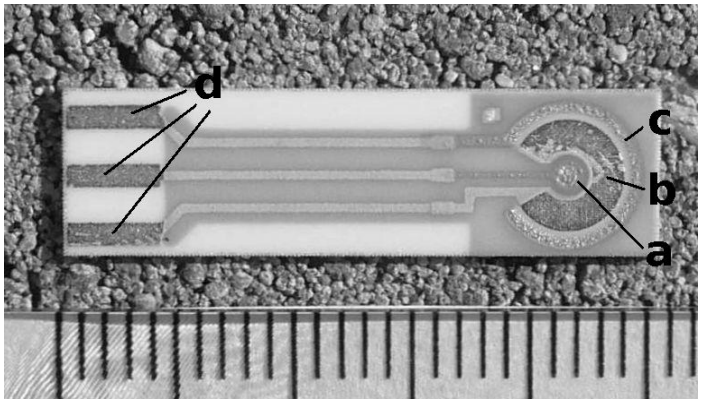

Fig. 3. Example of the three-electrode screen-printed sensor produced by BVT (Brno, Czech Rep.).

[The sensor body is made from ceramics. A gold working electrode (a) is surrounded by an Ag/AgCl reference electrode (b) and gold auxiliary electrode (c). Letter d means silver output contacts. The ruler in the bottom is in millimeter scale.]

The amperometric biosensors were often been reported to be used on a large scale for analytes such as glucose, lactate [30], and sialic acid [31]. Biological agents such as model *Bacillus cereus* and *Mycobacterium smegmatis* [32], the serological diagnosis of *Francisella tularensis* [33], a pharmacology study [34] and the detection of pesticides and nerve agents [35] have also been described. A metabolism apparatus of whole cells can also be used for certain analytes such as the measurement of phenol with immobilized *Pseudomonas* sp. cells [36]. Rapid detection of organophosphates and carbamates can be done by employing biosensors based on AChE and butyrylcholinesterase (BChE) [37] due to strong enzyme inhibition [38]. The dichlorvos assay could be done using AChE amperometric biosensor based on a nanoporous carbon matrix [39] and a similar device based on the screen-printed carbon electrode modified with Prussian blue was tested for aldicarb, paraoxon and parathion-methyl [40]. Amperometric biosensors were evaluated also for carrying out assays with nucleic acid acting as a marker and/or biorecognition component; uropathogens were assayed using their 16S rRNA [41]. Several commercial amperometric biosensors exist. The glucose biosensors are most well known and commonly available; examples include SIRE P201 (Chemel AB, Lund, Sweden), FreeStyle Freedom Blood Glucose Monitoring System, Precision Xtra (Abbot Diabetes Care, Alameda, CA, USA), and GlucoWatch Biographer (Cygnus, Redwood City, CA, USA). The device Midas Pro (Biosensori SpA, Milan, Italy) is widely employed for the analysis of surface waters [42].

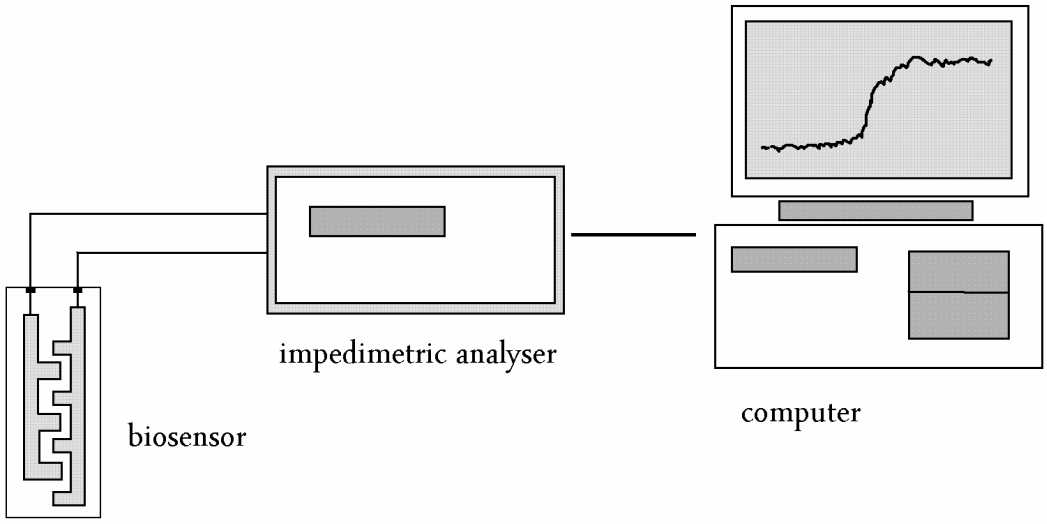

Fig. 4. Simplified scheme of analytical device based on impedimetric biosensor**.** Scheme picture screen printedtransducer with typical labyrinth electrodes.

IMPEDIMETRIC BIOSENSORS

Such devices follow either impedance (Z) or its components resistance (R) and capacitance (C); inductance typically has only a minimal influence in a typical electrochemical setup. Thus, the expression of impedance is as follows:

$$Z^2 = R^2 + \frac{1}{(2fC)^2}$$

The inverse value of resistance is called conductance hence for this reason such systems have also been named as as conductometric by some investigators. These biosensors consist of two electrodes with applied alternating voltage, amplitudes from a few to 100 mV are used. The impedance biosensor is commonly a functional part of the Wheatstone bridge. These systems are considered for the assay of urea when urease is used as a biorecognition component. The following reaction takes place in the medium:

(NH2)2CO + 3 H2O 6 2NH4+ HCO3- + OH- (4)

The principle is obvious; urea and water molecules on the left side of the equation exhibit only minimal influence on the measured impedance. A significant increase in the impedance is provided by the enzymatically produced. Alternatively, impedance biosensors have been successfully used for growth monitoring of microorganism based on the production of conductive metabolites [43]. One of the main disadvatges of such biosensors is the false positive results due to electrolytes from the. Impedimetric biosensors are less frequent compared to potentiometric and amperometric biosensors; nevertheless, there have been some promising approaches. Impedance assays have been used to monitor Hybridization of DNA fragments previously amplified by a polymerase chain reaction [44]. Antibody levels as low as 10 pg/ml present in a sample can be succefully detected througa model impedance immunosensor containing electrodeposited polypyrrole film with captured avidin connected through biotin to anti-human IgG [45]. Impedance biosensors based on immobilized yeasts were used for evaluation of ethanol level in some alcoholic beverages (*Saccharomyces cerevisiae;* [46]). The impedance-based commercial device Malthus 2000 (Malthus Instruments, Crawley, UK) was used for an assay of the pathogenic fungus Ichthyophonus hofery [47] and the *Erwinia carotovora* rot [48].

CONCLUSION

Electrochemical biosensors although are quite old but still hold a very promising future. Selectivity of biochemical recognition and sensitivity of electrochemical detections could be combined successfully to make this technology efficient for use. The current technological uplift has aided biosensor with miniaturized electrochemical instrumentation which has made them very advantageous for some sophisticated applications requiring portability, rapid measurement and use with a small volume of samples. Numerous commercial applications confirm the attractive advantages of electrochemical biosensors.

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