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REVIEW

Electrochemical biosensors – principles and applications

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Summary
The first scientifically proposed as well as successfully commercialized biosensors were those based on electrochemical sensors for multiple analytes. Electrochemical biosensors have been studied for a long time. Currently, transducers based on semiconductors and screen printed electrodes represent a typical platform for the construction of biosensors. Enzymes or enzyme labeled antibodies are the most common biorecognition components of biosensors. The principles of, and the most typical applications for electrochemical biosensors are described in this review. The relevant systems are divided into three types according to the operating principle governing their method of measurement: potentiometric, amperometric and impedimetric transducers, and the representative devices are described for each group. Some of the most typical assays are also mentioned in the text.

Keywords: enzyme electrode – immunosensor – potentiometric – amperometric – impedimetric transducer

INTRODUCTION

Electrochemical biosensors have been the subject of basic as well as applied research for nearly fifty years. Leland C. Clark introduced the principle of the first enzyme electrode with immobilized glucose oxidase at the New York Academy of Sciences Symposium in 1962 (Clark and Lyons was the YSI 23A Blood Glucose Analyzer; Yellow 1962). The first commercially produced biosensor Springs Instruments (Yellow Springs, OH, USA) placed on the market in 1975. This device was applied to the fast glucose assay in blood samples from diabetics. At present, there are many proposed and already commercialized devices based on the biosensor principle including those for pathogens and toxins, some even based on a multi-channel configuration (Pohanka et al. 2007a, b).

The most typical part of electrochemical biosensors is the presence of a suitable enzyme in the biorecognition layer providing electroactive substances for detection by the physico-chemical transducer providing the measurable signal. A native enzyme can be used as the biorecognition component; in this case the analyte is equal to the enzyme substrate; alternatively it may function as its inhibitor. In addition, enzymes can be used as

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labels bound to antibodies, antigens and oligonucleotides with a specific sequence, thus providing affinity-based sensors (Bakker 2004). A rather limited number of enzymes processed in biotechnology were chosen for the monitoring of clinical metabolites and, especially from the group of oxidoreductases: glucose oxidase (Kafi et al. 2006) and glucose dehydrogenase (Antiochia et al. 2007) for glucose assays, alcohol oxidase for ethanol (Yildiz and Toppare 2006), NADH dependent lactate dehydrogenase (D´Auria et al. 2000) and lactate:cytochrome c oxidoreductase for lactate (Stein and McShane 2003; Garjonyte et al. 2006; Pohanka and Zbořil 2007), urease for urea (Barhoumi et al. 2005) and cholesterol oxidase co-immobilized with cholesterol esterase for the cholesterol assay (Singh et al. 2007). Peroxidase and alkaline phosphatase are the most common enzyme labels for electrochemical affinity biosensors (Skládal 1997).

Based on their operating principle, the electrochemical biosensors can employ potentiometric, amperometric and impedimetric transducers converting the chemical information into a measurable amperometric signal.

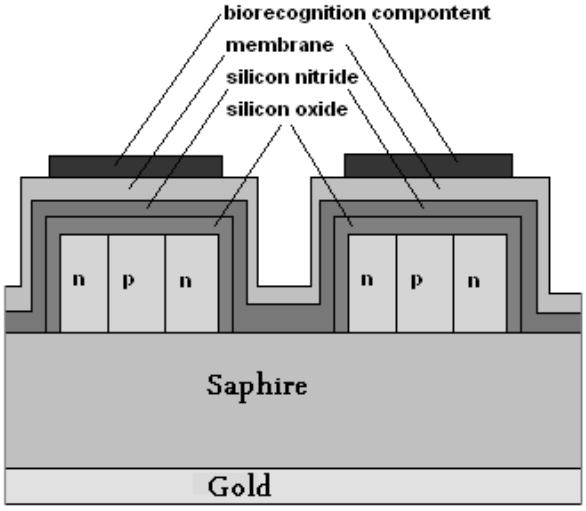

Fig. 1. **Schematic drawing of a field effect transistor (n-p-n type) based biosensor.**

POTENTIOMETRIC BIOSENSORS

Potentiometric biosensors are based on ion-selective electrodes (ISE) and ion-sensitive field effect transistors (ISFET). The primary outputting signal is possibly due to ions accumulated at the ion-selective membrane interface. Current flowing through the electrode is equal to or near zero. The electrode follows the presence of the monitored ion resulting from the enzyme reaction (Kauffmann and Guilbault 1991). For example, glucose oxidase can be immobilized on a surface of the pH electrode. Glucose has only minimal influence on pH in the working medium; however, the enzymatically formed gluconate 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 (Newman and Setford 2006). 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 (Buerk 1993):

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

(E potential, R the universal gas constant, T temperature, F Faraday constant, z_a followed and z_i interfering ion valence, a_a activity of measured and a_i activity of interfering ion and $K_{a,i}$ represents the selectivity coefficient).

Nowadays, semiconductor based physico-chemical transducers are more common. ISFETs and LAPS (light addressable potentiometric sensor) based systems especially are convenient for biosensor construction. The ISFET principle (Yuqing et al. 2003 and 2005) 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 in ISFET is covered by a selective membrane; for pH detection this could be made from compounds such as silicon nitride (Si_3N_4), alumina (Al_2O_3), zirconium oxide (ZrO_2) and tantalum oxide (Ta_2O_5).

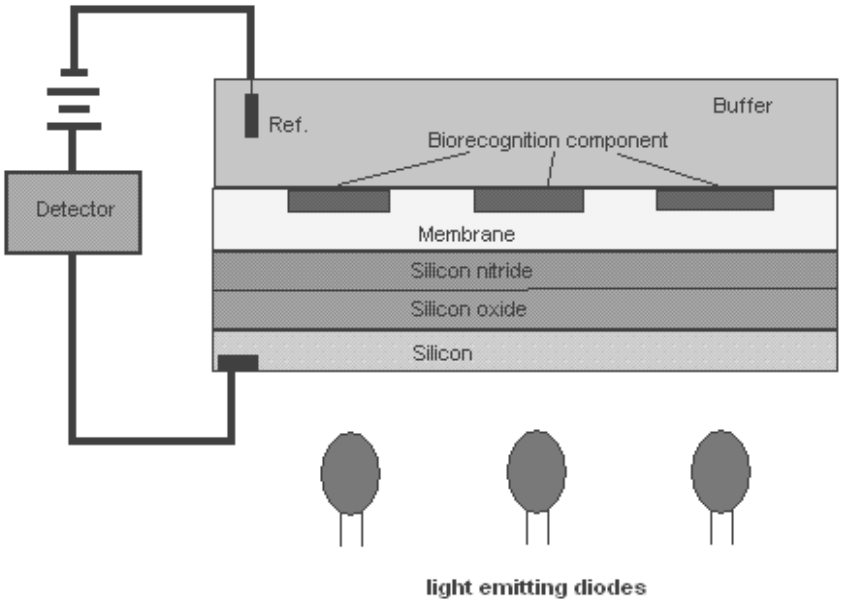

Fig. 2. **Block diagram of the light addressable potentiometric sensor with biorecognition component bound into membrane and with buffered reaction cell.**

The LAPS principle (Yoshinobu et al. 2005) 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. The LAPS measures a voltage change as a function of medium pH in the LED activated zone. This opens the way 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 (D´Agostino et al. 2006); molecularly imprinted polymer was also used for tracking the level of neurotransmitter serotonin (Kitade et al. 2004). Another potentiometric biosensor with co-immobilized urease and creatinase on the poly(vinylchloride) ammonium membrane was used for creatine analysis (Karakus et al. 2006).

ISFET with immobilized butyrylcholinesterase was employed for the glycoalkaloids assay (Korpan et al. 2006). A simple pH electrode modified with acetylcholinesterase (AChE) was used for the detection of organophosphate pesticides (Timur and Telefoncu 2004). 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 (Ercole et al. 2002). 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 (Ghindilis et al. 1998). The working electrode of the amperometric biosensor is usually either a noble metal or a screen-printed layer covered by the biorecognition component (Wang 1999). Carbon paste with an embedded enzyme is another economic option (Cui et al. 2005). 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 (Mehrvar and Abdi 2004). The principle of the previously mentioned YSI 23A (Magner 1998) can serve as an example:

$$\text{glucose} + \text{GOD(FAD)} \rightarrow \text{gluconolactone} + \text{GOD(FADH}_2\text{)} \quad (1)$$

$$\text{GOD(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOD(FAD)} + \text{H}_2\text{O}_2 \quad (2)$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad (3)$$

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.

Amperometric biosensors can work in two- or three-electrode configurations. The former case consists of reference and working (containing immobilized biorecognition component) electrodes. The main disadvantage of the two-electrode configuration is limited control of the potential on the working electrode surface with higher currents, and because of this, the linear range could be shortened. To solve this problem, a third auxiliary electrode is employed. 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.

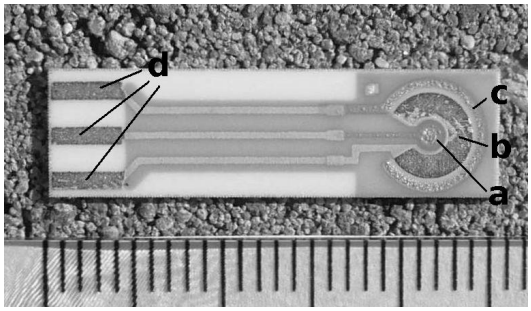

Fig. 3. **Example of the three-electrode screen-printed sensor produced by BVT (Brno, Czech Rep.).** The sensor body is made from ceramics. A gold working electrode (a) is surrounded by an Ag/AgCl reference electrode (b) and gold auxiliary electrode (c). Letter d means silver output contacts. The ruler in the bottom is in millimeter scale.

The amperometric biosensors are often used on a large scale for analytes such as glucose, lactate (Ohnuki et al. 2007), and sialic acid (Marzouk et al. 2007). Biological agents such as model *Bacillus cereus* and *Mycobacterium smegmatis* (Yemini et al. 2007), the serological diagnosis of *Francisella tularensis* (Pohanka and Skládal 2007), a pharmacology study (Pohanka et al. 2007c) and the detection of pesticides and nerve agents (Liu et al. 2006) have also been described. A metabolism apparatus of whole cells can be used for certain analytes such as the measurement of phenol with immobilized *Pseudomonas* sp. cells (Skládal et al. 2002). Biosensors based on AChE and butyrylcholinesterase (BChE) can be employed for rapid detection of organophosphates and carbamates (Skládal 1996) due to strong enzyme inhibition (Krejčová et al. 2005). The AChE amperometric biosensor based on a nanoporous carbon matrix was used for the dichlorvos assay (Sotiropoulou and Chaniotakis 2005) and a similar device based on the screen-printed carbon electrode

modified with Prussian blue was tested for aldicarb, paraoxon and parathion-methyl (Suprun et al. 2005). Amperometric biosensors were evaluated also for assays with nucleic acid acting as a marker and/or biorecognition component; uropathogens were assayed using their 16S rRNA (Liao et al. 2006).

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 (Rosseti et al. 2001).

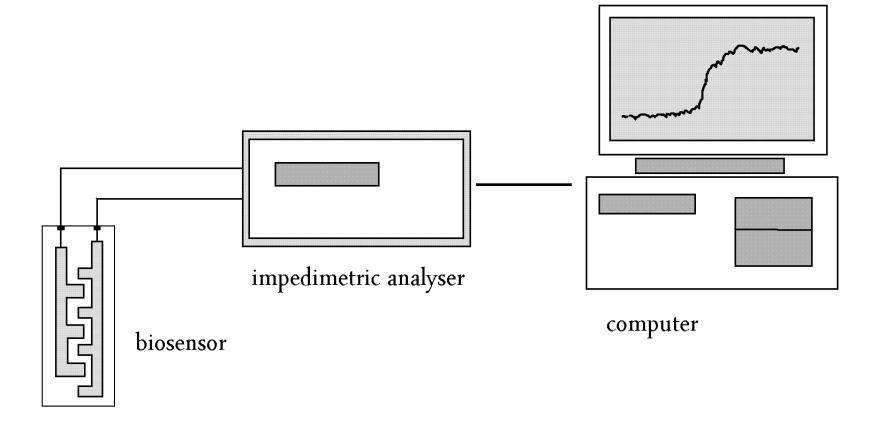

Fig. 4. **Simplified scheme of analytical device based on impedimetric biosensor.** Scheme picture screen printed transducer with typical labyrinth electrodes.

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:

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

The inverse value of resistance is called conductance and for this reason some investigators name such systems as conductometric. Impedance biosensors include 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:

$$(NH_2)_2CO + 3\ H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \qquad (4)$$

The principle is obvious; urea and water molecules on the left side of the equation exhibit only minimal influence on the measured impedance. The enzymatically produced ions are able to provide a significant increase of impedance. Alternatively, impedance biosensors have been successfully used for microorganism growth monitoring due to the production of conductive metabolites (Silley and Forsythe 1996). False positive results due to electrolytes from the samples are the main disadvantage of impedance biosensors.

Impedimetric biosensors are less frequent compared to potentiometric and amperometric biosensors; nevertheless, there have been some promising approaches. Hybridization of DNA fragments previously amplified by a polymerase chain reaction has been monitored by an impedance assay (Davis et al. 2007). A model impedance immunosensor containing electrodeposited polypyrrole film with captured avidin connected through biotin to anti-human IgG was able to detect antibodies as low as 10 pg/ml present in a sample (Ouerghi et al. 2002). The ethanol level in some alcoholic beverages was evaluated by an impedance

biosensor with immobilized yeast (*Saccharomyces cerevisiae;* Korpan et al. 1994). The impedance-based commercial device Malthus 2000 (Malthus Instruments, Crawley, UK) was used for an assay of the pathogenic fungus Ichthyophonus hofery (Spanggaard et al. 1994) and the *Erwinia carotovora* rot (Fraaje et al. 1997).

CONCLUSION

Electrochemical biosensors have existed for nearly fifty years and seem to possess great potential for the future. This technology gains practical usefulness from a combination of selective biochemical recognition with the high sensitivity of electrochemical detection. Thanks to current technological progress, such biosensors profit from miniaturized electrochemical instrumentation and are thus 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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