Amperometric enzyme biosensors: Past, present and future
Biocapteurs enzymatiques à transduction ampérométrique :
passé, présent, futur
S.V. Dzyadevych a,*, V.N. Arkhypova a, A.P. Soldatkin a, A.V. El’skaya a, C. Martelet b, N. Jaffrezic-Renault c

a Laboratory of Biomolecular Electronics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo Street, Kiev 03143, Ukraine
b Ampère, UMR CNRS 5005, école centrale de Lyon, 69134 Ecully cedex, France
c Laboratoire des sciences analytiques, UMR CNRS 5180, université Claude-Bernard Lyon-1, 69622 Villeurbanne cedex, France

Received 10 October 2007; accepted 12 November 2007
Available online 24 April 2008

Abstract

Three groups of the amperometric biosensors such as unmediated, mediated and based on direct transfer of electrons have been thoroughly described, and their advantages and disadvantages were shown. The amperometric biosensors are mostly utilized in commercial devices since they are studied to a greater extent and have some advantages. The modern commercial systems based on amperometric biosensors and its applications have been presented. The major field of employing biosensors is medical diagnostics where numerous commercial devices are currently functioning.

Résumé

Trois groupes de biocapteurs ampérométriques, sans médiateur, avec médiateur et avec transfert électronique direct, ont été décrits en détail et leurs avantages et inconvénients ont été montrés. Les biocapteurs ampérométriques sont les plus utilisés dans des dispositifs commerciaux parce qu’ils ont été très étudiés et qu’ils ont de nombreux avantages. Les systèmes commerciaux actuels, basés sur les biocapteurs ampérométriques, ainsi que leurs applications ont été présentés. Le domaine principal d’application des biocapteurs est le diagnostic médical pour lequel de nombreux dispositifs commerciaux fonctionnent couramment.

Keywords: Amperometric biosensor; Enzyme; Mediator; Electrode; Commercial system

Mots clés : Biocapteur ampérométrique ; Enzyme ; Médiateur ; Électrode ; Systèmes commerciaux

1. Introduction

The requirements and regulations in the fields of environmental protection, control of biotechnological processes and certification of food and water quality are becoming more and more urgent. At the same time stricter requirements regarding human and animal health have led to a rising number of clinical and veterinary tests. Therefore, there is a need in developing highly sensitive, fast and economic methods of analysis. The elaboration of biosensors is probably one of the most promising ways to solve some problems concerning sensitive, fast, repetitive and cheap measurements.

As a rule, a biosensor is a self-contained device consisting of two functional parts: a bioselective membrane in direct contact with a physical transducer (Fig. 1) [1].

A biosensor converts the modification of the physical or chemical properties of a biomatrix, which occurs as a result of biochemical interactions, into an electric or an optic signal...
whose amplitude depends on the concentration of defined analytes in the solution. Functionally, the device consists of two parts:

- a biomatrix, that is, a detecting layer of immobilized material (enzymes, antibodies, receptors, organelles, microorganisms);
- a transducer (potentiometric, impedimetric, amperometric, conductometric, acoustic, optic or colorimetric).

Amperometric biosensors are a class of the most widespread, numerous and successfully commercialized devices of biomolecular electronics. The importance of these biosensors consists in the fact that the development of the novel field of analytical biotechnology started from them.

The research in the field of biosensors and, amperometric transducers in particular, was initiated by Clark whose study on oxygen electrode was published in 1956 [2]. On the basis of these experiments he, together with Lyons, has presented a report at the Symposium of New York Academy of Sciences in which they proposed the way “to make electrochemical sensor more reasonable” by adding “an enzyme transducer in the form of a membrane sandwich”. This concept was illustrated with the following experiments: glucose oxidase was placed at the sensitive surface of oxygen “Clark electrode” covered with semi-permeable dialysis membrane, glucose oxidase being separated from the solution tested by additional dialysis membrane [3]. In the same work a concept of “enzyme electrode” was firstly introduced by Clark and Lyons. A number of authors arrogate it to Updike and Hicks [4] who actually developed this idea later on and applied it to biosensor with entrapped enzyme. They were also the first to describe the glucose-specific enzymatic electrode which appeared to be simpler and more stable in operation compared with the Clark’s one. These pioneer works have grounded successful development and subsequent commercialization of the amperometric biosensors.

Generally, the amperometry is based on measuring either value or density of current in an electrochemical cell at a constant applied potential value. The current density is a function of the presence in the solution of electrochemically active particles, whose oxidation or reduction takes place on the surface of a working electrode, proportional to its concentration. During electrolysis the working electrode may serve as either anode or cathode depending on the nature of the substance measured and the voltage value applied. Amperometry is widely used in analytical practice since under certain conditions the concentration of the substance detected may be lower than $10^{-8}$ M and dynamic range is of 3–4 orders.

The amperometric biosensors can be divided into three main classes:

- sensors based on measurement of concentration of natural substrates and products of enzyme reaction (mediatorless amperometric biosensors);
- sensors using mediators as carriers of electrons from active enzyme centre to electrode (mediator amperometric biosensors);
- amperometric biosensors using direct electron transfer between enzyme and electrode.

### 2. Amperometric mediatorless biosensors

The first group of amperometric biosensors is constituted of the sensors based on measurement of concentration of natural substrates or products of enzyme reaction. In the course of any reaction some products are generated and some substrates are decreased. If they are electroactive, their concentration can be measured directly by amperometric sensor. The enzymes generally catalyzing reactions of this kind are various oxidases (Table 1).

A scheme of the enzyme reactions in which oxidases take part can be presented as follows:

\[ S + E_{FAD} \leftrightarrow E_{FAD}S \Rightarrow E_{FADH_2} + P \]  
\[ E_{FADH_2} + O_2 \Rightarrow E_{FAD} + H_2O_2 \]  

Most of the sensors of this kind operate due to either oxygen absorption during biocatalytic reaction measured by means of controlling $O_2$ cathode reduction on the electrode at the potential of $-0.7$ V, or biocatalytic generation of hydrogen peroxide evaluated by monitoring of $H_2O_2$ anode oxidation on the electrode at the potential of $+0.65$ V (Fig. 2).

Dehydrogenases constitute the second class of enzymes widely used in amperometric biosensors of the first group (Table 2) [22].

### Table 1 Oxidases applied in amperometric biosensors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase</td>
<td>Glucose</td>
<td>[5–7]</td>
</tr>
<tr>
<td>Lactate oxidase</td>
<td>Lactate</td>
<td>[8,9]</td>
</tr>
<tr>
<td>Cholin oxidase</td>
<td>Choline</td>
<td>[10,11]</td>
</tr>
<tr>
<td>Alcohol oxidase</td>
<td>Ethanol</td>
<td>[12,13]</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>[14,15]</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde</td>
<td>[16]</td>
</tr>
<tr>
<td>Glutamate oxidase</td>
<td>Glutamate</td>
<td>[17]</td>
</tr>
<tr>
<td>Triptophan-2-monoxygenase</td>
<td>Triptophan</td>
<td>[18]</td>
</tr>
<tr>
<td>Lysine oxidase</td>
<td>Lysine</td>
<td>[19,20]</td>
</tr>
<tr>
<td>Xantine oxidase</td>
<td>Hypoxantine</td>
<td>[21]</td>
</tr>
</tbody>
</table>
Table 2
Dehydrogenases applied in amperometric biosensors

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>Aldehydes</td>
<td>[23,24]</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Ethanol</td>
<td>[25–27]</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Lactate</td>
<td>[27–29]</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>Glutamate</td>
<td>[28,30]</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td>Glucose</td>
<td>[5]</td>
</tr>
<tr>
<td>Glycerol dehydrogenase</td>
<td>Glycerol</td>
<td>[30]</td>
</tr>
</tbody>
</table>

The scheme of the enzyme reactions with dehydrogenase participation can be presented as follows:

\[
S + E + NAD^+ \Leftrightarrow S^*E^*NAD^+ \\
\Leftrightarrow E + P + NADH + H^+ \tag{3}
\]

\[
NADH \Rightarrow NAD^+ + H^+ + 2e^- \tag{4}
\]

Hydrolitic enzymes (e.g., alkaline phosphatase [31]) can be also used if electroactive particles are produced during reaction.

The sensors with co-immobilized enzymes are also referred to the first class of amperometric biosensors. In this system the product of one enzyme reaction functions as a substrate for another. The final product has to be electroactive substance though (Fig. 3). The examples of such systems are presented in Table 3.

The main disadvantages of amperometric biosensors of the first group are as follows:

- Necessity of electrode pretreatment for obtaining reproducible surface, electrode geometry effect on sensor response;
- Effect of particle mass transfer through biocatalytic and semipermeable membranes on biosensor response;
- Necessity of permanent correction of sensor calibration because of Faraday’s processes, especially at long-term practice;
- Permanent data correction due to the effect of background solution as a result of interference with nonspecific particles certainly present in the solution.

To avoid the first drawback, the surface is polished, processed to heat or chemical treatment, the cyclic voltamperometry is used. These techniques often result in improvement of response reproducibility. Yet, this effect vanishes at long-term exposition of an electrode to the tested solution. A promising approach to overcome this obstacle is chemical modification of the surface [23,42], using high-conductive organic materials [43,44] or redox-polymers [45]. In particular, conductive organic salts produced from N-methylphenazine (NMP) and tetracyanoquinodimethane (TCNQ) were successfully used in dehydrogenase-based sensors [44,46], while conductive organic salts on the basis of TCNQ and tetraethylfulvalene (TTF) were used as an electrode material in oxidase-based sensors [47].

One more important issue is an option of electrode geometry, especially for sensor miniaturization [48].

Particle mass transfer during reaction consists of the following processes:

Table 3
Multiienzymes systems for application in amperometric biosensors

<table>
<thead>
<tr>
<th>Enzyme system</th>
<th>Substrate</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytrate lyase – oxaloacetatecarboxilase – piruvate oxidase</td>
<td>Cytrate</td>
<td>[32,33]</td>
</tr>
<tr>
<td>Invertase – mutarotase – glucose oxidase</td>
<td>Sucrose</td>
<td>[34]</td>
</tr>
<tr>
<td>Amyloglucosidase – glucose oxidase</td>
<td>Maltose</td>
<td>[35,36]</td>
</tr>
<tr>
<td>β-Galactosidase – glucose oxidase</td>
<td>Lactose</td>
<td>[37,38]</td>
</tr>
<tr>
<td>Acetylcholinesterase – choline oxidase</td>
<td>Acetyl choline</td>
<td>[39]</td>
</tr>
<tr>
<td>Alcaline phosphatase – glucose oxidase</td>
<td>Phosphates</td>
<td>[40]</td>
</tr>
<tr>
<td>Phospholipase – choline oxidase</td>
<td>Lecitine</td>
<td>[41]</td>
</tr>
</tbody>
</table>
substrate diffusion toward the membrane surface;
substrate diffusion across membrane toward the enzyme active center;
hydrogen peroxide generation or oxygen absorption;
their diffusion toward electrode surface.

In practice, intensive stirring is often used to make the diffusion layer thinner, thus decreasing its effect on sensor response. Yet, the membrane influence is unavoidable and has to be taken into account at response analysis. Artificial changes in diffusion coefficients of substrates allows the regulating of the sensor operational range, response time and sensitivity [49].

However, the main problems of utilization of amperometric sensors of the first group are the necessity of permanent correction of calibration curve and signal as well as taking into account electroactive interfering particles, such as ascorbic acid, uric acid, glutathione, etc. Differential mode of measurement [22,50] which operates with the signal difference between enzyme electrode and identical electrode without enzyme is promising from this viewpoint. Various charged dialysis membranes can be also used [31,51], such as acetate cellulose and polycarbonate or polyvinylchloride films. The usage of dialysis membranes improved sensor stability and allowed working in real biological liquids both in vitro and in vivo.

3. Amperometric mediated biosensors

The second group of amperometric biosensors is constituted of the transducers which use alternative oxidizing agents – mediators – as electron carriers. As a result we can work with low potentials, thus the influences of oxygen (in case of oxydase) and of different interferents on response decrease. Some important redox-potentials for amperometric measurements are presented in Table 4.

In general, a mediator is a low-molecular weight particle, which transfers electrons between redox center of enzyme and working electrode. In this case the Reactions (2) and (4) will be as follows:

\[
E_{\text{FADH}} + \text{MED}_{\text{ox}} \rightarrow E_{\text{FAD}} + \text{MED}_{\text{red}} \tag{5}
\]

\[
\text{NADH} + \text{MED}_{\text{ox}} \rightarrow \text{NAD}^+ + 2\text{H}^+ + \text{MED}_{\text{red}} \tag{6}
\]

The principle of operation of amperometric mediated biosensor is shown in Fig. 4.

All mediators could be divided into two groups:

- natural;
- artificial electron carriers (Table 5).

The most common and well-known mediators are ferricyanide and ferrocene.

Mediators could be added to measuring solution or immobilized on electrode surface. The first variant is easier but it is not technological. Moreover, for example, organic colorants such as methylene blue, phthalocyanine or methyl violet are toxic, unstable to reduction, pH sensitive and often could be autooxidized. Optimal and more technological variant is immobilized mediators.

Some amperometric mediated biosensors are presented in Table 6.

Low stability of biosensors based on immobilized mediators restricts their further development. Various methods were used to overcome this problem. In the simplest case, a carbon electrode was produced by mixing mediator powder with carbon

---

**Table 4**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$E$ (V)</th>
<th>Reaction</th>
<th>$E$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate – acetalddehyde</td>
<td>$-0.6$</td>
<td>Oxaloacetate – t-malate</td>
<td>$-0.17$</td>
</tr>
<tr>
<td>Acetone – propan-2-ol</td>
<td>$-0.43$</td>
<td>Fumarate – succinate</td>
<td>$+0.03$</td>
</tr>
<tr>
<td>$\text{H}^+ – \text{H}_2$</td>
<td>$-0.42$</td>
<td>Dehydroascorbate – ascorbate</td>
<td>$+0.06$</td>
</tr>
<tr>
<td>Xanthisine – hypoxanthine</td>
<td>$-0.37$</td>
<td>Ubiquinone – reduced ubiquinone</td>
<td>$+0.10$</td>
</tr>
<tr>
<td>(\text{NAD}^+ – \text{NADH})</td>
<td>$-0.32$</td>
<td>Ferrocene</td>
<td>$+0.17$</td>
</tr>
<tr>
<td>(\text{Oxidised glutathione} – \text{reduced glutathione})</td>
<td>$-0.23$</td>
<td>(\text{O}_2 – \text{H}_2\text{O}_2)</td>
<td>$+0.31$</td>
</tr>
<tr>
<td>Cystine – cysteine</td>
<td>$-0.22$</td>
<td>[Fe(CN)$_6$]$^{3-}$ – [Fe(CN)$_6$]$^{4-}$</td>
<td>$+0.45$</td>
</tr>
<tr>
<td>Acetate aldehyde – ethanol</td>
<td>$-0.20$</td>
<td>Fe$^{3+}$ – Fe$^{2+}$</td>
<td>$+0.53$</td>
</tr>
<tr>
<td>Piruvate – t-malate</td>
<td>$-0.19$</td>
<td>$\text{O}_2 – \text{H}_2$</td>
<td>$+0.82$</td>
</tr>
</tbody>
</table>

**Table 5**

<table>
<thead>
<tr>
<th>Natural mediators</th>
<th>$E$ (V)</th>
<th>Artificial mediators</th>
<th>$E$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome $a_3$</td>
<td>$+0.29$</td>
<td>Ferricyanide (hexacyanoferrate (III))</td>
<td>$+0.45$</td>
</tr>
<tr>
<td>Cytochrome $c_1$</td>
<td>$+0.24$</td>
<td>2,6-dichlorophenol</td>
<td>$+0.24$</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>$+0.10$</td>
<td>Indophenol</td>
<td>$+0.24$</td>
</tr>
<tr>
<td>Cytochrome $b$</td>
<td>$+0.08$</td>
<td>Ferrocene</td>
<td>$+0.17$</td>
</tr>
<tr>
<td>Vitamin $K_2$</td>
<td>$-0.03$</td>
<td>Phenazine</td>
<td>$+0.07$</td>
</tr>
<tr>
<td>Rubredoxin</td>
<td>$-0.05$</td>
<td>Methylenblu</td>
<td>$+0.04$</td>
</tr>
<tr>
<td>Flavoproteins</td>
<td>$-0.4$</td>
<td>Phtalocyanine</td>
<td>$-0.02$</td>
</tr>
<tr>
<td>FAD–FADH$_2$</td>
<td>$-0.23$</td>
<td>Phenosafranine</td>
<td>$-0.23$</td>
</tr>
<tr>
<td>FMN–FMNH$_2$</td>
<td>$-0.23$</td>
<td>Benzyl violet</td>
<td>$-0.36$</td>
</tr>
<tr>
<td>NAD$^+-$NADH</td>
<td>$-0.32$</td>
<td>Methyl violet</td>
<td>$-0.46$</td>
</tr>
<tr>
<td>NADP$^+$–NADPH</td>
<td>$-0.32$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>$-0.43$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
paste (consisting of liquid paraffin and graphite powder) with subsequent enzyme adsorption [64]. A dialysis membrane protected the mixture components from washing out into solution. The methods of direct mediator adsorption onto the electrode surface were also used. For example, a drop of dimethylferrocene solution in toluene was deposited on the electrode surface and then, after toluene evaporation, glucose oxidase was immobilized [52]. However, the mediator solubility can influence the sensor characteristics in this case. Actually, when the sensor is dipped into solution, the insoluble mediator stays at the surface. At applying voltage, the ferricinium ions are generated; being highly soluble in aqueous solutions they are washed out, thus reducing mediator volume on the electrode surface, which results in decreasing response.

In order to remove the mentioned disadvantage some conductive polymers, modified by the mediators, have been developed [65]. Polypyrrole film was applied on the gold electrode surface with subsequent covalent binding of ferrocenecarboxylchloride and glucose oxidase. The electrode obtained was stable in glucose solution for five days.

Another method to prevent the washing of the mediator out of the membrane is the introduction of the mediator and enzyme into colloidal graphite emulsion over which the cationic membrane is fixed [66]. The positively charged ferricinium ions are thus kept at the electrode surface. The electrode is simply manufactured, the response is fast, and the range of glucose determination is sufficiently wide. The enzyme residual activity after six-month storage under dry conditions was dropped up to 70%.

To select the proper mediator, the factors under consideration should be as follows:

- applied voltage should not exceed the oxygen reduction potential;
- reduced mediator should not react with oxygen;
- electron transfer between the mediator and enzyme should be very fast;
- mediator should not be influenced by pH;
- mediator should be nontoxic.

These challenges being satisfied, the amperometric biosensor can be proceeded with analytical characteristics provided for reliable prolonged operation under actual conditions.

### Table 6

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme</th>
<th>Mediator</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Glucose oxidase</td>
<td>Ferrocene</td>
<td>[52–54]</td>
</tr>
<tr>
<td>Fructose</td>
<td>α-fructose dehydrogenase</td>
<td>Ferricyanide</td>
<td>[55,56]</td>
</tr>
<tr>
<td>Lactate</td>
<td>Lactate oxidase</td>
<td>Ferrocene</td>
<td>[57,58]</td>
</tr>
<tr>
<td>Glutamate</td>
<td>L-glutamate oxidase</td>
<td>Ferrocene</td>
<td>[59,60]</td>
</tr>
<tr>
<td>Lysine</td>
<td>L-lysine dehydrogenase</td>
<td>Ferricyanide</td>
<td>[61]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Alcohol dehydrogenase</td>
<td>Ferricyanide</td>
<td>[62]</td>
</tr>
<tr>
<td>Morphine</td>
<td>Morphine dehydrogenase</td>
<td>Phenazine methasulfate</td>
<td>[63]</td>
</tr>
</tbody>
</table>

### 4. Amperometric biosensors based on direct electron transfer

Amperometric biosensors of the third group are based on direct electron transfer between enzyme and electrode. This phenomenon is often called bioelectrocatalysis. The key features of these biosensors setting them apart from other amperometric sensors are catalytic nature of the process as a whole as well as direct electron transfer from the electrode toward the substrate molecule and vice versa across active centre of enzyme without any carriers.

According to the theory of electron transfer [67,68], the rate constant of direct electrochemical interaction between donor and acceptor is determined by potential drop between the enzyme redox-centre and electrode, transformation energy at electron transfer, and mostly by a distance between enzyme active site and electrode surface. Peptide environment separates spatially enzyme redox-site, thus practically isolating active centre from electric contact with electrode surface. Therefore, to make electric contact between redox-protein and electrode, such mediators as electron carriers [52–63] and different modified electrode surfaces [23,42–47] were used. These methods have been above described in detail when mediator and mediatorless amperometric biosensors are presented. However, some authors sometimes relate these sensors mistakenly to the amperometric biosensors based on direct electron transfer. Meanwhile, in case of bioelectrocatalysis an electron itself is a cosubstrate of the reaction, hence enzyme and electrode reactions cannot be independent from one another.

By cyclic voltamperometry the direct electron transfer between cytochrome c and electrode was first demonstrated on the electrode made of tin-doped indium oxide [69] and gold electrode modified with 4,4’-bipyridine [70]. At the same time, cytochrome c3 from Desulfovibrio vulgaris was shown to be able of exchanging electrons with a mercury electrode [71]. From this moment on a number of works devoted to the direct electron transfer between cytochrome c and other redox-proteins, on the one hand, and various both modified and nonmodified electrodes, on the other, have been published. These works are thoroughly reviewed by Armstrong [72,73]. However, from the viewpoint of biosensor creation the direct electron transfer between redox-enzymes and electrode is more interesting.

In 1979, Tarasevich et al. were the first to study the mediatorless bioelectrocatalysis of fungal laccase, blue copper-containing oxidase [74]. They obtained the oxygen reduction on a carbon electrode with immobilized laccase at the potential of +1.2 V versus chlorine–silver electrode, pH 5.0:

\[
O_2 + 4e^- + 4H^+ \xrightarrow{\text{laccase}} 2H_2O +1.2 \text{ V vs. Ag/AgCl} \quad (7)
\]

Later on, this phenomenon was analyzed in detail considering a possible mechanism of biocatalytic reaction which appeared to be quite complicated [75,76]. In 1984, the catalytic current of oxygen reduction in water on laccase graphite electrode was also obtained by Lee et al. [77]. Biocatalytic mode was similar, but not entirely identical to that observed by Tarasevich et al. The potential of catalytic oxygen reduction was +0.74 V versus
calomel electrode at pH 4.7, which clearly differs from 1.2 V obtained earlier [78–80], this difference can be associated with different electrode materials.

Electrochemical reduction of horse-radish peroxidase was first studied by Yaropolov et al. in 1979 on a carbon electrode at potential smaller than +1.2 V versus calomel electrode [81]:

\[
\text{H}_2\text{O}_2 + 2\text{e}^- + 2\text{H}^+ \overset{+1.2 \text{ V vs. Hg/HgCl}}{\rightarrow} 2\text{H}_2\text{O} \tag{8}
\]

From this point, the research on peroxidase biocatalysis and its application in development of biosensors for hydrogen peroxide determination, in which various electrode materials (gold, platinum, pyrolytic graphite, carbon paste, soot or activated carbon) were utilized, was presented by a lot of authors [79–83]. In [83] the peroxidase-catalytic reduction of peroxide formed as a result of reactions with oxidases was of special attention in connection with the development of amperometric sensors. The scheme of direct peroxidase reduction on the activated carbon electrode [84] is presented in Fig. 5.

These electrodes demonstrated excellent electrocatalytic characteristics and high current density in the presence of peroxide at low potential without any subsidiary electrochemical and physical pretreatment of the electrode surface. Direct electron transfer between the electrode and peroxidase resulted in electroenzymatic reduction of hydrogen peroxide which occurred at the potential of +480 V versus Ag/AgCl reference electrode.

A possibility of direct electron transfer was also shown for cytochrome c peroxidase [85–87].

To obtain the electric contact between the enzyme redox-centre and electrode surface the chemical modification of redox-proteins by redox-associated components was proposed and examined [88,89]. The authors altered the glucose oxidase structure by covalent binding of 12 ferrocene-carboxyl oxygen groups to enzyme aminogroups. This procedure ensures decreasing distance of electron transfer since these groups function as electron relays between the FAD/FADH2 enzyme redox-centres and the electrode.

Another method for the electrical binding of enzyme redox-centre with electrode is immobilization into redox-polymeric matrix [90,91]. In such a way, glucose oxidase was immobilized in ferrocene-modified polymer or in osmium(II)-polypyrindine-substituted polymer, which resulted in glucose biocatalysis [91]. Similarly, the immobilization of nitrate-reductase into N,N'-bipyridine-modified polythiophene [92] was used.

Electrochemical redox-enzyme activation and subsequent direct electron transfer between the enzyme and electrode are especially important for the development of amperometric biosensors. Their key advantages are as follows:

- high sensitivity because of large current density which enables electrode miniaturization;
- remarkable decreasing of nonspecific interfering responses due to the effective electric activation of redox-enzyme providing high sensor selectivity and sensitivity.

5. Commercial systems based on amperometric biosensors

The major fields of practical application of amperometric biosensors are clinical diagnostics, food industry, biotechnological production and ecological monitoring. Clinical diagnostics
### Table 8
Portable analyzers for home diagnostics

<table>
<thead>
<tr>
<th>Company</th>
<th>Country</th>
<th>Model</th>
<th>Substance tested</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Laboratories, Illinois, IL</td>
<td>USA</td>
<td>MediSense ExacTech, MediSense Precision</td>
<td>Glucose, Glucose</td>
<td>[103]</td>
</tr>
<tr>
<td>Bayer Corporation, Leverkusen</td>
<td>Germany</td>
<td>Glucometer Elite</td>
<td>Glucose</td>
<td>[104]</td>
</tr>
<tr>
<td>i-STAT Corporation, Princeton, NJ</td>
<td>USA</td>
<td>i-STAT G3+</td>
<td>Glucose, oxygen</td>
<td>[105]</td>
</tr>
<tr>
<td>Roche Diagnostics, Basel</td>
<td>Switzerland</td>
<td>Advantage Meter</td>
<td>Glucose</td>
<td>[106]</td>
</tr>
<tr>
<td>Johnson &amp; Johnson, Brunswick, NJ</td>
<td>USA</td>
<td>LifeScan OneTouch</td>
<td>Glucose</td>
<td>[107]</td>
</tr>
<tr>
<td>Home Diagnostics Inc., Fort Lauderdale FL</td>
<td>USA</td>
<td>Prestige IQ</td>
<td>Glucose</td>
<td>[108]</td>
</tr>
<tr>
<td>MedTest Systems, College Park, MD</td>
<td>USA</td>
<td>Medisensor 2001</td>
<td>Glucose, uric acid</td>
<td>[99]</td>
</tr>
<tr>
<td>Inverness Medical Technology Inc., Inverness</td>
<td>Scotland</td>
<td>Excel G</td>
<td>Glucose</td>
<td>[109]</td>
</tr>
</tbody>
</table>

is put deliberately at the first place since more than 80% of commercial devices are utilized in this domain.

The analyzers for clinical applications based on amperometric biosensors presented in Table 7 were manufactured by different companies starting with the first commercial apparatus for glucose determination produced by Yellow Springs Instr. (1975) [93].

At present, YSI Incorporated produces the YSI 2300 Stat-Plus analyzer for analysis of glucose in whole blood, serum and plasma as well as of lactate in cerebrospinal fluid (Fig. 6).

Twenty-five microlitres samples are analyzed, the result is displayed in a minute, and the interval between measurements is about 2 min. The enzyme (glucose or lactate oxidase) is immobilized between two membranes – polycarbonate and acetate cellulose. The first membrane restricts the substrate diffusion into the enzyme layer, the second one prevents the interference with other electrochemically active components. The lifetime of glucose sensitive membrane is 21 days, while of the lactate one – 14 days.

The analyzers presented in Table 7 have analogous characteristics since basically they use similar techniques and principles. Up to now they are the simplest and the cheapest laboratory analytical methods. All the data of measurement are stored in the analyzer memory, can be printed and directed to the external data base. Most of analyzer parameters can be varied according to the given programme, for example, boundaries of pathologic values (for repeated measurements automatically), protocols, operational communication language, etc. The analyzers enable continuous dynamic observation (e.g., “sugar profile”) and are vital in resuscitation units for express diagnostics.

A variety of portable analyzers based on amperometric biosensors are presented in Table 8.

Food industry and biotechnology are the fields where biosensors application recently proceeds though not as intensively as in medical diagnostics. In the first place, the known commercial systems used in medicine are adapted to the processes control, besides, some novelties are in progress.

There are two main variants of biosensor usage in production control – in situ and on line (not considering off-line analysis). At in situ, that is, the biosensor placed inside bioreactors, the following conditions should be taken into account:

- sensor should keep its efficiency even after sterilization;
- value of concentrations to be tested in bioreactors often exceeds the sensor range of measurement;
- presence of a lot of interfering particles of high concentration in the bioreactor;
- high temperature in the bioreactor can cause the sensor biomaterial inactivation.

Because of the requirements mentioned, the development of commercial in situ sensor so far is no success. The enzyme electrode for glucose in situ analysis during dough fermentation is described in [110]. There were two parts in the analyzer. The enzyme sensor, placed inside, consisted of four working electrodes modified with 1.1-dimethylferrocene and glucose oxidase immobilized on three of them. Sterility was provided by the external polycarbonate membrane with 0.015 μm pores on electrode surfaces and metal membrane with 2 μm pores covered with an outer housing. The sensor was in situ calibrated by the calibration solution flowing between the housing and enzyme electrodes and demonstrated operational stability for the period of four days, during which it dropped only by 15%.

Basically, all the systems used at present operate in so-called quasi-continuous mode. The analyzer is attached to the sampling system periodically supplying the probes from bioreactor.

A number of commercial analyzers for food industry and biotechnology are presented in Table 9.

In environmental monitoring, microbial amperometric sensors are mostly used [114], in particular, in sewage analysis for determination of biochemically oxidized components (BOC).
These sensors, however, are unsuitable for continuous control since the estimation takes about five days. The sensors based on immobilized *Bacillus subtilis* and *Trichosporon cutaneum* cells were developed for BOC express determination [115]. The sensor for determination of bacterial composition of solutions [116] is based on the mediators (e.g., p-quinone) ability to take electrons away from microorganism breathing cycle.

The mediator reoxidation on the electrode can be used as direct indication of bacterial activity in the solution. The commercial version of such biosensor, Midas Pro, is produced by Biosensori Iritche Spa (Rome, Italy) [117].

6. Conclusions

Three groups of the amperometric biosensors developed up-to-date are thoroughly described. The division of them into three groups is quite reasonable. It is motivated not only by the basic principles; moreover, they are three different generations of analytical devices.

Electroactivity of substrates and products of enzyme reaction is the key principle of the first generation of amperometric biosensors. Their main disadvantages are high applied potential and interference with nonspecific electroactive particles.

The biosensors of the second generation are based on using mediators as electron carriers. This approach allowed overcoming the mentioned disadvantages. However, other shortcomings occur. First, the mediators should be connected with electrode, which complicates the technology, and second, sometimes mediators take part in other interfering reactions.

The biosensors of the third generation use direct electron transfer between the electrode and enzyme and hence their principle attractiveness is the absence of any mediators. They are positively featured in high selectivity and sensitivity, and in the absence of interfering particles and interaction.

The amperometric biosensors are mostly utilized in commercial devices since they are studied to a greater extent and have some advantages. The major field of employing biosensors is medical diagnostics where numerous commercial devices are currently functioning. There is still insufficient application of biosensors in food industry and ecology, therefore, it can be considered as a serious challenge.

Acknowledgements

Part of this work was financially supported by National Academy of Sciences of Ukraine in the frame of Scientific and Technical Program “Sensors systems for medical-ecological and industrial-technological problems”.

References

Quing-Shan Li, Bang-Ce Ye, Bai-Xiang Liu, Jian-Jiang Zhong. Improvement of the performance of \( \text{H}_2\text{O}_2 \) oxidation at low working potential by incorporating TTF-TCNQ into a platinum wire electrode for glucose determination. Biosens Bioelectron 1999;14:327–34.


[92] NOVA Biomedical (http://www.bioresearchonline.com).
[93] LifeScan from Johnson and Johnson (http://www.lifescan.com).
[107] ExtraCare from Roche diagnostics (http://www.extracare.co.nz).
[108] LifeScan from Johnson and Johnson (http://www.lifescan.com).
[110] Inverness medical technology (http://invernessmedical.com).