**Review**

**Glucose Biosensors: 40 Years of Advances and Challenges**

*Joseph Wang*

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA;
e-mail: joewang@nmsu.edu

Received: November 7, 2000
Final version: November 27, 2000

**Abstract**

Forty years have passed since Clark and Lyons proposed the concept of glucose enzyme electrodes. Excellent economic prospects and fascinating potential for basic research have led to many sensor designs and detection principles for the biosensing of glucose. Indeed, the entire field of biosensors can trace its origin to this glucose enzyme electrode. This review examines the history of electrochemical glucose biosensors, discusses their current status and assesses future prospects in connection primarily to the control and management of diabetes.

**Keywords:** Glucose, Biosensor, Diabetes, Enzyme electrodes

---

**1. Introduction**

Diabetes is a world-wide public health problem. It is one of the leading causes of death and disability in the world. The diagnosis and management of diabetes mellitus requires a tight monitoring of blood glucose levels. The challenge of providing such tight and reliable glycemic control remains the subject of enormous amount of research [1, 2]. Electrochemical biosensors for glucose play a leading role in this direction. Amperometric enzyme electrodes, based on glucose oxidase (GOx) bound to electrode transducers, have thus been the target of substantial research [1, 2].

Since Clark and Lyons first proposed the initial concept of glucose enzyme electrodes in 1962 [3] we have witnessed tremendous activity towards the development of reliable devices for diabetes control. A variety of approaches have been explored in the operation of glucose enzyme electrodes. In addition to diabetes control, such devices offer great promise for other important applications, ranging from food analysis to bioprocess monitoring. The great importance of glucose has generated an enormous number of publications, the flow of which shows no sign of diminishing. Yet, despite of impressive advances in glucose biosensors, there are still many challenges related to the achievement of clinically accurate tight glycemic monitoring.

The goal of this review article is to examine the history of electrochemical glucose biosensors, assess their current status, and discuss future challenges.

---

**2. Forty Years of Progress**

The idea of a glucose enzyme electrode was proposed in 1962 by Clark and Lyons from the Children Hospital in Cincinnati [3]. Their first device relied on a thin layer of GOx entrapped over an oxygen electrode (via a semipermeable dialysis membrane), and monitoring the oxygen consumed by the enzyme-catalyzed reaction:

\[
\text{glucose + oxygen} \rightarrow \text{gluconic acid + hydrogen peroxide} \quad (1)
\]

Clark’s original patent [4] covers the use of one or more enzymes for converting electroinactive substrates to electroactive products. The effect of interferences was corrected by using two electrodes (one covered with GOx) and measuring the differential current. Clark’s technology was subsequently transferred to Yellow Spring Instrument Company that launched in 1975 the first dedicated glucose analyzer (the Model 23 YSI analyzer) for the direct measurement of glucose in 25 μL samples of whole blood. Updike and Hicks [5] developed further this principle by using two oxygen working electrodes (one covered with the enzyme) and measuring the differential current for correcting for the oxygen background variation in samples. Guilbault and Lubrano [6] described in 1973 an enzyme electrode for the determination of blood glucose based on amperometric (anodic) monitoring of the liberated hydrogen peroxide:

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

Good precision and accuracy were obtained in connection to 100 μL blood samples. A wide range of amperometric enzyme electrodes, differing in the electrode design or material, membrane composition, or immobilization approach have since been described.

During the 1980s biosensors became a ‘hot’ topic, reflecting the growing emphasis on biotechnology. Intense efforts during this decade focused on the development of mediator-based ‘second-generation’ glucose biosensors [7, 8], the introduction of commercial strips for self-monitoring of blood glucose [9, 10], and the use of modified electrodes for enhancing the sensor performance [11]. In the 1990s we witnessed intense activity towards the establishment of electrical communication between the GOx redox center and the electrode surface [12, 13], and the development of minimally-invasive subcutaneously implantable devices [14–16]. Table 1 summarizes major historical landmarks in the development of electrochemical glucose biosensors.

---

**3. First-Generation Glucose Biosensors**

First-generation devices have relied on the use of the natural oxygen cosubstrate, and the production and detection of hydrogen peroxide (Equations 1 and 2). Such measurements of...
peroxide formation has the advantage of being simpler, especially when miniaturized sensors are concerned. A very common configuration is the YSI probe, involving the entrapment of GOx between an outer diffusion-limiting/biocompatible polycarbonate membrane and an inner anti-interference cellulose acetate one (Fig. 1).

3.1. Redox Interferences

The amperometric measurement of hydrogen peroxide requires application of a potential at which coexisting species, such as ascorbic and uric acids or acetaminophen, are also electroactive. The anodic contributions of these and other oxidizable constituents of biological fluids can compromise the selectivity and hence the overall accuracy. Extensive efforts during the 1980s were devoted for minimizing the error of electroactive interferences in glucose electrodes. One useful strategy is to employ a permselective coating that minimizes access of such constituent to the transducer surface. Different polymers, multilayers and mixed layers, with transport properties based on size, charge, or polarity, have thus been used for discriminating against coexisting electroactive compounds [17–19]. Such films also exclude surface-active macromolecules, hence imparting higher stability. Electropolymerized films, particularly poly(phenylenediamine) and overoxidized polypyrrole, have been shown particularly useful for imparting high selectivity (based on size exclusion) while confining the GOx onto the surface [17, 18]. Other widely used coatings include the negatively charged (sulfonated) Nafion or Kodak AQ ionomers, size-exclusion cellulose acetate films, and hydrophobic alkanethiol or lipid layers. The use of multi-(overlaid) layers, combining the properties of different films, offers additional advantages. For example, alternate deposition of cellulose acetate and Nafion was used for eliminating the interference of the neutral acetaminophen and negatively charged ascorbic and uric acids, respectively [19].

Efforts during the 1990s focused on the preferential electrocatalytic detection of the liberated hydrogen peroxide [20–23]. This has allowed tuning of the detection potential to the optimal region (ca. 0.0 to −0.20 V vs. Ag/AgCl) where most unwanted background reactions are negligible. The remarkably high selectivity thus obtained was coupled to a fast and sensitive response. Metalized (Rh,Ru)-carbon [20, 21] and metal-hexacyanoferrate [22, 23] based transducers have been particularly useful for enhancing the selectivity towards the target glucose substrate. Additional improvements can be achieved by combining this preferential catalytic activity with a discriminative layer, e.g., by dispersing rhodium particles within a Nafion film [24].

3.2. Oxygen Dependence

Since oxidase-based devices rely on the use of oxygen as the physiological electron acceptor, they are subject to errors accrued from fluctuations in the oxygen tension and the stoichiometric limitation of oxygen. Such limitation (known as the “oxygen deficit”) reflects the fact that normal oxygen concentrations are about an order of magnitude lower than the physiological level of glucose.

Several routes have been proposed for addressing this oxygen limitation. One strategy relies on the use of mass-transport limiting films (such as polyurethane or polycarbonate) for tailoring the flux of glucose and oxygen, i.e., increasing the oxygen/glucose permeability ratio [1, 25]. A two-dimensional electrode, designed by Gough’s group [25], has been particularly attractive for addressing the oxygen deficit by allowing oxygen to diffuse into the enzyme region of the sensor from both directions and glucose diffusion only from one direction. We have recently addressed the oxygen limitation of glucose biosensors by designing an oxygen-rich carbon paste enzyme electrode [26]. The new biosensor is based on a fluorocarbon (Kel-F oil) pasting liquid, which has very high oxygen solubility, allowing it to act as an internal source of oxygen. The internal flux of oxygen can thus support the enzymatic reaction even in oxygen-free glucose solutions. It is possible also to circumvent the oxygen demand issue by replacing the GOx with glucose dehydrogenase (GDH) that does not require an oxygen cofactor [27].
4. Second-Generation Glucose Biosensors

4.1. Electron Transfer between GOx and Electrode Surfaces

Further improvements (and attention to the above errors) can be achieved by replacing the oxygen with a nonphysiological (synthetic) electron acceptor, which is able to shuttle electrons from the redox center of the enzyme to the surface of the electrode. Glucose oxidase does not directly transfer electrons to conventional electrodes because a thick protein layer surrounds its flavin redox center. Such thick protein shell introduces a spatial separation of the electron donor-acceptor pair, and hence an intrinsic barrier to direct electron transfer, in accordance to the distance dependence of the electron transfer rate [28]:

\[ K_{et} = 10^{13}e^{-0.91(d-3)\frac{d}{RT} + \frac{d}{R}} \]

where \( \Delta G \) and \( \lambda \) correspond to the free and reorganization energies accompanying the electron transfer, respectively, and \( d \) the actual electron transfer distance. The minimization of the electron-transfer distance (between the immobilized GOx and the electrode surface) is thus crucial for ensuring optimal performance. Accordingly, various innovative strategies have been suggested for establishing and tailoring the electrical contact between the redox center of GOx and electrode surfaces.

4.2. Use of Artificial Mediators

Particularly useful has been the use of artificial mediators that shuttle electrons between the FAD center and the surface by the following scheme:

\[ \text{glucose} + \text{GOx}_{\text{ox}} \rightarrow \text{gluconic acid} + \text{GOx}_{\text{red}} \]  
\[ \text{GOx}_{\text{red}} + 2\text{M}_{\text{ox}} \rightarrow \text{GOx}_{\text{ox}} + 2\text{M}_{\text{red}} + 2\text{H}^+ \]  
\[ 2\text{M}_{\text{red}} \rightarrow 2\text{M}_{\text{ox}} + 2e^- \]

where \( \text{M}_{\text{ox}} \) and \( \text{M}_{\text{red}} \) are the oxidized and reduced forms of the mediator. Such mediation cycle produces a current dependent on the glucose concentration. Diffusional electron mediators, such as ferrocene derivatives, ferricyanide, conducting organic salts (particularly tetrathiafulvalene-tetracyanoquinodimethane, TTF-TCNQ), phenothiazine and phenoxazine compounds, or quinone compounds have thus been widely used to electrically contact GOx [7, 8] (Fig. 2). As a result of using these electron-carrying mediators, measurements become largely independent of oxygen partial pressure and can be carried out at lower potentials that do not provoke interfering reactions from coexisting electroactive species (Equation 6). In order to function effectively, the mediator should react rapidly with the reduced enzyme (to minimize competition with oxygen), possess good electrochemical properties (such as a low redox potential), have low solubility in aqueous medium, and must be nontoxic and chemically stable (in both reduced and oxidized forms). Commercial blood glucose self-testing meters, described in the following section, commonly rely on the use of ferrocene or ferricyanide mediators. Most in vivo devices, however, are mediatorless due to potential leaching and toxicity of the mediator.

4.3. Attachment of Electron-Transfer Relays

Heller’s group [12] developed an elegant nondiffusional route for establishing a communication link between GOx and electrodes based on ‘wiring’ the enzyme to the surface with a long flexible poly-pyridine polymer having a dense array of osmium-complex electron relays. The resulting three-dimensional redox-polymer/enzyme networks offer high current outputs and stabilize the mediator to electrode surfaces.

Chemical modification of GOx with electron-relay groups represents another novel avenue for facilitating the electron transfer between its redox center and the electrode surface. Willner and co-workers [13] reported on an elegant approach for modifying GOx with electron relays (Fig. 3). For this purpose, the FAD active center of the enzyme was removed to allow positioning of an electron-mediating ferrocene unit prior to the reconstitution of the enzyme. The attachment of electron-transfer relays at the enzyme periphery has also been considered for yielding short electron-transfer distances [29]. More sophisticated bioelectronic systems for enhancing the electrical response, based on patterned monolayer or multilayer assemblies and organized enzyme networks on solid electrodes, have been developed for contacting GOx with the electrode support [29]. Functionalized alkanethiol modified gold surfaces have been particularly attractive for such layer-by-layer creation of GOx/mediator networks.

5. In Vitro Glucose Testing

Electrochemical biosensors are well suited for satisfying the needs of home (personal) glucose testing. The majority of personal blood glucose meters are based on disposable (screen-printed) enzyme electrode test strips. Such single-use disposable electrode strips are mass produced by the thick-film (screen-printing) microfabrication technology. Each strip contains the printed working and reference electrodes, with the working one coated with the necessary reagents (i.e., enzyme, mediator, stabilizer, linking agent). Such reagents are commonly being dispensed by an ink-jet printing technology. A counter and an additional (‘baseline’) working electrode may also be included. Such single-use devices obviate problems of carry over, contamination, or drift.

The control meter is typically light and small (pocket-size), battery operated, and relies on a potential-step (chronoamperometric) operation. Such devices offer considerable promise for obtaining the desired clinical information in a faster, simpler (“user-friendly”), and cheaper manner compared to traditional assays. The first product was a pen-style device (the Exactech), launched by Medisense Inc. in 1987, that relied on the use of a ferrocene-derivative mediator. Various commercial strips and pocket-sized test meters, for self-monitoring of blood glucose –
based on the use of ferricyanide or ferrocene mediators – have since been introduced (Table 2) [30]. In most cases, the diabetic patient pricks the finger, places the small blood droplet on the sensor strip, and obtains the blood glucose concentration (on a LC display) within 15–30 s. Recent efforts have led to new strips, requiring sub-micrometer blood volumes and enabling “less-painful” sampling from the arm. In addition to small size, fast response, and minimal sample requirements, such modern personal glucose meters have features such as extended memory capacity and computer downloading capabilities.

6. Continuous In Vivo Monitoring

Although self testing is considered a major advance in glucose monitoring it is limited by the number of tests per a 24 h period. Such testing neglect nighttime variations and may result in poor approximation of blood glucose variations. Tighter glycemic control, through more frequent measurements or continuous monitoring, is desired for triggering proper alarm in cases of hypo- and hyperglycemia, and for making valid therapeutic decisions [15]. A wide range of possible in vivo glucose biosensors has thus been studied for maintaining glucose levels close to normal. The first application of such devices for in vivo glucose monitoring was demonstrated first by Shichiri et al. in 1982 [31]. Continuous ex vivo monitoring of blood glucose was proposed already in 1974 [32].

6.1. Requirements

The major requirements of clinically accurate in vivo glucose sensors have been discussed in various review articles [1, 15]. These include proper attention to the issues of biocompatibility/biofouling, miniaturization, long-term stability of the enzyme and transducer, oxygen deficit, baseline drift, short stabilization times, in vivo calibration, safety, and convenience. The sensor must be of a size and shape that can be easily implanted and cause minimal discomfort. Under biocompatibility one must consider the effect of the sensor upon the in vivo environment as well as the environment effect upon the sensor performance. Problems with biocompatibility have proved to be the major barriers to the development of reliable implantable devices. Most glucose biosensors lack the biocompatibility necessary for a prolonged and reliable operation in whole blood. Alternative sensing sites, particularly the subcutaneous tissue, have thus received growing attention. While the above issues represent a major challenge, significant progress has been made towards the continuous monitoring of glucose.

6.2. Subcutaneous Monitoring

Most of the recent attention has been given to the development of subcutaneously implantable needle-type electrodes (Fig. 4) [14–16]. Such devices are designed to operate for a few days and be replaced by the patient. Success in this direction has reached the level of short-term human implantation: continuously functioning devices, possessing adequate (>1 week) stability, are expected in the near future. Such devices would enable a swift and appropriate corrective action (through a closed-loop insulin delivery system, i.e., an artificial pancreas). Algorithms correcting for the transient difference (time lag) between blood and tissue glucose concentrations have been developed [16]. The recently introduced CGMS unit of Minimed. Inc. (Sylmar, CA) offers a 72 h of such subcutaneous monitoring, with measurement of tissue glucose every 5 min and data storage in the monitor’s memory. After 72 h, the sensor is removed, and the

---

Table 2. Commercial electrochemical systems for self-monitoring of blood glucose.

<table>
<thead>
<tr>
<th>Source</th>
<th>Trade name</th>
<th>Enzyme</th>
<th>Mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbot/Medisense</td>
<td>Precision</td>
<td>GOx</td>
<td>Ferrocene</td>
</tr>
<tr>
<td>Bayer</td>
<td>Elite</td>
<td>GOx</td>
<td>Ferricyanide</td>
</tr>
<tr>
<td>LifeScan</td>
<td>SureStep</td>
<td>GOx</td>
<td>Ferricyanide</td>
</tr>
<tr>
<td>Roche-Diagnostics</td>
<td>Accu-Check</td>
<td>GOx</td>
<td>Ferricyanide</td>
</tr>
<tr>
<td>Therasense</td>
<td>FreeStyle</td>
<td>GOx</td>
<td>Osmium ‘wire’</td>
</tr>
</tbody>
</table>

---

Fig. 3. Electrical contacting of a flavoenzyme by its reconstitution with a relay-FAD semisynthetic cofactor. (Reproduced with permission [29b]).
information is transferred to a computer for identifying patterns of glucose variations. In addition to easily removable short-term implants, efforts are continuing towards chronically implanted devices (aimed at functioning reliably 6–12 months).

6.3. Towards Noninvasive Glucose Monitoring

Noninvasive approaches for continuous glucose monitoring represent a promising route for obviating the challenges of implantable devices. In particular, Cygnus Inc. has developed an attractive wearable glucose monitor, based on the coupling of reverse iontophoretic collection of glucose and biosensor functions [33]. The new GlucoWatch biographer (shown in Fig. 5) provides up to three glucose readings per hour for up to 12 h (i.e., 36 readings within a 12 h period). The system has been shown to be capable of measuring the electroosmotically extracted glucose with a clinically acceptable level of accuracy. An alarm capability is included to alert the individual of very low or high glucose levels. Other routes for “collecting” the glucose through the skin and for noninvasive glucose testing are currently being examined by various groups and companies.

7. Conclusions and Prospects

Over the past forty years we have witnessed an intense activity and tremendous progress towards the development of electrochemical glucose biosensors. Major advances have been made for enhancing the capabilities and improving the reliability of glucose measuring devices. Such intensive activity has been attributed to tremendous economic prospects and fascinating research opportunities. The success of glucose blood monitors has stimulated considerable interest in in vitro and in vivo devices for monitoring other physiologically important compounds. Despite the impressive advances in glucose biosensors, there are still many challenges related to the achievement of tight, stable and reliable glycemic monitoring. The development of new and improved glucose biosensors thus remains the prime focus of many researchers.

As this field enters the fifth decade of intense research we expect significant efforts coupling fundamental sciences with technological advances. Such stretching of the ingenuity of researchers will result in greatly improved electrical contact between the redox center of GOx and electrode surfaces, enhanced “genetically engineered” GOx, new “painless” in vitro testing, advanced biocompatible membrane materials, the coupling of minimally invasive monitoring with compact insulin delivery system, new innovative approaches for noninvasive monitoring, and miniaturized long-term implants. These, and similar developments, will greatly improve the control and management of diabetes.

8. Acknowledgement

Financial support from the National Institute of Health (NIH grant RO1 RR 14173-03) is gratefully acknowledged.

9. References