Trends in electrochemical biosensors†

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The development of electrochemically based biosensors is discussed in the context of what has been learned from the successful development of glucose biosensors. Some future trends are discussed.

Much has been written of the puissance of electrochemical biosensors (and of biosensors in general) to transform traditional methods of analysis, and in particular of the ability of such sensors to perform fast and specific on-site monitoring of a wide range of analytes. In the last two decades there has been a huge effort in academic and in industrial laboratories devoted to developing biosensors for an array of analytes. As with almost every new type of technology, extravagant claims for such devices have been made. Yet, after the expenditure of an enormous amount of effort, only one commercially successful product (albeit in a number of different forms) has been produced. The development of a biosensor for glucose is, therefore, unique. In this perspective the development of electrochemical glucose biosensors is examined with the aim of explaining why they are the only commercially successful product to date.

The origins of the first successful electrochemical biosensor for glucose can be traced to the efforts of Leland Clark in the 1950s.1 At that time Clark was working on the development of the first heart–lung machine and required a reliable method of measuring oxygen levels in blood. To overcome the deleterious effects encountered in using the complex matrix blood, he placed a cellophane membrane in front of a platinum electrode and measured the oxygen concentration by monitoring the current resulting from the application of a potential bias to the electrode. By placing enzyme, glucose oxidase (GOx), in solution between the membrane and the electrode, Clark found that the concentration of glucose could be monitored by detecting the decrease in oxygen tension resulting from the enzymatic oxidation of glucose to gluconic acid and hydrogen peroxide (Scheme 1).2 Alternatively, application of a potential of ca. 0.6 V enabled measurement of hydrogen peroxide to be carried out. Upon further refinement, this latter approach became the basis for a commercially viable instrument developed by Yellow Springs Instruments (YSI)3 in the early 1970s, which was the first commercially available electrochemical biosensor (Fig. 1). A biosensor device, using a similar principle of operation was successfully developed and marketed by Scheller et al. some five years later.4

The glucose sensor developed by YSI proved to be highly successful, gaining acceptance in both clinical and in hospital laboratories as a reliable and accurate means of directly measuring blood glucose concentrations. This analysis is one of the most commonly performed procedures in hospital laboratories.

Diabetes is the most frequently encountered disorder of carbohydrate metabolism and results in elevated blood glucose concentrations.5 If uncontrolled, it can be a life threatening condition. As such, it is the sixth leading cause of death in the US. Diabetics are unable to regulate the concentration of glucose in their blood and require an external method of monitoring blood glucose levels. In 1993 the National Institutes of Health published the results of a large-scale trial, the Diabetes Control and Complications Trial,6 which showed that diabetic patients, who maintained their blood glucose concentrations at or near normal levels, substantially reduced complications due to diabetes. A recommendation of this study was that diabetics should monitor their blood glucose levels at least four times per day.

The YSI sensor, while extremely reliable, was too expensive (currently one unit costs ca. $8,000) and much too large (dimensions of ca. 30 × 20 × 15 cm) to be used as a portable sensor. Portable devices to measure blood glucose levels have been available since the 1950s, when urine test strips, developed by Ames, Inc., became available. These tests were cumbersome to perform (the timing of the test had to be controlled manually by the patient) and were not sufficiently accurate. The growing demand for a more practical and reliable test spurred on development of devices that could give direct readings of blood glucose concentrations in a timely, simple manner. It should be emphasised that the large market for such devices was an essential component to their successful development. In 1995, diabetes and complications arising from the condition were estimated by the National Institutes of Health to cost the US economy $140 billion or approximately 14% of the entire US health care budget. Such a huge market ensures an adequate return for the development of a test that can require an initial investment of up to $100 million. Currently the world market for home use glucose testing is approximately $2 billion and is growing at a rate of 15% per annum. Sales of electrochemical


Scheme 1 Reaction scheme for the YSI glucose test.

\[
\begin{align*}
\text{glucose + GOx(FAD)} & \rightarrow \text{gluconolactone + GOx(FADH2)} \\
\text{GOx(FADH2) + O}_2 & \rightarrow \text{GOx(FADH2)} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 & \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-
\end{align*}
\]

Fig. 1 Schematic diagram of the glucose sensor developed by Yellow Springs Instruments, Inc. (diagram courtesy of YSI).
The development of a successful test for blood glucose presented a number of challenges in addition to those normally demanded of a clinical method. A successful test should:

1. Be small, cheap and portable. The sensor is designed to be used by diabetics who need to test their blood glucose levels on a daily basis. Ideally the sensor should be the size of a wallet or smaller, powered by batteries and cost less than $100.

2. Require a small sample volume. For practical reasons, blood is collected from the fingertips, one of the most sensitive parts of the body. Early versions of glucose biosensors required 15–20 μl of blood, which is a significant volume for a finger stick analysis, considering that it has to be performed a number of times each day. Current versions of glucose biosensors require as little as 3 μl of blood, a significant improvement over earlier sensors. However, obtaining a blood sample (even if only 3 μl) is still the single most important impediment to more frequent testing of blood glucose by diabetics. Simply put, it is painful to extract blood. Frequent testing leads to heavily calloused hands, making it even more difficult to obtain blood.

3. Employ undiluted whole blood. The test must be simple to run and thus should involve neither sample dilution nor separation of red cells from plasma, unless the latter is performed directly on the test strip by incorporation of some form of separation membrane.

4. Be a disposable, single use test. Multiple use strips would have to possess features that would ensure that contamination from a previous sample (from a measurement and from a safety point of view) would not interfere with the test results. Such a strip would be expensive to fabricate and difficult, if not impossible, to maintain in good working order. From the manufacturer’s point of view, this is an important consideration, as disposable single use tests are much more profitable than multiple use tests.

5. Be stable, with a shelf life of at least twelve months at room temperature. The test has to be convenient and simple, for use by a lay person as part of his or her normal daily routine. This precludes storage in, for example, a refrigerator where the shelf life would be presumably much longer. It is also important that the sensor be able to withstand temperature fluctuations that can occur during storage and transportation.

6. Possess stable calibration. The calibration should remain stable for the shelf life of the test. This is essential, as any recalibration would have to be performed by the end user. This would be too complicated to carry out in normal daily practice.

7. Have all required components incorporated. Again this is to ensure ease of use by the end user who, as known from experience with the first urine based test strips, demanded a test which required as few steps as possible. Therefore steps such as addition of reagent, wiping the test area, etc. have to be avoided.

8. Be capable of mass production. From a manufacturing point of view this is essential. Electrochemical glucose test strips are presently produced at rates of millions per day. Such levels of production require that the number of manufacturing steps be kept to a minimum and that the complexity of each step be kept as simple as possible.

9. Be low in cost. This should be transparently obvious. It should be remembered also that reimbursement rates for test strips for medically diagnosed diabetics are set (either by governments or by insurance companies) at a certain price, which varies from country to country. These rates in turn determine the price that can be charged by the manufacturer.

10. Be easy to use. It is necessary that the test can be used by diabetics ranging in age from as young as four years old to the elderly. It should therefore be technically very simple. Because older diabetics can have poorer vision and motor control than normal people, there are a number of design constraints. For instance the liquid crystal panel used to display the results should be of sufficient size that it can be read by a person with poor eyesight. In addition, the test strip should not be so small nor be packaged in such a way that it is difficult for a person with poor motor control to use it.

11. Work as well in the cool, damp environment encountered in Limerick as in the hot, dry environment of Arizona. In addition, it is desirable that the test can be performed while the user is standing, i.e., it should not need to be positioned on a flat, stable surface in order to work properly.

The glucose biosensor developed by YSI uses a relatively high potential bias of 0.6 V, which is necessary in order to efficiently oxidise hydrogen peroxide. Contamination of the electrode surface by protein fouling, and interference from substances such as ascorbate, can be eliminated by a composite membrane which is placed over the electrode surface. It is neither feasible nor cost efficient to use such a membrane on a disposable electrode. This fact precludes detection of hydrogen peroxide as a means of quantifying glucose. Since direct oxidation of glucose oxidase is not feasible at an electrode, it is necessary to add a mediator to the solution to shuttle electrons from the FAD group of the enzyme to the electrode surface.

The first version of an electrochemically based glucose biosensor specifically designed for home use came on the market in 1987 (released by MediSense, Inc.). This test is based on the work carried out in Hill’s laboratory at the University of Oxford. It uses a ferrocene derivative (1,1' -dimethylferrocene ethanolamine) as a mediator to oxidise glucose oxidase. Test strips are manufactured by screen printing a series of layers on to a poly(vinyl chloride) strip (Fig. 2). The strip itself consists of a three-electrode cell (Scheme 2). There are two working electrodes, the first containing enzyme and mediator (in addition to a number of additives) and the second

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**Scheme 2 Reaction scheme for the MediSense glucose test.**

**Working electrode (1)**

\[ \text{GOx(FADH)} + \text{glucose} \rightarrow \text{GOx(FADH)} + \text{glucoconolactone} \]

**Working electrode (2)**

\[ \text{Fe} \rightarrow \text{Fe}^+ + 2\text{e}^- \]

**Reference/Counter electrode**

\[ \text{AgCl} + e^- \rightarrow \text{Ag} + \text{Cl}^- \]
containing mediator (and additives). The function of the latter electrode is to quantify the level of interfering substances such as ascorbate that may be present in the sample. The glucose concentration is measured by subtracting the current obtained at the second electrode from that obtained at the first electrode. A silver/silver chloride electrode is used as the combined reference/counter electrode.

Subsequent to the launch of this test,* two other electrochemically based glucose biosensors (sold by Bayer® and by Boehringer-Mannheim®) became available for home use. Both of these tests utilise ferricyanide as a mediator.

The Boehringer electrode is manufactured in a continuous, web fed process. Palladium electrodes are laminated onto a polysulfone substrate. A solution containing enzyme, ferricyanide and a number of additives is dispensed onto the electrodes and allowed to dry. A high concentration of glucose oxidase is used (5–10 units per strip which compares to ca. 0.02 units on the ferrocene strips). This ensures that most, if not all, of the glucose in the sample is oxidised. A specified period of time after sample addition, a potential bias of ca. 300 mV is applied between the two electrodes. Oxidation of enzymatically generated ferrocyanide occurs at the working electrode and reduction of ferrocyanide to ferricyanide occurs at the second electrode (which acts as a pseudo-reference electrode). The concentration of glucose is then proportional to the measured current. A high concentration of ferricyanide is used, ca. 100 mM, both to overcome the relatively sluggish kinetics associated with the oxidation of glucose oxidase by ferricyanide and to provide a sufficient reservoir of reagent for the pseudo-reference electrode. The overall reaction scheme is shown in Scheme 3. The test manufactured by Bayer utilises the same reaction scheme but employs screen printed carbon electrodes rather than palladium. Each of these test strips performs reasonably well as a home use test for glucose. First versions of the tests possessed some, but not all of the features described above. Later versions (utilising the same sensor technology) incorporated improvements such as smaller sample size, etc. None, in terms of accuracy and precision, are as good as the YSI test, though the performance of each of the home use tests is steadily improving as the technology is refined. Future refinements will no doubt further increase the reliability and precision of each of the tests. The tests are however good enough to give diabetics information on their blood glucose level simply and quickly.

The tests suffer from a significant hematocrit (HCT) bias, in the range –0.5 to –1.5%/%HCT. Usually the tests are calibrated using blood samples containing normal levels of hematocrit, ca. 45%. A test with a –1.0%/%HCT bias will then give a reading that is 10% lower when the hematocrit is 55% and 10% higher when the hematocrit is 35%. Glucose is distributed between plasma and the red cells and accurate measurement of the concentration of glucose requires measurement of the total amount of glucose. In a test such as the YSI, the sample is diluted by a factor of 25 into isotonic buffer. The resultant large concentration gradient ensures that virtually all of the glucose in the red cells is transferred to the solution phase. Dilution is not feasible on tests designed for home use. In such whole blood sensors, the red cells screen a proportion of the glucose from the assay, producing a negative bias in the measurement. Even if all of the glucose in the sample was oxidised, a bias (in comparison to standard plasma assays) would remain due to differences in the water concentration of plasma (0.93 kg dm⁻³) and that of red cells (0.84 kg dm⁻³). In addition to this screening effect, red cells can also cause fouling of the electrode surface, a problem which does not arise with the YSI test since the membrane protects the electrode.

Each manufactured test has some advantage over the other. For instance, the ferrocene test has, by virtue of its second working electrode, the capability of subtracting out interfering substances such as ascorbate and uric acid. The other two tests do not need a reference electrode, an essential component for the ferrocene assay, eliminating the costs of the associated manufacturing steps. There is a trade off between the two approaches, and not only in terms of cost. The ferricyanide based tests are essentially titrations in which most of the glucose in the sample is oxidised. Completion of the reaction depends on the enzyme kinetics and will be limited by them. This is not the case with the ferrocene-based assay, which in principle can be made significantly faster, since it performs a kinetic, rather than an end-point measurement. Currently the ferrocene assay requires 20 s while the ferricyanide assays require 15–45 s (Boehringer test, with the length of time required being dependant on the concentration of glucose) and 30 s (Bayer test).

The huge advantage that these tests enjoy when compared to laboratory based tests is their portability, which allows the diabetic to incorporate testing of blood glucose levels into his or her normal daily routine. Because there is such a large number of diabetics and consequently such a large market, there is a considerable financial incentive to develop and market home use blood glucose tests. This large demand ensures that glucose sensors are unique and are likely to remain so for the foreseeable future. At present there is no other analyte that requires multiple daily testing by such a large number of people. Such a demand is vital for the successful commercialisation of a biosensor-based assay. In response to this demand, a number of companies are attempting to develop tests which would allow for the continuous monitoring of glucose levels, either by continually extracting fluid from the skin¹³ or by implanting a sensor subcutaneously.¹⁴ The advantage of such tests would be that they would offer a continuous profile of the concentration of glucose, instead of the snapshot provided currently. It remains to be seen if these tests will be successful.

While glucose sensors are unique, the development of biosensors for other clinical analytes would be favourably received. Some examples are listed in Table 1, along with the appropriate detection levels that are required. Demand for such tests ranges from small (fructosamines) to large (troponin-T).

A biosensor for troponin-T, which is used as a marker for myocardial infarction, would find an immediate market. The need for this type of assay is quite different from that of a glucose sensor, with the assay being conducted when the

<p>| Table 1 List of some target analytes for biosensors |</p>
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Disease/condition</th>
<th>Normal concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin</td>
<td>Myocardial infarction</td>
<td>10–92 µg L⁻¹ (ref. 18)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Myocardial infarction</td>
<td>20–38 U L⁻¹ (ref. 18)</td>
</tr>
<tr>
<td>Troponin-T</td>
<td>Myocardial infarction</td>
<td>0.1 ng ml⁻¹ (ref. 17)</td>
</tr>
<tr>
<td>Glycated haemoglobin</td>
<td>Diabetes</td>
<td>4.5–5.7% of total Hb (ref. 18)</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>Diabetes</td>
<td>1.6–2.7 mM (ref. 18)</td>
</tr>
</tbody>
</table>

* Electrochemical tests for glucose and lactate are now available on a number of clinical instruments (e.g., instruments manufactured by i-Stat (Princeton, NJ, USA), Radiometer (Copenhagen, Denmark), Chiron Diagnostics (Walpole, MA, USA) and Nova Biomedical (Waltham, MA, USA). Such instruments are designed for use in hospital laboratories, accident and emergency rooms, etc., and are outside the scope of this article.
occurrence of cardiac infarction is suspected. In such a situation, in addition to accuracy, a speedy diagnosis is often essential for a successful outcome. Ideally, such a test could be conducted in an ambulance en route to the hospital, eliminating any waiting time for test results after arrival of the patient at the hospital. The main difficulty in developing a test for troponin-T is that its concentration is low, less than 0.1 ng ml\(^{-1}\) or 1.4 pM.\(^{18}\) It is not feasible to detect such low levels by direct electrochemical methods. A successful test will require the incorporation of an appropriate recognition element such as an antibody together with an efficient means of amplifying the signal. Such tests may not require all of the features required for a glucose test. For example, small sample volumes of 5 µl may not be required. In addition the cost constraints that apply to glucose tests would not pertain, the need for additional materials such as antibodies, etc., would make the test more expensive to manufacture.

The development of a successful, portable immuno electrode would be of immense value since such a system would form a platform for the analysis of a wide range of analytes. In principle, different sensors could be constructed by changing the antibody. Such a device has yet to be constructed. In practice, variations in antibody stability, in the degree of cross reactivity exhibited by the antibody, the type of sample used, e.g., whole blood vs. plasma or fresh water vs. marine water, make it likely that each test will have to be developed and implemented on an individual basis.

Other areas where biosensors could play a successful role are in environmental analysis and in process control. Examples include the analysis of pesticides and herbicides in aquatic samples and in the monitoring of fermentation processes. In environmental analysis, the advantage of immediate on-site analysis is of great advantage when attempting to ascertain the extent of pollution in, for example, a lake. Laboratory based techniques require that samples be obtained over a wide area in order to delineate the area of contamination. In situ analysis would ensure that the extent of pollution would be known almost immediately, eliminating unnecessary sample analysis outside the polluted area as well as the cost of transporting samples back to the laboratory for analysis. Similarly, direct monitoring of the concentrations of species such as glucose, lactate, carbon dioxide and oxygen would be of great benefit in fermentation processes. The successful introduction of these devices requires that they incorporate many of the features listed above for glucose sensors. Some additional features will be necessary. In a fermentor, continuous monitoring is required, meaning that the sensor has to be stable for at least the duration of the fermentation cycle. Ideally, it should be stable for many such cycles and should be able to withstand autoclaving without deterioration in performance. While there are devices specifically designed for use in conjunction with fermentors (e.g., the instrument sold by YSI for measurement of the concentrations of glucose, glutamine and ammonia\(^{3}\)), these devices work offline. Thus far there are no commercially available, electrochemically-based biosensors for on-line monitoring. (BOD biosensors, based on whole organisms, are commercially available.)

It is worth reiterating that the main driving force behind the development of electrochemical glucose biosensors was the existence of the diabetic market. Although it is difficult to predict the size of the market for environmental or fermentation biosensors, in monetary terms these markets do not currently come anywhere near approaching the diabetic market. While stricter environmental legislation will no doubt create a larger market for environmental biosensors and there is widespread interest in developing such sensors (e.g., immunosensors for pesticides\(^{19-21}\) and organic pollutants\(^{22}\), the successful exploitation of this market awaits the development of a cheap, reliable and robust biosensor.

This article has focussed on the commercial development of electrochemical biosensors and has not discussed the use of such sensors for research purposes. It should be remembered that it is now possible to directly monitor the concentration of a number of metabolites using biosensors that have been developed solely for research purposes, e.g., a glutamate sensor.\(^{23}\) It will probably never be necessary to develop a glutamate biosensor for home use, but the use of such a sensor in research laboratories can help to answer fundamental questions on the metabolic pathways of glutamate and its role in ischaemia. Commercial benefits may accrue in the form of new drugs, the effect of which may be monitored \emph{in vivo} by direct measurement of glutamate levels.

A salient point is that the development of a glucose biosensor was far from the original intention of Leland Clark when he initially worked on the development of the heart–lung machine nearly fifty years ago. Yet the oxygen sensor that he invented was eventually adapted into what remains today the most accurate and reliable electrochemical glucose biosensor available.

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