Amperometric test strips for point of care biosensors: an overview

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***Abstract*—Amperometric biosensor strips are a fast and inexpensive way to measure blood metabolites. Although many different compounds can be measured amperometrically by exploiting the same measuring algorithms, the blood glucose monitoring still accounts for the 85% of the biosensors market. The competition between the main industry players promoted the development of sophisticated glucose sensors capable to deliver accurate results while limiting at minimum the user errors. This article presents an overview of the amperometric test strips for point-of-care diagnostics, describing the production and the measurement principle and reviewing some of the most important innovations in the field.**

Introduction

Thanks to the knowledge increase in interdisciplinary sciences, in the last decades it has arisen the possibility to combine electronics with biology. A *biosensor* is a device combining a *biological element*, capable to detect a chemical, physical or biological property of a specific substance, and an electronic *transducer*, which translates the interaction between analyte and bioelement into a signal which can be more easily measured and quantified. Biosensors have become some of most widely used electronic devices in biology, with applications spanning from medical sciences and bio-defense, to environmental surveillance and food industry. In fact, the total market for biosensors, valued at $11.39 Billion in 2013, is expected to reach $22.68 Billion by 2020, at an estimated CAGR of 10.00% from 2014 to 2020[5].

The Point-of-care (POC) diagnostics has the largest impact among all the end-user markets of biosensors, with the main business being the management of diabetes mellitus, which accounts for about 85% of the entire biosensor market[29]. This is not surprising, considering that millions of diabetics must test their blood glucose multiple times per day. While for diabetes management the next major breakthrough will be the commercialization of a fully implantable systems capable to track the glucose levels for long time periods [16], the POC systems marketed today are based on the combination of a disposable biosensor strip and an electronic reader. To succeed in this market, industries compete in the realization of systems granting faster analysis, greater accuracy, reduced user error, and limited analysis pain, mainly focusing on the perfecting of existing test strips. This article presents an overview of the amperometric test strips for point-of-care diagnostics, describing the production and the measurement principle and reviewing some of the most important innovations in the field.

Test strip production

Test strips are initially fabricated from a roll of plastic coated on one side with a thin layer of metal or carbon. A laser carves into the layer an intricate pattern, defining the strip's electrodes. Typically, at least a reference and a working electrode are needed for the chronoamperometric measurement, with the working one carrying the necessary reagents (i.e., enzyme, mediator, stabilizer, surfactant, linking, and binding agents) needed to turn the analyte into electricity. Depending by the strip producer additional electrodes for more complex measuring algorithms or for quality control may also be printed. The biosensor reagents are commonly deposited by inkjet printing in a dry form. How the chemicals are dried is a crucial part of the process, since a minimum amount of hydration is necessary to keep the enzyme active, while too much may degrade the components. After drying, the roll is transferred to another machine that carves out a space for the blood sample and applies a sticker to protect the strip. Finally, the roll is cut into 4 or 5 million individual strips. [14]

Detection principle

Test strips evolved sensibly over the past few decades; when the first home glucose testing system were launched on the market in the 1980s, the technology behind the test strips was fundamentally different from today. The first strips measured glycemia by using an enzymatic system capable to convert the blood glucose into a proportional amount of

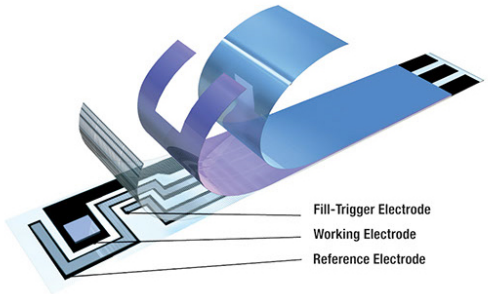

Figure 1. Example diagram of a blood glucose test strip from Abbott [1].

dye. The meter quantified the glucose by shining the test spot with an LED and detecting how much light was reflected by the dye. This technique, called *reflectance photometry*, is still employed today in several commercial POC biosensors (i.e. cholesterol [4], HbA1C [3] and urinalysis [2]), but is impractical for glucose sensing, since requires fairly large blood amounts (tens of microliters) and a long measurement time (several minutes). Amperometric test strips, the world standard today, employ enzymes to convert the analyte into an electrical current which is read by the meter and correlated to a specific concentration. Modern strips enable faster and less painful analysis, measuring blood compound in as little as 5 seconds, while using only few hundred nanoliters of blood.

Amperometric test strips exploit enzymes to convert the analyte of interest into an electrical current. These biosensors are mainly based on an enzyme class called *oxidases*, which catalyzes the transfer of electrons between two molecules according to the following steps:

1) The enzyme binds molecular oxygen and the target compound into its active site and oxidizes the analyte, extracting two electrons in the process.
2) During the oxidation, the electrons are collected by the enzyme's cofactor[1], which enters in a reduced state.
3) In the last stage, the enzyme's cofactor returns to its native state by transferring the the electrons to a molecule of oxygen and generating hydrogen peroxide as product, allowing a new cycle to start.

The precise quantification of the electron transfer enables to understand the amount of substance present in the sample. Initially, this electron transfer was assessed by measuring the amount of H_2O_2 produced by the enzyme using a technique called *chronoamperometry* [17]. In chronoamperometry, an electrode is polarized with a specific potential; depending by the substance, in proximity of the electrode the electric field may be strong enough for the compound to discharge its electrons to the electrode surface, generating an electrical signal. With respect to the hydrogen peroxide, this electron transfer occurs at potentials above +600 mV (vs Ag/AgCl).

The measurement of hydrogen peroxide by chronoamperometry has two inconvenient: the first is that the signal produced by the enzyme depends by the concentration of oxygen in the sample, therefore, fluctuations in oxygen partial pressure, and limited concentrations of oxygen in the sample can decrease the linearity of the sensor and introduce unpredictable changes in its response[29]; the second is that at such high electrode potentials, other compounds in the blood (i.e. ascorbic acid, uric acid and acetaminophen) can transfer their electrons to the electrode, compromising the selectivity of the system and generating inaccurate readings. Modern strips solved this problem by integrating in their composition a *mediator:* a chemical compound acting as an electron shuttle between the enzyme's cofactor and the electrode. A mediator reacts rapidly with the reduced enzyme and has a high electron transfer rate. As result, electrons are preferentially transferred from the enzyme to the mediator, bypassing the oxygen [8].

[1]a cofactor is a non proteic chemical compound required for the biological activity of the enzyme.

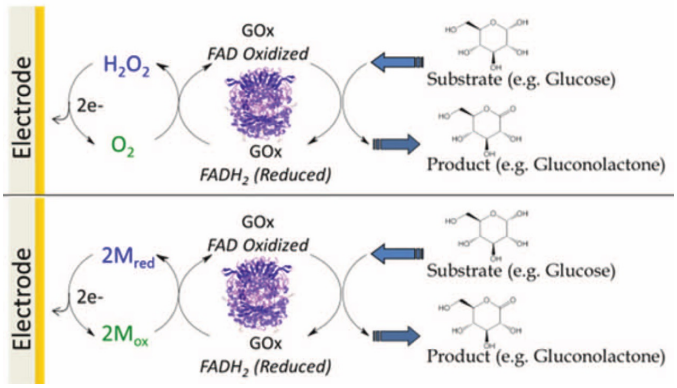

Figure 2. Comparison between the reaction of the first generation amperometric biosensors (H_2O_2 sensing) and the second generation biosensors (with a mediator). GOx: glucose oxidase; $FAD/FADH_2$ flavin adenine dinucleotide (enzyme's cofactor) M: mediator. Image adapted from [18]

The inclusion of mediators in the sensor strips conferred two important advantages:

1) Measurements become largely independent of oxygen partial pressure, improving in linearity and stability;
2) A mediator reduces the interference of electroactive compounds by transferring electrons to the electrode surface at much lower potentials, typically around +200 mV.

It is noteworthy to say that while this article describes the innovations brought to glucose strips, also other compounds can be measured with the same detection principle and the same measuring technique by simply changing the oxidase enzyme. For, commercial amperometric biosensors strips have been developed for lactate, uric acid , ketones, creatinine, hemoglobin and total cholesterol, while many other biosensors based on oxidases and chronoamperometry have been documented in the scientific literature [28].

Strategies to improve measurement accuracy

As said in the introduction, a great part of competition among industrial players orbits around the production of more sophisticated strips, capable to grant accurate results and to limit the errors by the user. Several sources of errors can be related to the strips, with the main being:

- Hematocrit interference;
- Contaminant interference;
- Difference in strip batches;
- incomplete blood filling;
- Strip *rotting*;

Hematocrit interference

The hematocrit (Hct) is the volume percentage of red cells in blood. Variations in blood Hct have been known to affect the accuracy of the electrochemical analysis, generating values higher or lower than the real concentration [25, 6, 27]. Several hypotheses have been proposed to explain the impact of abnormal hematocrit values on blood glucose testing, such as altered viscosity of blood, prevention of plasma from reaching the reaction surface of the test strip, change in diffusion

kinetics, and/or increased packed red cell volume, leading to insufficient plasma volume for accurate testing [19]. Different methods have been proposed to reduce or eliminate the Hct bias effect, generally based on one of two approaches: a) *compensatory*, in which sample properties such as impedance, resistivity and conductivity are directly interrogated in order to derive a measure of Hct loading and consequently the application of compensation factors [11, 22]; b) *structural*, in which the strip comes with a designs or formulations aimed to impede Hct interaction with the sensing mechanism . Examples include the use of an erythrocyte exclusion layer [7]; printable reagent/blood separation paste formulated with a silica filler [20] or the inclusion of fatty acids and quaternary ammonium salts in the reagent layer [10]

Contaminant interference

Amperometric measurements may be sensitive to the presence of electroactive compounds interfering with the measurement and yielding incorrect results. With respect to the glucose sensors, common contaminants are ascorbic acid, uric acid, acetaminophen, maltose, galactose, xylose and lactose [23]. A straightforward technique exclude contaminants is based on the introduction of permselective coatings [**?**]: the deposition of different polymer layers with transport properties based on the charge, size, or polarity examples are nafion and polyurethane coatings [21, 26]. This strategy is however unpractical to apply in the disposable biosensor industry. The introduction of electrochemical mediators partially solved the problem of interferents, enabling measurements at lower potentials. In the more sophisticated strips, mediators and enzymes have been entrapped in *chemistry matrices*, with increased specificity for the analyte and capable of minimizing interference from parasite substances [32]. A third method for excluding interferents is based on the introduction of differential measurements [33]. In this technique, the strip has two sensing electrodes: one containing the enzyme specific for the compound to measure, and another without enzyme, as control. The interferents are sensed on both electrodes; but the difference between the current recorded by enzyme electrode and the control one, enables an accurate determination of the analyte of interest.

Differences in strip batches

Due to slightly differences in raw materials and fabrication conditions., test strip's response vary slightly from batch to batch. These variations are accounted by a batch-specific "code", which identifies a set of parameters defining the relationship between the signal change induced on the test strip and the analyte concentration. Uncoded meters yield inaccurate results which may lead to potentially harmful medical decisions. To solve this problem, test strips were sold with a memory chip capable to program the meter with the right calibration parameters [30]. Improved fabrication conditions granted addition control over the strip production; the following generation of meters were preloaded with all the possible calibration curves, and users were only required the insert a short numeric code into the meter [9]. However, this methods were subject to significant user errors, and a study demonstrated that 16% of patients miscode the reader [24]. The most modern strips are code-free: the meter can read a resistive element screen-printed on the strip at the moment of production and autoload the right calibration parameters without any user input [13].

Incomplete blood filling

The accuracy of the biosensor readings is also dependent on the test strip being completely filled with sufficient sample volume. The blood necessary to provide a correct reading has been constantly decreased, reaching in many devices volumes below 1 μl, and increasing patient's compliance with easier and less painful blood sampling. The blood chamber of modern test strips is transparent, allowing the user to visually inspect if a sufficient volume of blood has been provided, and may contain venting holes, to counter the effect of air pressure and favor rapid sample filling by capillarity [33]. Additionally, some systems have algorithms for the detection of incomplete filling, either using as a trigger a resistive change across two electrodes put at the beginning and at the end of the filling chamber [12], or by sending to the strip a potential pulse, and detecting if the current generated is bigger than a given threshold value [31]. Without these internal triggers the measure does not start, and the meter displays an error message.

Strip rotting

The storage of the sensor strips in detrimental conditions, such as excessive heat, light, and/or moisture may induce the *strip rotting*: a slowing of the reaction times due to the loss of enzyme activity, and an increase in the background current of the sensors due to the a premature reduction of the mediator [32]. Solutions to address strip rotting included the inclusion of engineered enzymes with high specific activities, or the introduction of a *burn-off* step in the measurement procedure. In a burn-off procedure, two DC signals are applied to the sensor. The first is used to consume or oxidize any species responsible for the blank current, while the second is used to measure the analyte [15].

CONCLUSIONS

Amperometric biosensor strips are a fast and inexpensive way to measure blood metabolites. Compared to the optical methods still in use in POC sensors, the latest amperometric strips are capable deliver results in few seconds using only few nanoliters of blood, and increasing patient's comfort and compliance. Although many different metabolites can be measured amperometrically by exploiting the same measuring algorithms, the blood glucose monitoring still accounts for the 85% of biosensor market. The competition between the main industry players promoted the development of sophisticated glucose sensors capable to give accurate results and limit at minimum the user errors. However, due to a reduced market demand and to a less severe medical requirements, these innovations are in gran part limited to the biosensing of glucose.

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