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Research Paper

A robust and practically free of charge intermittent use glucose biosensor

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ABSTRACT

In the past decades, great effort has been put in finding new electrode surface modifiers and enzyme immobilizer agents that prevent the enzyme leakage, minimize the effect of interfering species, retain the enzyme bioactivity, and enhance the sensor sensitivity. In this work, a sandwich-type glucose biosensor that keeps its sensitivity and operational linear range for more than a year is presented. After 5 months of intermittent use, where the biosensor was exposed to more than 500 standard additions, it presented a limit of detection of 5 μM, and the linear behavior was from 5 μM to 3 mM with a value of $r^2 = 0.999$. Besides, after 7 months of its assembling, the biosensor was employed for assessing the glucose concentration of real serum samples and its performance was compared with the response of a commercial autoanalyzer. A year later, the biosensor still exhibited very good performance of its analytical parameters.

The performance of identical sandwich-type biosensors is analyzed when they are exposed to three different storage conditions. Simulated curves are compared with experimental data to explain the dependence of sensitivity and response-time on the aging and storage conditions of the biosensors.

1. Introduction

Biosensors can be defined according to the way in which they are utilized. On the one hand, they can be used for qualitative, semi-quantitative, or quantitative analysis, but on the other hand, they can be used for a single, intermittent or continuous measurement process [1]. Within this last classification, single use biosensors are the most common type of sensor when the analysis of glucose in blood is required [1]. These sensors are user friendly because they contain the selective and the transducer elements in a cell, which is the single use test strip. Since they cannot be calibrated while they are in use, they are mechanically created to provide sufficient sensor-to-sensor reproducibility. This kind of sensors is the most used for periodical testing of diabetes, a disease that has been declared as a global epidemic by World Health Organisation [2,3].

Unfortunately, the possibility of a cure for diabetes seems to be unrealistic in the short term [3]. There is a highly lucrative market though, which is the main driving force in the area of commercial devices for blood glucose monitoring [3]. In this regard, it is important to consider that there are wide gaps between academic achievements, commercial developments, and social needs related to sensor research [1]. Academic researchers and large diagnostic companies are both focused on the improvement of easy-to-use strips and continuous monitoring devices. To achieve their goals, they are using different strategies commonly related to nanotechnology. However, while academic researchers are focused on the sensitivity and detection limit of their sensors, they do not put too much attention on the cost per strip or the cost per analysis, which is one of the main concerns of diagnostic companies [3]. Typically it is considered that the price of a sensing strip is low enough if it is around or below U$ 1. This asseveration is true when it is compared with the price of a strip developed in an academic research lab. However, the cost per analysis is still quite expensive and not too accurate when it is compared to other laboratory methods [1].

The market of strips for glucose analysis is segmented with Roche, Minimed, LifeScan, Dexcom, Bayer, and Abbott as the key players. Their commercial devices fulfill the accuracy, precision, and reliability required by the International Organization for Standardization (ISO). In this regard, the ISO 15197:2003 specifies that 95% of the individual glucose results must be within $\pm 15\ mg\ dL^{-1}$ for samples with glucose concentration $<75\ mg\ dL^{-1}$ and that the error must be within 20% if the glucose concentration is $\geq 75\ mg\ dL^{-1}$ [3]. After reading those requirements, a simple question should come up in the mind of most researchers: Why are not those parameters more rigorous? Perhaps the physicians do not require more accuracy or perhaps this is the actual state of art for

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Table 1
Comparison of the analytical performance of different GOx electrodes.

Electrode matrix	Immobilization method	Detection range/mM	Effect of interfering species	Storage Stability	Electroactive species	References
Glucose oxidase (GOx)/polyaninline (PANI)	Electrochemical doping	0.005–10	Low	95% activity after 30 days	H_2O_2	[9]
Chitosan(Chit) Grafted Pd nanoparticles (NPs)		0.002–1	Low	91% activity after 60 injections or 94% 3 weeks	H_2O_2	[10]
GOx/PANI/ /polyisoprene	Entrapment	0.01–12	Negligible	5 months	H_2O_2	[11]
GOx/Pt NPs/mesoporous silica NPs	Crosslinking	0.001–26	Negligible	90% activity after 1 month (used > 100 times)	H_2O_2	[12]
GOx/PANI/polyacrylonitrile	Entrapment	0.002–12	Negligible	100 days	H_2O_2	[13]
Polymethyl methacrylate/bovine serum albumin (BSA) core-shell NPs	Crosslinking	0.2–9.1	Very low	1 month	H_2O_2	[14]
multi-walled carbon nanotubes (MWNTs)/Chit/BSA/Ferrocene/GOx	crosslinking	0.01–30	Negligible	95% activity after 350 injections or 99% 30 days	Ferrocene	[15]
GOx/cytochrome C/Au NPs/PANI nanospheres	Immobilization of GOx via Nafion	0.01–3.2	Very low	92% activity after 30 days	H_2O_2	[16]
GOx/Chit hydrogel/Au NPs	Physical entrapment	0.012–3	Negligible	91% activity after 4 weeks	Ferrocene	[17]
Au NPs on eggshell membrane	crosslinking	0.008–1	Very low	87% activity after 10 weeks	Oxygen	[18]
Nafion/GOx/(MWNTs)/PANI/Prussian blue	Physical entrapment	1–11	Very low	90% after 30 days (used 15 times)	H_2O_2	[19]
Polycarbonate/Mucine/BSA/GOx	crosslinking	0.005–3	Low	Close to 100% after 1 year and > 500 injections	H_2O_2	This manuscript

most single use biosensors. Table 1 summarizes some biosensors listed in recent published reviews [3–8].

As it can be observed most of these biosensors offer excellent limit of detection (LOD) and linear range. Also, they have quite good stability and present low or negligible effect of interfering species. However, it is not clear if they can assure the same LOD or linear range after a week or a month of assembling. Furthermore, nor the sensor-to-sensor reproducibility neither the linear range are typically analyzed after a month of assembling. Eventually, some of those reasons would be associated with the definition of ISO 15197:2003 [3].

Intermittent use biosensors correspond to another commercially available methodology of analysis in which a flow stream is commonly used to refill the cell and reuse the sensor. This other kind of biosensors typically exhibits much better precision, accuracy, and sensitivity than single use biosensors. Moreover, the cost per data point of intermittent use biosensors is very modest, since they are commonly used for weeks or months instead of for few minutes [1]. Even though this kind of sensors is very promising, they have at least two important disadvantages. First, they are not as easy-to-use as the well-known glucose test strips and second, they do not provide the same economical profits [1]. Sandwich-type biosensors are a kind of intermittent use biosensor where the enzyme is stored into a hydrogel placed between two diffusion membranes. The diffusion membranes are hydrophilic and they can be modified to stop the passage of interfering species [20,21]. The performance of enzymatic biosensors is dramatically affected by the physicochemical characteristics of the microenvironment that surrounds the enzyme [22–24]. The activity and stability of the enzyme, as well as the response-time of the sensor are the outcome of the immobilization process, the sensor geometry, and of the electrode material that was selected for building up the sensor. Usually, the stability of these biomolecules is increased when they are in contact with molecules presenting glycosidic groups [23,25].

In this opportunity, it is analyzed the performance of a sandwich-type glucose biosensor that has been intermittently used for more than a year. The cost of each sensing membrane would be around a quarter (U$ 0.26) and it can be stored in buffer or into an empty vial. Chronoamperometric experimental curves have been simulated to explain how different storage conditions affect the behavior and performance of the biosensor. Finally, the glucose concentrations of real serum samples are compared with the

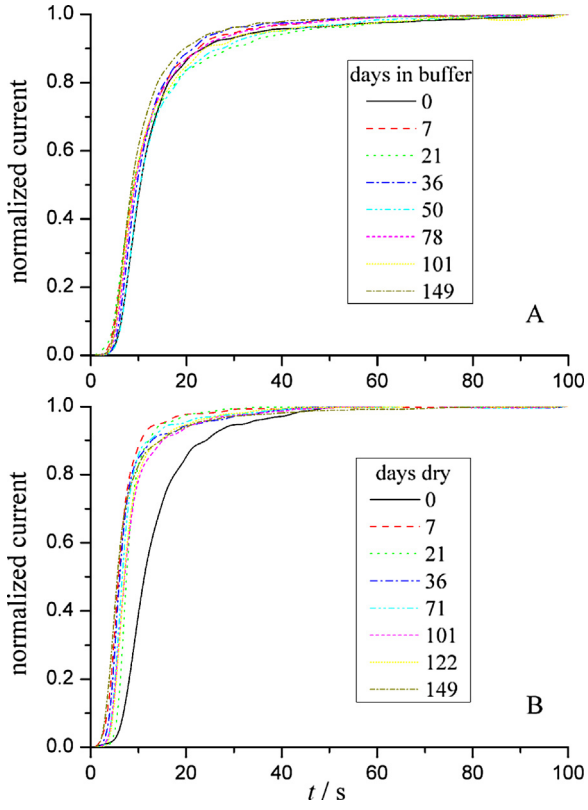

Fig. 1. Normalized chronoamperometric profiles of two glucose biosensors after the addition of 0.2 mM glucose. The curves correspond to the response of each biosensor after different days of assembling. The biosensors were stored in buffer (A) and in an empty vial at 4 °C (B).

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