Detection of glucose in potentiometric paper-based enzymatic biosensors using glucose dehydrogenase

MASTER'S DEGREE FINAL PROJECT

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Abstract

A novel sensitive paper-based sensor for the potentiometric detection of glucose response is presented. Working and reference electrodes were built into a potentiometric cell in order to measure glucose levels through a new proposed reaction using co-enzymes and mediators. The working electrode consists on a platinum sputtered filter paper coated with a membrane of Nafion that entraps the glucose dehydrogenase enzyme with the NAD coenzyme. The reference electrode was developed by drop-casting of polyvinylbutyral-based membrane on a conductive paper coated with a layer of NaCl. Hydrogen peroxide was detected by the working electrode under influence enzymatic reaction. The response obtained was proportional to the logarithm of the concentration of glucose, with a sensitivity -95,9 ± 3,6 mV/decade, a linear range that spans from 0,3 mM to 10 mM and a limit of detection of 0,2 mM of glucose. This approach opens up the way for fabrication the new devices and prospects for diagnostic results.

1. Introduction

From several decades, the humanity strives with the huge rise in the number of people affected by chronic and metabolic diseases. Diabetes on the worldwide, it is leading cause of morbidity and mortality [1]. Estimations show that the prevalence of diabetes is continued to increase [2]. According to a recent report by the World Health Organization (WHO) [3], at approximately 170 million people in 2000 will be affected by diabetes. diabetes. It is predicted that by 2030 more than 430 million people will be affected by this disease [4]. Monitoring blood glucose levels has been established as a valuable and almost mandatory tool in the management of diabetes; i.e. the well-known glucometer is nowadays available at the global level. However, the cost of the device and particularly the strips still hinders the real adoption for general perspective (from low-income countries to poorest people in developed countries) [5]. For this purpose, the development of inexpensive sensors and devices could have a significant impact for glucose monitoring at the global level.

Biosensor technology has grown rapidly and plays nowadays a key role in different applications, particularly in medicine. Enzymatic biosensors show good performance parameters such as good reproducibility,

high specificity, low limit of detection, stability and a simple detection scheme [6]. In the 21st century, the field of biosensors is dominated by the amperometric glucose biosensors [1, 7]. However, it was recently demonstrated that the detection framework could even be simplified by using potentiometry. Indeed, potentiometry shows low power consumption, robustness, simplicity of operation and instrumentation, and ease of miniaturization [8, 9]. The first enzyme-based biosensors based on the potentiometric detection were proposed several decades ago [8]. More recently, the group of Willander have been pioneered with the use of nanomaterials such ZnO and CuO for the enzymatic detection of glucose and cholesterol [10, 11]. In addition, our research group have shown a novel approach to produce enzymatic-based potentiometric sensors. In this approach, a platinized paper acting as a redox sensitive substrate was employed for hydrogen peroxide detection. The sensor detects the hydrogen peroxide (H_2O_2) generated by the following reaction: [12]:

$$\text{Glucose} + H_2O + O_2 \longrightarrow \text{Gluconolactone} + H_2O_2$$

Thereafter, the H_2O_2 is monitored by the platinum surface:

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

Furthermore, a Nafion membrane drops casted on top of the platinized paper enhancing the sensitivity of the detection. The final construction scheme is depicted in Fig. 1, where the enzyme glucose-oxidase (GOx) is entrapped in a Nafion layer on top of a Pt paper. The Nafion has three functions: firstly it acts like a matrix where the enzyme is immobilized; secondly it dramatically enhances the sensitivity from -18,5 mV/decade to -125,1 mV/decade for Pt only and Pt with Nafion [9] and in addition it reduces the interferences produced by other redox-active species (such as ascorbic acid, uric acid) [13, 14] which are negatively charged. The negatively charged Nafion indeed bears sulfonate groups over the backbone of the polymer which may prevent negatively charged interferences from approaching to the sensing part [15]. This new configuration of biosensors fulfils the requirements for real sample application and recently blood glucose detection in a whole-paper potentiometric cell was demonstrated [12]. In addition, the use of paper as a substrate to develop analytical tools is a promising solution to build powerful, simple and low-cost analytical platforms [16, 17]. The cost of fabrication for both working and reference paper-based electrodes are reduced by several orders of magnitude (from hundreds of thousands € to cents) [12]. Therefore, the use of potentiometry together with paper substrate is the basis for constructing new low-cost platforms with good analytical performance, robustness and simplicity.

However, these types of biosensors are limited so far to the use of oxidase enzymes. We propose herein to take the latter advantages to develop sensors based on other types of enzymes. We have selected dehydrogenase enzymes because they cover analytically targets in real samples. In this way enzymes such as malic acid dehydrogenase for wine samples, or phenylalanine, leucine and alanine dehydrogenase for amino acid detection in health issues, might be used. Moreover, the advantage of dehydrogenase enzymes over oxidases is that they are more abundant, oxygen-independent and more substrate specific [18]. Here, we selected the glucose dehydrogenase (GDH) as model for the development and optimization of a dehydrogenase-based potentiometric sensor.

The model system of glucose dehydrogenase (GDH) requires the use of a cofactor which assists in the biochemical process. Besides, if we want to detect hydrogen peroxide, an appropriate mediator is also required [19]. The following reactions are involved in the detection process: glucose in presence of GDH and NAD as a cofactor is oxidized to glucolactone; then NADH in presence of an appropriate mediator (PMS in its oxidised form) allows reducing oxygen to form H_2O_2:

$$\text{Glucose} + \text{NAD} \xrightarrow{\text{GDH}} \text{Gluconolactone} + \text{NADH} + H^+$$

$$NADH + H^+ + PMS \longrightarrow NAD + PMSH$$

$$PMSH + O_2 \longrightarrow PMS + H_2O_2$$

Then we have to first, optimize the selection of the cofactor by screening four different ones (nicotinamide adenine dinucleotide (NAD), β-nicotinamide adenine dinucleotide phosphate hydrate (NADP), flavin adenine dinucleotide disodium salt hydrate (FAD) and methoxatin disodium salt (PQQ)) together with phenazine methosulfate (PMS) as a mediator. Different configurations have been optimized for the generation of hydrogen peroxide (H_2O_2). After optimization of cofactors, working paper-based electrode was fabricated. Finally a potentiometric cell was constructed with the working and reference paper-based electrodes.

In this work, the fabrication and optimization of a GDH-NAD biosensor based on paper-based technology is presented on the Fig. 1.The analytical parameters such as sensitivity, linear range and limit of detection were fully characterized to demonstrate the novel use of dehydrogenase enzyme in potentiometric paper-based sensors.

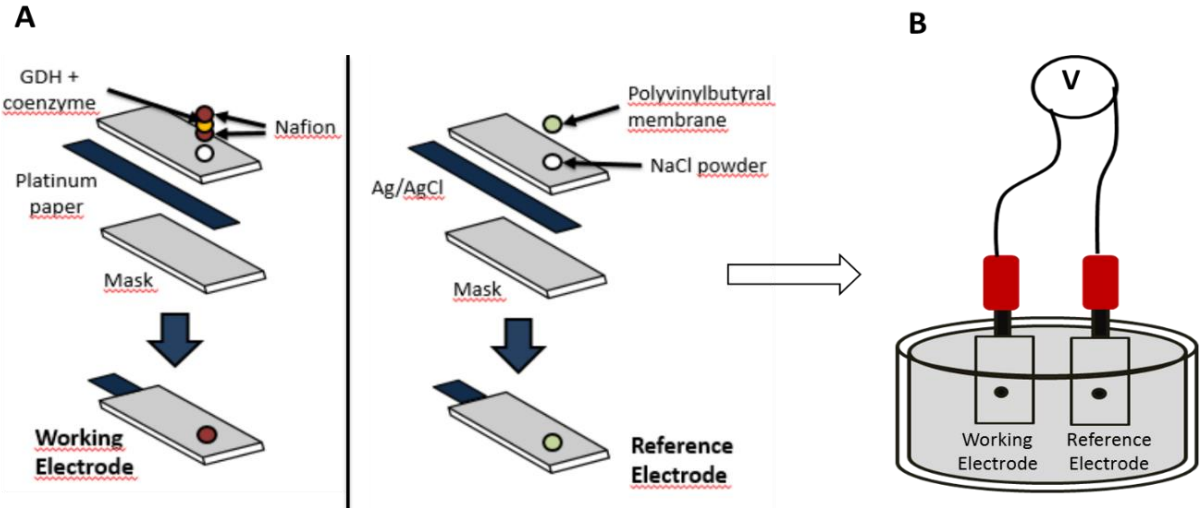

Figure 1. (A) Scheme for the construction of the working and reference electrodes. Working electrode: a layer of Nafion; a layer of GDH enzyme with NAD coenzyme; a layer of Nafion. Reference electrode: layer of NaCl and reference membrane. (B) Picture containing sensors in the cells connected with potentiometer.

2. Experimental

Reagents

Whatman ® Grade 5 qualitative filter paper was used for the development of the electrodes. Nafion ® 117 solution (ca. 5% in a mixture of lower aliphatic alcohols and water, 45% water), glucose dehydrogenase (GDH) from *Pseudomonas sp.,* ≥ 200 units per mg, nicotinamide adenine dinucleotide (NAD, free acid, grade I, approx 100%), %), methoxatin disodium salt (PQQ) ≥97.0% (HPLC), β-nicotinamide adenine dinucleotide phosphate hydrate (NADP), flavin adenine dinucleotide disodium salt hydrate (FAD) ≥95% (HPLC), powder; phenazine methosulfate (PMS) ≥ 90% (UV), D-(+)-glucose, methanol and sodium ascorbate were purchased from Sigma-Aldrich. Phosphate buffer saline (PBS) was prepared from potassium phosphate monobasic, potassium chloride, sodium chloride and sodium phosphate dibasic. All salts were purchased from Sigma-Aldrich. 0,1 M PBS was prepared at pH = 7,4 and used in all further experiments. All solutions were prepared using 18,2 MΩ cm^{-1} double deionized water (MilliQ water system, Merck Millipore). Butvar B-98 (PVB) was obtained from Quimidroga S.A. (Barcelona, Spain). Plastic mask (Arcare 8565) was provided by Adhesives Research Ins., Limerick, Ireland. Silver/silver chloride (Ag/AgCl) ink was obtained from Creative Materials Inc. (Massachusetts, USA).

Instrumentation and measurements

Electromotive force (EMF) was measured with a high input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc. Malvern) at room temperature (24°C) in a stirred distilled water solution. A double junction Ag/AgCl in 3 M KCl reference electrode (type 6.0726.100, Metrohm AG) containing a 1 M LiAcO electrode bridge was used. Potentiometric measurements were performed using a 4 mL cell in 0,1 M PBS (pH 7.4). The EMF values were corrected using the Henderson equation for the liquid-junction potential.

Platinum sputtering was performed using a radiofrequency sputtering process (ATC Orion 8—HV, AJA International) operated at 3 mTorr, for 65 s at 200 W.

Fabrication of the enzymatic paper-based potentiometric cell

To develop the working electrodes, a 100 nm layer of platinum (Pt) was sputtered on one side of a filter paper. For the reference electrode, a filter paper was coated with a conductive Ag/AgCl and treated for 10 min at 90 °C. Then these cured papers were cut into rectangular pieces of 10 mm × 5 mm (Fig. 1). In order to prepare the sensing part electrodes, two plastic masks were used as a sandwich between the two sides of the conductive paper. The top mask has a circular orifice with an area of approximately 3 mm diameter to expose the electroactive area. On the circular window, a membrane was coated by drop-cast (for both the working and reference electrodes).

To build the working electrode, the circular orifice of each electrode was rinsed with double-distilled water and air-dried. Then, a 7 µl drop of Nafion solution was cast and it was dried for 60 min at room temperature. Thereafter, 10 µl of a 25 mg mL^{-1} solution of glucose dehydrogenase (GDH) and nicotinamide adenine dinucleotide (NAD) were cast on top of the Nafion membrane and left to dry overnight at 4 °C. Finally, 7 µl of the Nafion solution was applied in order to make the second layer that entraps the enzymatic layer. After that process, working electrode was let dry overnight at 4 °C.

For the reference electrode [20], one layer of sodium chloride (NaCl) powder was coated on the top of the electroactive surface. Thereafter, 30 µl of a reference membrane consisting of 10 mg NaCl and 118,6 mg PVB in 1 mL of methanol was drop-cast (3 aliquots of 10 µl each, with 30 min drying at room temperature in between) and let it dry during 90 min. Finally, conditioning step of overnight in 1 M KCl left reference electrode ready to use.

Optimization of in situ measurements 0,01 M of NAD, PQQ, FAD and NADP coenzymes was done in 20 mL of PBS solution. Each of cofactors play key role in enzymatic reaction with use 0,001 M PMS as mediator to generation of H_2O_2. During optimization of coenzymes also were optimized metals such as gold, silver, copper, platinum and glassy carbon for detection of glucose. Everything was optimized in one cell with PBS solution. Calibration curve was done with different concentrations of glucose with increasing volume of PMS mediator in the cell (data not shown). After these experiments, coenzyme and metal with the best of sensitivity of glucose were chosen for the next steps.

3. Results and discussion

Optimization of coenzyme deposition

The fabrication of platinum and gold paper-based electrodes is one of the main advantages to obtain low cost sensors for the detection of hydrogen peroxide. The mechanism of detection is not yet fully known, the Au and Pt layers create some electrical response under the presence of H_2O_2. In our group, we have demonstrated and validated the response of Pt electrodes to glucose based on the detection of peroxide through the enzymatic reaction with glucose oxidase (GOx) [12]. In addition, the Nafion membrane have been shown to have a significant impact on the sensitivity. Glucose oxidase is one of the most

popular enzymes used for glucose detection because the result of its reaction is based on the oxygen consumed, which detection of oxygen is widely described in the bibliography. Glucose measurements can also involve interaction with other enzymes such as glucose-1-dehydrogenaze (GDH), which do not depend upon dissolved oxygen [5]. When compared with GOx, GDH requires a coenzyme and a mediator to generate hydrogen peroxide. In a previous work [12], it was shown that the biosensor is built as a sandwich-type; the enzyme is entrapped between two layers of Nafion. As it was already explained, the layer of Nafion plays 3 significant roles: it enhances the sensitivity of the detection, it increases the selectivity by restricting the effect of redox-anions and it offers a better substrate for direct immobilization of the enzyme.

The construction of the electrodes is based on the immobilization of the GDH with the coenzyme (either NAD, NADP, FAD or PQQ) by direct drop-casting of the enzyme solution on top of the first layer of Nafion. After drying the enzyme solution, a second layer of Nafion is drop-casted on top, in order to improve the immobilization and also to help to isolate the generation of H_2O_2 (Fig. 1). These experiments were done in 4 mL of PBS solution with 0,001 M of PMS mediator. PMS allows reducing molecular oxygen forming H_2O_2. Thus, experiments were performed with increasing concentration of glucose and the resulting sensitivity and reproducibility were monitored (see Table 1). From a previous experiments with used a four different kinds of coenzymes, NAD and NADP gave satisfactory response. In two different experiments was entrapment of GDH enzyme with each one of two coenzymes NAD and NADP on the platinum and gold electrodes. Sensitivity of gold electrodes was higher than platinum electrodes, when used with NAD and NADP coenzymes. However, during calibration of glucose, gold electrodes showed a drift with high concentration of glucose and the response time was also longer during detection of glucose by gold electrodes.

The results displayed in Fig. 2 A and B display the calibration curves of potential with increasing concentration of glucose after stabilization of the potential signal. During these calibration curves, FAD and PQQ coenzymes did not take part in enzymatic reaction, because do not give any signal. However, the use of NAD and NADP coenzymes based on gold electrodes, show negative potential (-68 mV and -90 mV, respectively) with increasing concentration of glucose. For this reason, standard deviation for gold electrodes were very high for these two coenzymes. It is worth mentioning that the reproducibility with NAD coenzyme of the platinum paper electrodes was highly satisfactory between 10^{-2} and 10^{-3} M.

	Pt			Au		
Coenzyme	**Sensitivity (mV/decade)**	**Linear range (M)**	**LOD (M)**	**Sensitivity (mV/decade)**	**Linear range (M)**	**LOD (M)**
NAD	-64.2 ± 16.3	10^{-3} to 10^{-2}	$10^{-3.7}$	-140.9 ± 26.7	10^{-4} to $10^{-2.5}$	$10^{-4.4}$
NADP	-55.7 ± 9.7	10^{-3} to 10^{-2}	$10^{-3.5}$	-168.0 ± 96.2	10^{-4} to $10^{-2.5}$	$10^{-4.2}$

Table 1. Analytical parameters of platinum and gold electrodes after optimization of NAD and NADP coenzymes.

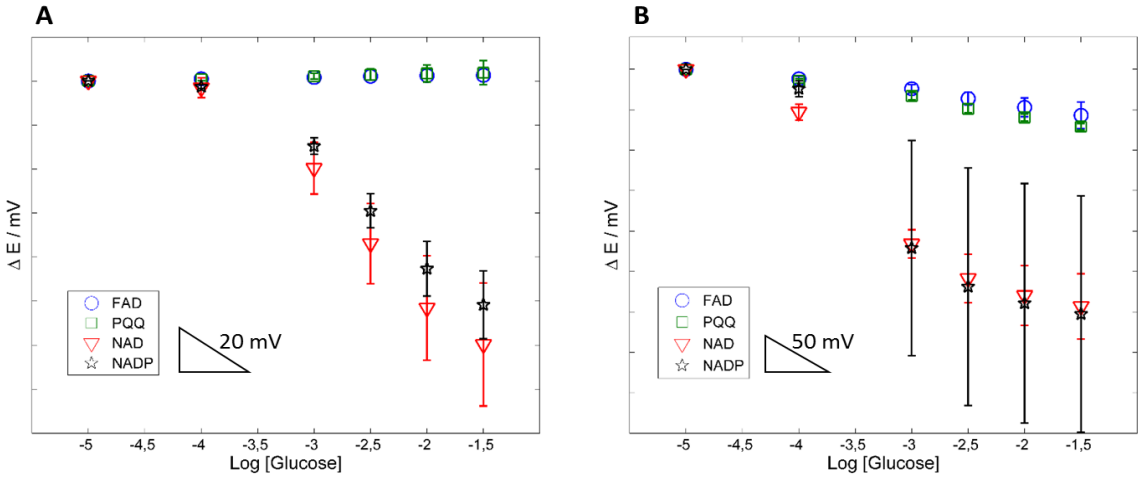

Figure 2. Calibration curve of optimization of coenzymes with increasing concentration of glucose for (A) platinum electrodes and (B) gold electrodes.

Optimization of biosensor parameters

Concentration of NAD coenzyme

In the initial phase, the concentrations of enzymes and coenzymes of the biosensor were optimized. Preliminary experiments show that the best response for glucose detection was obtained from the immobilization of GDH enzyme with NAD cofactor on the platinum electrodes. Cofactor NAD with different concentrations was immobilized on the platinum layer in order to obtain the best sensitivity and reproducibility for glucose detection (Table 2). It is easy to observe that sensitivity for platinum electrodes is higher with increased concentrations of NAD coenzyme. A reduction in sensitivity (from 61,3 ± 15,5 mV to -50,0 ± 3,6 mV/decade) but similar linear ranges and LODs was found in the two highest concentrations of coenzyme (Tab. 3). In previous experiments, gold and platinum electrodes were used without a layer of Nafion. As previously demonstrated [12], the results show that the sensitivity of naked electrodes is lower than with Nafion-coated electrodes (data not shown). As the sensitivity increases, the linear range shifts towards lower concentrations, which results in an improvement of the limit of detection for H_2O_2. Fig. 3 A and B show both time-traces and calibration curves with increasing concentration of glucose. The time of response was increased to approximately 500 s with decreasing concentration of cofactor. During calibration of glucose, lower concentration of NAD coenzyme (10^{-4} M) does not give significant response; it means that platinum electrodes could not detect hydrogen peroxide at this level of concentration. Repeatability test was carried out by performing several consecutive calibration curves for three different electrodes from 10^{-5} M to $10^{-1.5}$ M for each concentration of coenzyme. The reproducibility of paper-based electrodes for 10^{-2} M of NAD coenzyme concentration was highly satisfactory. From the plots of Fig. 3 it is evident that a Nafion-coated platinum electrodes display an enhanced sensitivity.

Parameters	NAD coenzyme		
	10^{-2} M	10^{-3} M	10^{-4} M
Sensitivity (mV/decade)	-61.3 ± 15.5	-50.0 ± 3.6	-
LOD (M)	$10^{-3.7}$	$10^{-3.7}$	-
Linear range (M)	$10^{-3.5}$ - 10^{-2}	$10^{-3.5}$ - 10^{-2}	-

Table 2. Analytical parameters of NAD coenzyme in three different concentrations after optimization.

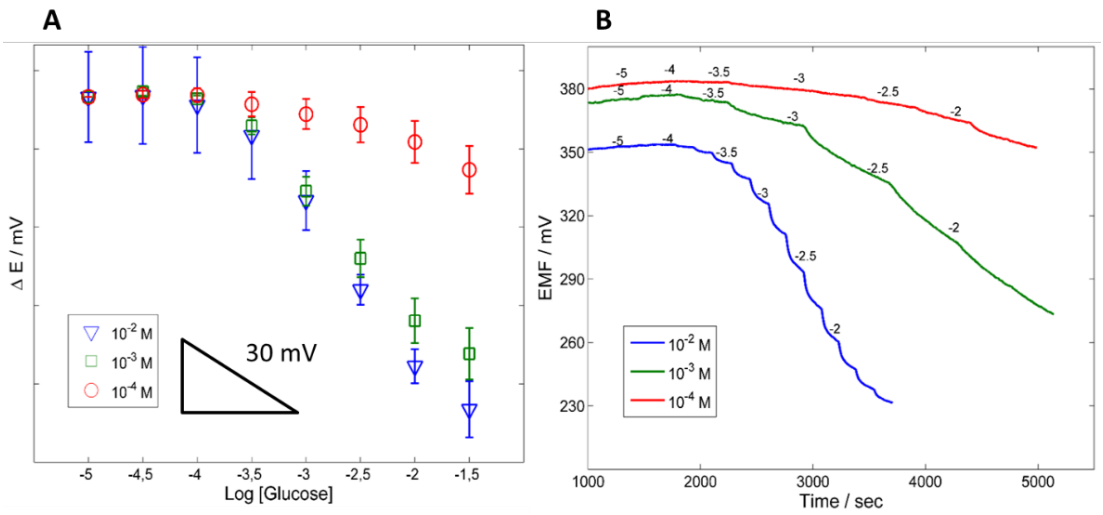

Figure 3. Comparison of the sensitivity and reproducibility of platinum electrodes with optimization of different concentration of NAD coenzyme, (A) calibration curve and (B) time-trace.

Concentration of PMS mediator

Phenazine methosulfate (PMS) is widely used as an electron-transfer catalyst in well-established enzyme assays [21]. PMS is photosensitive [22, 23], when it is exposed to room light, the colour changes from yellow to green. The use of PMS in the oxidised form as a mediator, allows reducing molecular oxygen forming H_2O_2.

For a better detection of glucose with platinum electrodes, the concentration of PMS mediator was studied to reach the best detection parameters. Table 3 summarizes the most relevant parameters of the analytical performance for the four different concentrations. Difference of potential between concentration of 10^{-3} M and 10^{-4} M of PMS mediator, was around -7 mV. The high value of sensitivity for 10^{-4} M concentration suggests that with this concentration a high amount of hydrogen peroxide is produced, which was detected by platinum electrodes. However, with a concentration of 10^{-4} M of mediator, during calibration of glucose the response requires a too long time (700 s) to be stabilized in comparison with the concentration of 10^{-3} M (500 s). In Fig. 4, the lowest concentration of PMS mediator did not give a clear response for the detection of glucose during the calibration curve. For this reason, with a higher concentration of PMS equal 10^{-3} M, a better detection is displayed. Repeatability tests were performed using three electrodes and glucose concentrations from 10^{-5} M to $10^{-1.5}$ M. Initial potential for a concentration of 10^{-3} M of PMS was recovered (data not shown). Fig. 4 also shows the reproducibility of paper electrodes, where for high concentrations of mediator the reproducibility was highly satisfactory. In the time-trace figure, with concentration of 10^{-4} M of PMS, response from platinum electrode was not stable during the addition of glucose (data-shown-on-the-sub-index).

Parameters	PMS mediator			
	$10^{-2.5}$ M	10^{-3} M	$10^{-3.5}$ M	10^{-4} M
Sensitivity (mV/decade)	-65.4 ± 7.2	-81.4 ± 5.6	-66.4 ± 12.0	-88.3 ± 2.6
LOD (M)	$10^{-4.2}$	$10^{-4.2}$	$10^{-4.7}$	$10^{-4.2}$
Linear range (M)	10^{-4} - 10^{-2}	10^{-4} - $10^{-2.5}$	10^{-4} - 10^{-2}	10^{-4} - $10^{-2.5}$

Table 2. Analytical parameters of PMS mediator in four different concentrations after optimization.

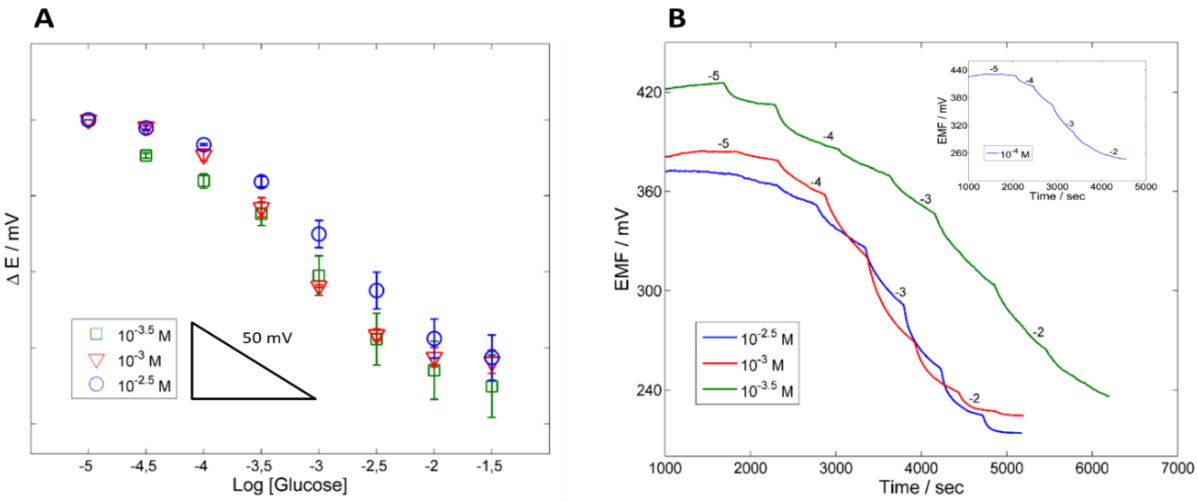

Figure 4. Comparison of the sensitivity and reproducibility of platinum electrodes with optimization of different concentration of PMS mediator, (A) calibration curve and (B) time-trace.

Fabrication of reference paper-based electrode

The main challenge of the fabrication of the reference (RE) paper-based electrode was to obtain a low variability in the experimental measurements. A good behaviour was obtained after a first conditioning of the sensor with 1 M KCl solution for 8 hours. Potentiometric measurements were carried out in order to check the reference electrode behaviour. The analytical performance of this home-made RE was assessed by adding increasing concentrations of hydrogen peroxide. A successful behaviour of RE electrodes was obtained after 30 min. The signal was stabilized and noise levels were significantly reduced. The initial potential of the RE electrode was equal to -8 mV/decade. This step has to be carried out only the first time the electrode is used. The RE electrode should be always wet before experiments.

Potentiometric cells and future work

In order to develop a new and inexpensive potentiometric sensor to detect glucose in an enzymatic reaction with use of GDH enzyme, electrochemical experiments were carried out in cells. The solution to perform the calibration curve was done with a 0,001 M PMS mediator in PBS buffer (0,1 M). After optimization of NAD coenzyme in the working electrode and the conditioning of RE electrode, both, were ready to use. Both electrodes were combined and introduced into the cells. The calibration curve was done with 8 points of glucose at different concentrations between the ranges $10^{-1,5}$ M and 10^{-5} M.

Fig. 5 A and B shows both time-traces and calibration curves for detection of glucose. The time-trace curve shows the calibration of platinum electrodes and the initial potential was close to 380 mV. With the use of reference paper-based electrodes, the time of response was a little bit shorter compared to commercial reference electrodes. The calibration curve shows the average from three platinum electrodes. The reproducibility of platinum electrodes tends to increase with increasing of concentration of glucose.

Table 4 presents the comparison of analytical parameters using two different kinds of enzymes for glucose detection by platinum paper-based electrodes. Sensitivity with GDH enzyme was a little bit lower than with the use of GOx [12], that can happens because the enzymatic reaction for the generation of H_2O_2 needs a cofactor and a mediator and probably this is the impact on the lower sensitivity of platinum electrodes. Furthermore, it should be take into account that the activity of GOx is much higher than GDH. The response time for glucose detection was much lower with the use of GOx enzyme. The limit of detection for both coenzymes

had the same value but the linear range was wider with GDH.

Picture 1. Working and reference electrodes which were combined to build a potentiometric cell. In this design a hydrophilic plastic mask was placed over the electrodes in order to create a channel enough for the required measuring volume down to 150 µL. The goal of that design was to reduce the measuring volume needed and to improve the performance of detection of glucose by platinum electrode. Furthermore, the overwhelming simplicity of operation makes the system easy to use. This system requires an optimization of parameters and also, one of the current work focuses, the improvement on the control of the sensitivity and linear ranges of the system. Developing the new platform based on the paper-based electrodes with using different kind of coenzymes could become a valuable in the growing fields of telemedicine, fermentation of wines and health issues.

	Platinum				
	Sensitivity (mV/dec)	**Linear range (mM)**	**LOD (mM)**	**Response time (s)**	**References**
GDH	-95.9 ± 3,6	0.3 - 10	0.2	500	Current work
GOx	-107.1 ± 7,2	0.3 - 3	0.2	50	Canovas et al., 2017

Table 3. Comparison of analytical parameters two different enzymes – GDH and GOx.

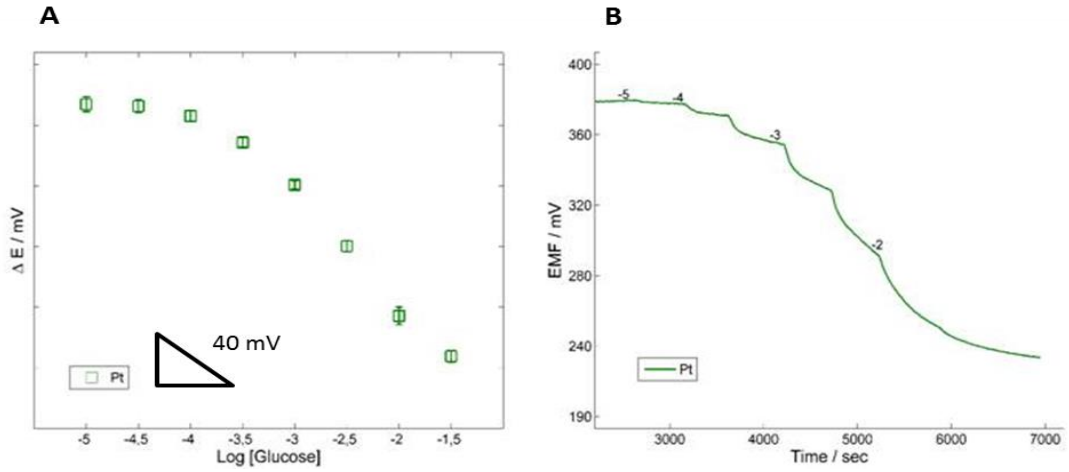

Figure 5. Potentiometric response of paper-based electrodes with detection of glucose: (A) calibration and (B) corresponding time-trace curves of paper-based reference electrode and platinum working electrode (in a 4 mL cell).

Picture 1. Picture showing the new ”channel” design, connected with potentiometer and detection of glucose.

Conclusions

A simple, low-cost and robust paper-based enzymatic electrode with high sensitivity for the determination of glucose has been presented. The use of the perm-selective Nafion membrane provides a highly convenient way to detect the peroxide generated by the enzymatic reaction. Moreover, the use of Nafion allows the enhancement of the potentiometric detection. The sensor exhibits excellent sensitivity and high reproducibility for the detection of glucose in a linear range from $10^{-3.5}$ M to 10^{-2} M with a limit of detection $10^{-3.7}$ M. A dehydrogenase enzyme was tested for the first time for the detection of glucose based on the potentiometric sensors. This kind of enzyme fulfils the targets that these sensors could detect, i.e. amino acids such as leucine and alanine dehydrogenase for health issues and also the malic acid dehydrogenase for wine purpose. Furthermore, dehydrogenase enzyme is oxygen-independent, more abundant and substrate specific in comparison to oxidase enzyme. The main disadvantage found in the study is that this kind of enzyme has high level of interferences with other components such as ascorbic and uric acid but in the group a lot of efforts have been driven to solve it.

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References:

1. Yoo, E. H., & Lee, S. Y. (2010). *Sensors 10*(5), 4558-4576.
2. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). *Diabetes research and clinical practice 87*(1), 4-14.
3. World Health Organization. (2016). *Global report on diabetes*.
4. Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). *Diabetes Care* 27, 1047–1053.
5. Witkowska Nery, Emilia, et al. (2016). *Analytical chemistry* 11271-11282.
6. Anzai, Jun-ichi, et al. (1998). *Analytical chemistry* 70(4), 811-817.
7. Heller, Adam. (1996). *Current opinion in biotechnology,* 7(1), 50-54.
8. Parrilla, Marc, Rocío Cánovas, and Francisco J. Andrade. (2017). *Biosensors and Bioelectronics* 90, 110-116.
9. Parrilla, Marc, Rocío Cánovas, and Francisco J. Andrade. (2017). *Electroanalysis* 29(1), 223-230.
10. Willander, Magnus, Kimleang Khun, and Zafar Hussain Ibupoto. (2014). *Sensors* 14(5), 8605-8632.
11. Israr, Muhammed Qadir, et al. (2010). *Thin Solid Films* 519(3), 1106-1109.
12. Cánovas, Rocío, et al. (2017). *Lab on a Chip*.
13. Manowitz, Paul, Paul W. Stoecker, and Alexander M. Yacynych. (1995). *Biosensors and Bioelectronics* 10(3-4), 359-370.
14. Wynne, Andrea M., and Niall J. Finnerty. (2015). *Chemosensors* 3(2), 55-69.
15. Vaidya, R., P. Atanasov, and E. Wilkins. (1995). *Medical engineering & physics* 17(6), 416-424.
16. Martinez, Andres W., et al. (2007). *Angewandte Chemie International Edition* 46(8), 1318-1320.
17. Delaney, Jacqui L., et al. (2011). *Analytical chemistry* 83(4), 1300-1306.
18. Zhang, Maogen, Conor Mullens, and Waldemar Gorski. (2007). *Analytical chemistry* 79(6), 2446-2450.
19. Esti, M., et al. (2004). *Analytica chimica acta* 513(1), 357-364.
20. Guinovart, T., Crespo, G. A., Rius, F. X., & Andrade, F. J. (2014). *Analytica chimica acta 821*, 72-80.
21. Halaka, F. G., G. T. Babcock, and James L. Dye. (1982). *Journal of Biological Chemistry* 257(3), 1458-1461.
22. McIlwain, H. (1937). *Journal of the Chemical Society (Resumed)* 359, 1704-1711.
23. Seidler, E. (1980). *Acta histochemica 66*(2), 273-275.