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A novel electrochemical immunosensor for *in situ* antibody quantification

Master's Thesis

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MSc in Nanoscience, materials, and processes: Chemical Technology at the Frontier

UNIVERSITAT ROVIRA i VIRGILI

Tarragona, Spain

2022

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Abstract. The COVID-19 pandemic has catalysed a shift of paradigm in the global healthcare system. There has been an increase in the use and demand of portable devices to obtain biochemical information out of the patient in the point of need, i.e. Point-of-Care (POC) testing devices. This has introduced the possibility of quantitatively measuring key healthcare indicators *in situ* and thus, monitoring chronic and infectious diseases. Electrochemical sensors have been proven to be of great potential for such large-scale demands. In this work we introduce the first steps towards the development of a novel POC device – an electrochemical immunosensor – able to quantify *in situ* immunoglobulin gamma (IgG) in biological samples. We report a simple and low-cost methodology of construction of paper support-based Platinum sensors that use potentiometry for the labelled quantification of IgG. The method for IgG recognition is based on Enzyme-Linked Immunosorbent Assay (ELISA), a robust biochemical technique used in clinical diagnosis. Antibodies against IgG linked to glucose oxidase (GOX) were used. In this manner, potentiometric measurements of the hydrogen peroxide (H_2O_2) generated by GOX were given based on the mixed potential mechanism of the sensors. The change in electrical potential is proportional to the IgG concentration in the solution. In this work we provide preliminary evidence showing that, under optimal condition, this method can achieve IgG quantification with a sensitivity of up to -251,9 mV per µg/mL. Moreover, an explanation of the factors affecting the sensors analytical performance, sensitivity and selectivity is provided.

INTRODUCTION

The COVID-19 pandemic has caused a healthcare crisis at global scale which has led to numerous efforts to reduce and control infections. Among these efforts we find Point-of-Care (POC) diagnostic tests. POC tests are portable devices that allow diagnosis in the proximity of patient care, providing real-time, precise, *in situ* detection of the target biomolecule [1,2]. The increasingly extended use of POC devices has opened new possibilities, such as using them to monitor the levels of biomarkers associated to certain diseases. This would be especially beneficial because the monitorization of chronic diseases and early diagnosis of infections allows for better treatment and, in turn, contributes to an optimal clinical outcome [3]. However, there is currently an unsatisfied need for tools that quantitatively measure biochemical parameters in human samples.

One such biochemical parameter that should be quantified in diagnostic analysis is immunoglobulin gamma (IgG). Immunoglobulins are glycoproteins associated to the immune system response that are found circulating in blood or attached to B cells. In function of their structure, there are five types of immunoglobulins – IgG, IgM, IgA, IgD and IgE –, IgG being the most abundant [4]. They play an important role in immunoreactions, acting as antibodies against exogenous antigens. When found in anomalous levels, they serve as biomarkers for immune system diseases, infectious or even cancerous pathologies [5]. A plethora of different biochemical techniques, i.e., *immunoassays*, and analytical devices – known as immunosensors – have been developed to detect immunoreactions [6,7]. Enzyme-Linked Immunosorbent Assay (ELISA) is among the most used laboratory techniques for this purpose [8].

The working principle of ELISA profits from the specificity of the interaction between antigens and their respective antibodies. In Direct ELISA, the antigen of interest is detected using specific antibodies labelled with an enzyme. Once the immunoreaction takes place, and with the addition of the enzyme's substrate, a colorimetric product is generated, and thus, the signal is produced [8,9]. Despite its effectiveness, ELISA presents some important limitations. The laboratory-based nature of the technique makes it difficult to conduct in the point-of-need, due to the complex instrumentalization [10,11]. An alternative is to use electrochemical ELISA-based immunosensors [12]. The structure of an immunosensor can be divided in two regions: the molecular recognition layer, where the antibodies are immobilized and the immunoreaction takes place; and the signal transducer [13,14]. State-of-the-Art ELISA-based immunosensors are mostly designed with optical approaches for transducers, providing colorimetric signals, i.e., only qualitative results [6,10].

Electrochemistry-based analytical techniques are a promising alternative for a transducer in immunosensors development, as they allow to quantify the target antigens through the measurement of an electrical signal [13,15]. One example is potentiometry [16]. Potentiometric immunosensors measure the difference of electrical potential between a reference electrode (RE) and a working electrode (WE) when no current flow is present. This difference is called electromotive force (EMF). The RE keeps a constant potential independently of the content of the sample, while the WE, which contains the molecular recognition layer, experiences a change in potential when the antigen-antibody binding event takes place. The higher the concentration of antigen in the sample, the more binding with antibodies takes place and thus, the more the change in EMF is detected [17,18]. In this sense, there is a proportionality between the EMF and the quantity or activity of the target antigen. Nevertheless, potentiometric sensors based on the direct detection of the immunoreaction event are limited in performance and present many shortcomings [16].

In this work we propose the development of an immunosensor with a methodology based on ELISA recognition of IgG, and an electrochemical

(potentiometric) detection mechanism to measure IgG. For this, we use specific anti-IgG antibodies labelled with glucose oxidase (GOX), which catalyses the reaction of glucose oxidation as seen in Reaction 1. The by-product of the GOX reaction is hydrogen peroxide (H_2O_2), which is decomposed in a redox reaction (see Reaction 2) during calibration and, in turn, produces a change in the electrochemical potential of the Pt electrode. The foundation of the potentiometric measurements is the mixed potential of the electrode, a method proven to work successfully for the quantification of glucose in whole blood [19,20]. We used paper-based sensors consisting of Platinum (Pt) electrode covered with a layer of polyelectrolyte Nafion ™ for an enhanced detection [21,22]. Moreover, sensors were produced using paper as a substrate in order to minimize time and cost of production.

$$\text{D-Glucose} + O_2 \rightarrow \text{D-Glucono-1,5-lactone} + H_2O_2$$

Reaction 1. Glucose oxidation reaction catalysed via Glucose Oxidase (GOX).

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

Reaction 2. Hydrogen peroxide oxidation

EXPERIMENTAL SECTION

Reagents and materials

The materials used for the fabrication of sensors were adhesive support paper, Whatman® Grade 5 qualitative filter paper and glue. The redox-sensitive Pt electrode is built using a Radiofrequency Sputtering (ATC-Orion 8-HV, AJA International) working at 3mTorr during 65s. The Pt surface is covered with Nafion® perfluorinated resin solution (5 wt% in a mixture of lower aliphatic alcohols and water, 45% water) (510211, SigmaAldrich).

For ELISA method implementation, the reagents required were native mouse IgG (Ab198772, Abcam) 1,77 mg/mL, primary anti-IgG antibody (Ab6709, Abcam), Glucose Oxidase (GOX) labelled anti-IgG antibody (GOX-Ab) (Ab136783, Abcam); Bovine Serum Albumin 1% (BSA), skimmed powder milk and Casein (C7078-500G, SigmaAldrich) as blocking agents; and buffer solution PBS 0,1 M pH=7.4.

All analytical grade salts for PBS preparation (Na_2HPO_4, NaCl, KH_2PO_4, KCl) are from SigmaAldrich. All solutions were prepared with 18.2 $M\Omega cm^{-1}$ double deionized water (Mili-Q water systems, Merck Millipore).

Sensor's design and development

Details of the sensor construction can be found elsewhere [19,21,23]. The support paper and Pt-sputtered filter paper were cut and arranged to dimensions depicted in Figure 1. A layer of 5 µL of Nafion® was deposited on the *window* (2,5 mm hole on the structure). Sensors were kept at 4 ºC for one hour to incubate Nafion. All posterior additions were also made on the window.

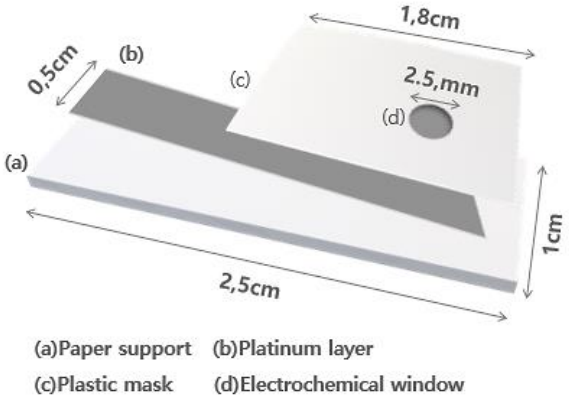

Figure 1. Schematic overview of the sensors final structure.

ELISA implementation methodology

Two different ELISA assays were implemented: Direct and Sandwich ELISA. All addition steps were followed by an incubation at 4 ºC, periods of incubation (2 vs 1,5 hours) were altered throughout the experiments for optimization purposes. As seen in Figure 2, for Direct ELISA: (1) 5 µL of IgG were drop casted on Pt *window* and incubated, (2) then washed thrice with PBS 0,1 M pH=7,4, and (3) incubated with 5 µL of blocking agent (BSA 1% or skimmed powder milk 5% w/v in PBS 0,1 M pH=7,4; or casein 0,1% in boric acid 0,05 M pH=8,5). After blocking, an addition of 5 µL of GOX-labelled antibodies (GOX-Ab) was made. Finally, (4) a step of washing was performed to remove remnant excess of reagents. In the case of Sandwich ELISA, the procedure presents a few alterations: (1) first an addition of 5 µL of primary anti-IgG antibody was performed, (2) then sensors were washed thrice with PBS prior to (3) addition of 5 µL blocking agent of choice. Then (4) 5 µL IgG were deposited and incubated, (5) sensors were washed once again with PBS and (6) incubated with 5 µL of GOX-Ab. Lastly, a step of washing thrice (7) for excess removal was done.

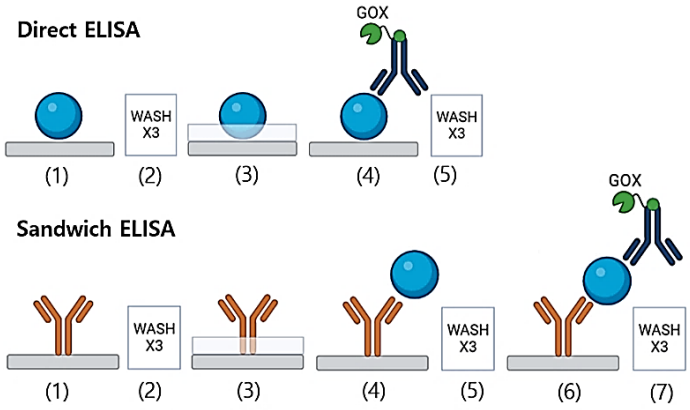

Figure 2. Schematic overview of Direct ELISA methodology (top figures) and of Sandwich ELISA (bottom figures). For details on the numbering see main text.

Potentiometric measurements

Calibrations were conducted using a Malvern high input impedance (10^{15} Ohm) multichannel EMF15 device (Lawson Laboratories, Inc. Malvern, USA). The sensor (2.5 mm diameter exposed electrochemical window of Pt electrode) was submerged in a 30 mL solution of PBS 0,1 M pH=7,4 in continuous stirring. All calibrations were conducted by duplicate at room temperature. Three replicas of each sensor were used at each experiment. The calibrations were conducted adding glucose standards, following a calibration curve comprising concentrations of glucose of 10^{-3} and 10^{-2} M initially and, after optimization experiments, $10^{-1,5}$ M. For the sake of simplicity, concentrations are expressed as -Log[Glucose] = -3, -2 and -1,5 respectively.

RESULTS AND DISCUSSION

Immunoassay design and optimization: ELISA methodology

Two types of ELISA assays were compared: one set of sensors was fabricated following direct ELISA methodology, and another set following sandwich ELISA methodology. Sensors were incubated with the following IgG concentrations: 0 (negative control, only PBS), 0,1 µg/mL, 0,5 µg/mL and 1 µg/mL of IgG. All other conditions were kept constant: incubation at 4 ºC for 2 hours each step, BSA 1% in PBS as blocking agent, and same aliquot of GOX-Ab. The calibration was performed with glucose concentration 10^{-2} M (-Log[Glucose] = -2). As seen in the results in Figure 3, direct ELISA sensors showed a greater change in EMF in response to glucose addition than sandwich

ELISA. The greater slope is translated into higher sensitivity of detection in the Linear Range (LR), which comprises [IgG] = 0 to 0,1 µg/mL as shown in Table 1 (*Annex*).

Sandwich ELISA is a method of higher specificity of action than direct ELISA, because of the use of two antibodies for each target antigen [8]. However, a higher specificity is often associated to detection within lower ranges of concentration. The results depicted in Figure 3 can be explained via this phenomenon: a lower sensitivity has been obtained because the range of detection is not the most suitable. A narrower range of IgG concentrations would have most probably ensured better results.

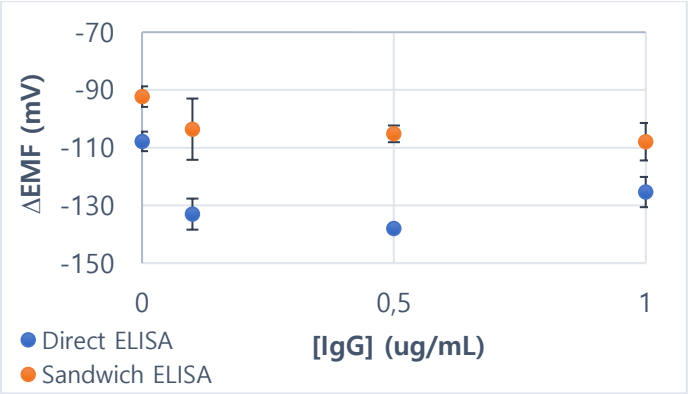

Figure 3. Change in electrochemical potential (ΔEMF) as function of IgG concentration: A comparison between Direct ELISA sensors and Sandwich ELISA sensors (sensitivity).

Direct ELISA sensors presented 50% higher sensitivity compared to Sandwich ELISA. For this, Direct ELISA will be used in the following experiments. However, LR is very narrow (0 to 1 µg/mL) and thus other parameters need to be optimized (blocking agent, incubation conditions, glucose concentration).

Immunoassay design and optimization: Blocking

The role of the blocking agent is crucial in ELISA, as it prevents unspecific interactions between GOX-Ab and the surface of the sensor. An experiment was conducted to select the optimal blocking agent out of three options: BSA 1%, skimmed milk 5% and Casein 0,1% [24,25]. A set of sensors was fabricated using each one. Based on previous results, sensors were incubated with a range of IgG concentrations of 0 to 1 µg/mL for BSA, 0 to 200 µg/mL for skimmed milk and 0 to 100 µg/mL for casein. This distinction was made because BSA has proven to work in narrower LR, and other works were taken as reference for skimmed milk and casein [24,25]. All other parameters were kept constant, and calibration was performed using glucose concentration of 10^{-2} M.

When comparing results shown in Figures 4-5, we find that sensors blocked with skimmed milk have the highest drop in EMF, reaching up to 79,74 mV as seen in Table 2 (*Annex*). The highest sensitivity in LR is obtained with BSA but it presents a narrow range of detection. Skimmed milk, however, presents a broader LR (0 to 50 µg/mL). This effect may be due to interactions taking place between the blocking agent and the Nafion coating of the sensors surface. Due to its partially hydrophilic nature, Nafion increases its conductivity when in contact with liquid or humified environments. Further research is needed, but Nafion may be interacting with any polar component of the skimmed milk solution and enhancing detection [26]. Therefore, skimmed milk is used as blocking agent in the following calibrations.

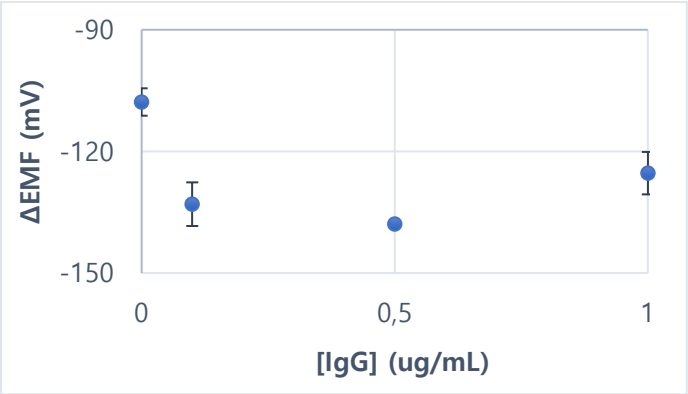

Figure 4. ΔEMF as function of IgG concentration for Direct ELISA sensors blocked with BSA 1%.

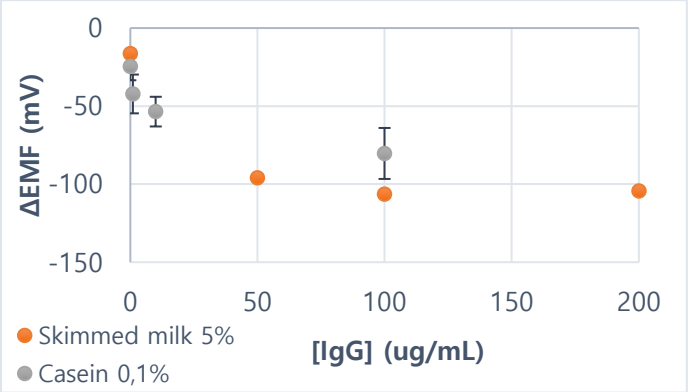

Figure 5. ΔEMF as function of IgG concentration for Direct ELISA sensors blocked with skimmed milk and casein.

Immunoassay design and optimization: Incubation

To minimize time and cost of production of sensors, an approach was taken to reduce periods of incubation of each step (incubation with IgG, blocking agent and GOX-Ab). One set of sensors was fabricated with two hours incubation steps and another set with one hour and a half. The IgG concentrations added were 0, 10, 20 and 30 µg/mL. The calibration was performed with glucose concentration of 10^{-2} M. As observed in the results in Figure 6, sensors incubated for 1,5 h present a more marked slope in the LR. As seen in Table 3 (*Annex*), LR of 1,5 h sensors is shifted to higher IgG concentrations (10 to 30 µg/mL). Moreover, the sensitivity is a 95,93% higher in sensors incubated for 1,5 h compared to those incubated for 2 h. The results demonstrated that incubating for a shorter period not only reduces production time but also increases sensitivity of detection and quality of results. This unexpected improvement in performance may be due to the fact that sensors spent less time incubated with reagents and this facilitated removal of excess of antibodies. An excess GOX-Ab may be one of the reasons why interferences in the detection of IgG take place. To avoid this a combination of an appropriate blocking agent and an adequate incubation timing is key.

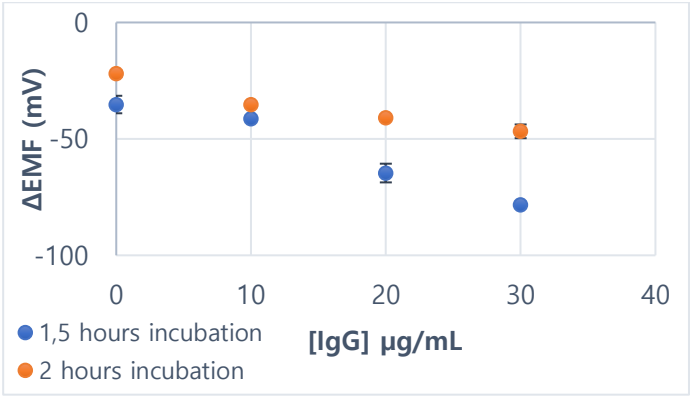

Figure 6. ΔEMF as function of IgG concentration: A comparison between sensors incubated for 1,5 h and incubated for 2 h.

Potentiometric measurements: Calibration with glucose standard

To obtain the signal in the form of change in electrical potential (ΔEMF) it is necessary to add the optimal amount of GOX substrate, glucose. A calibration was conducted to determine the appropriate addition of glucose standard to obtain the optimal response. A calibration was performed introducing three points, -Log[Glucose] = -2,5; -2 and -1,5. See Figure 7 for the overview of the time trace, and Figure 8 for the ΔEMF per [IgG]. As shown in Figure 8, when using -Log[Glucose] = -1,5 a more pronounced detection pattern, i.e. higher slope, is achieved. The results depicted in Table 1

show that with -Log[Glucose] = -1,5 the sensitivity of detection is -2,65 mV/mg·mL^{-1}. This is higher than the sensitivity of the addition previously used (-2). It is important to note that even though a high concentration of glucose is present in the cell (-Log[Glucose] = -1,5) the response seen in negative control sensors (0 µg/mL of IgG) is not too high. Detection is enhanced but unspecific interactions are not. This minimization of non-specific response due to interactions between GOX-Ab and the surface of the sensor means our system is correctly being optimized. This is a significant refinement of the IgG detection. For these reasons, the next experiments will be conducted using glucose concentration -Log[Glucose] = -1,5.

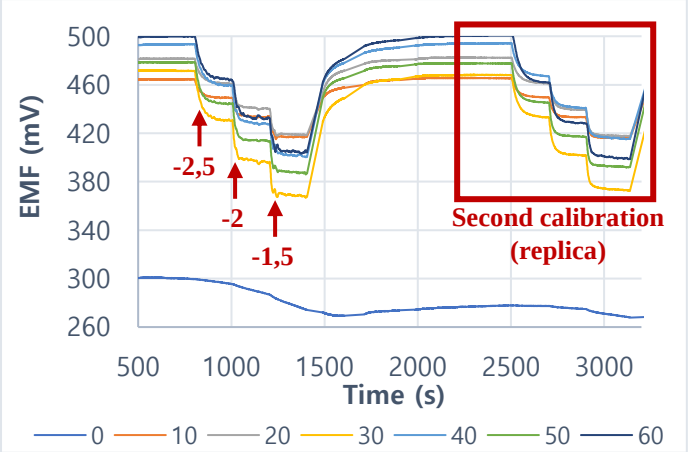

Figure 7. Calibration Time trace of Direct ELISA sensors incubated with [IgG] = 0, 10, 20, 30, 40, 50 and 60 µg/mL. Additions of glucose standard were done to achieve -Log[Glucose] = -2,5; -2 and -1,5, in this order.

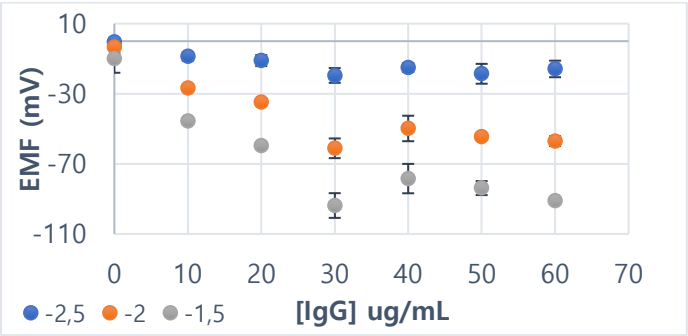

Figure 8. Change in electrochemical potential (ΔEMF) as function of IgG concentration: A comparison between glucose concentrations -Log[Glucose]=-3, -2 and -1,5.

Table 1. ΔEMF (mV) in function of IgG concentration in µg/mL, Linear Range, and Sensitivity of detection. Glucose additions optimization, -Log[Glucose] = -3, -2 and -1,5.

[IgG] (µg/mL)	ΔEMF (mV) per -Log[Glucose]		
	-3	**-2**	**-1,5**
0	-0,4±0,6	-3,3±3,5	-10,0±8,1
10	-8,7±2,4	-26,8±0,5	-45,6±0,1
20	-11,1±3,1	-34,9±0,7	-59,6±0,6
30	-19,6±4,2	-61,1±5,6	-93,8±7,1
40	-15,0±2,5	-49,8±7,3	-78,4±8,4
50	-18,6±5,7	-54,6±0,9	-83,8±4,0
60	-15,9±4,7	-57,0±2,8	-91,1±1,3
Linear Range ([IgG] in µg/mL)	0 to 10	0 to 20	0 to 30
Sensitivity in Linear Range (mV/µg·mL^{-1}):	-0,8	-1,6	-2,7
ΔEMF in Linear Range (mv)	8,4	31,5	83,8

Potentiometric measurements: Selectivity

To test the selectivity for IgG of our system, an experiment was conducted using two additional different analytes. One set of sensors was incubated with IgG, another one with IgA and a third one with human haemoglobin (Hb). The concentrations were the same for IgG, IgA and haemoglobin: 0, 5, 10, 15, 20 and 30 µg/mL. IgA was selected of its similarity in structure with IgG, and haemoglobin was used as control since it is of completely different nature. As seen in Figure 9, no detection pattern is found in sensors incubated with IgA and haemoglobin. As per sensors incubated with IgG, a marked slope with LR in 0 – 10 µg/mL is visible. As seen in Table 2, sensors with IgG have a sensitivity of -1,90mV/µg·mL^{-1}, which is lower than expected. A similarity in results between IgG and IgA sensors was expected, however from the data depicted in Table 2 we see that there is a

considerable overlap of detection. This is particularly true at low concentrations of IgG. Both this and the low sensitivity of IgG sensors may be due to degradation of the GOX-Ab aliquot. These are preliminary results; further work needs to be conducted to extract any conclusions.

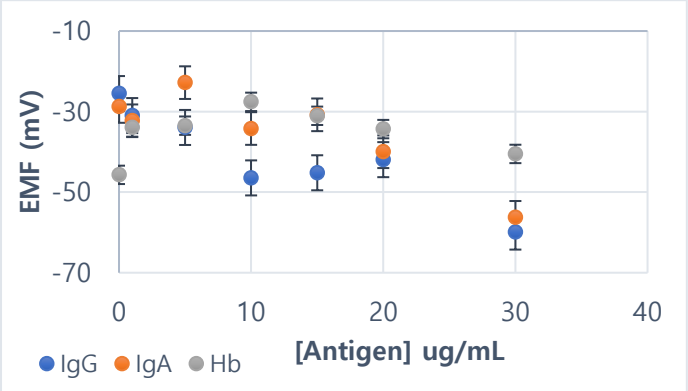

Figure 9. ΔEMF as function of Antigen concentration: IgG, IgA and Haemoglobin. Selectivity test.

Table 2. Values of Linear Range, Sensitivity of detection and ΔEMF in LR. Selectivity test, comparison of IgG, IgA and Haemoglobin detection.

	IgG	IgA	Haemoglobin
Linear Range ([IgG] in µg/mL)	0 to 10		15 to 30
Sensitivity in Linear Range (mV/µg·mL^{-1}):	-1,9	-0,3	-0,6
ΔEMF in Linear Range (mv)	-20,9	-5,5	-9,5

Potentiometric measurements: Analytical performance

Once all key parameters were optimized, a final experiment was conducted to test the performance of sensors and study the effect of each step of the methodology. A set of sensors was fabricated, and the process was stopped at IgG incubation for one, at skimmed milk incubation for another, and the process continued to GOX-Ab incubation for a third sensor. All sensors were calibrated under the same conditions. Results are depicted in Figure 10 and data is summarized in Table 3.

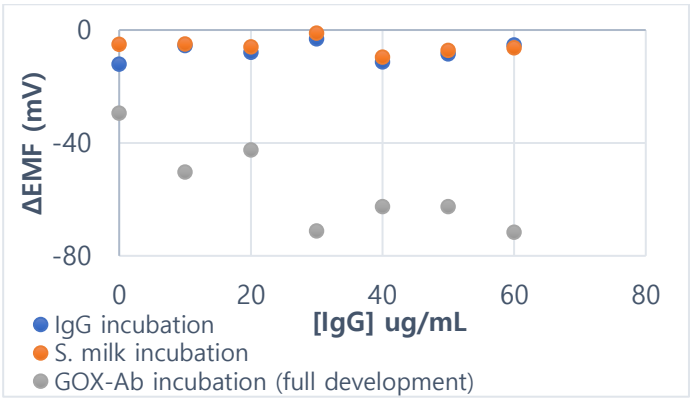

Figure 10. ΔEMF as a function of IgG concentration. Comparison of sensors stopped at IgG incubation, sensors stopped at skimmed milk incubation and sensors fully developed up to GOX-Ab incubation.

*Table 3. Values of Linear Range and Sensitivity of detection. Analytical performance test.*Sensitivity was not calculated within LR as no LR was defined.*

Process stopped at incubation of:	IgG	Skimmed milk	GOX-Ab (full development)
Linear Range ([IgG] in µg/mL)	0 to 10	-	-
Sensitivity in Linear Range (mV/µg·mL^{-1}):	-2,1	0,1*	-0,1*

The results indicate that the ELISA method for recognition is well implemented. No detection is produced in sensors lacking GOX-Ab. This demonstrates all parameters were optimized accordingly (blocking agent, incubation conditions, glucose concentration) for the immunoassay to work.

A calibration with H_2O_2 was also performed to assess the correct functioning of sensors, with -Log[H_2O_2] = -5, -4, -3 and -2. In Figure 1 and 2 *(Annex)* are shown the time traces of the calibration of sensors with [IgG] = 0 µg/mL and 50 µg/mL respectively. The results prove that the Platinum

electrode responds in the expected manner to H_2O_2 additions.

CONCLUSIONS

The proof of principle for the development of compact and portable electrochemical devices based on biochemical techniques such as ELISA has been demonstrated. In this work we have successfully developed a potentiometric paper support-based sensor for the detection and quantification of IgG based on the enzyme-linked mechanism of detection. Sensors were made of Platinum electrode covered with Nafion, which enhances the detection and is also an excellent platform for protein anchoring. For the IgG recognition method, Direct ELISA has proven to be a reliable system in terms of sensitivity of detection and selectivity for the target antigen. Skimmed powder milk was utilized as blocking agent, reducing significantly the amount of unspecific interactions between GOX-Ab and Pt surface.

Finally, the work methodology was successfully optimized to last 1,5 hours each incubation step. Together with the use of paper for a substrate, this contributed to making more efficient the process of production and minimizing the environmental impact of the device.

As per future work, this system presents several advantages that make it especially attractive to implement in Lateral Flow Assay platforms. For instance, the rapid and economic methodology of fabrication of sensors, the selective and sensitive mechanism of antibody detection through immunoassay, or the simplicity of quantification of the target analyte thanks to the use of an electrochemical transducer. However, it is important to note that this method presents a few limitations, such as the time requirement for the implementation of the ELISA system, the dependence on temperature for the integrity of the proteins, or the loss of reproducibility after successive uses (due to degradation of GOX-Ab). Further research needs to be conducted to solve these issues.

ACKNOWLEDGEMENTS

This work has been supported by the Spanish Ministry of Education through a Collaboration grant with the Department of Analytical Chemistry and Organic Chemistry. I want to give a special acknowledgement to Dr. Pascal Blondeau and Dr. Francisco Javier Andrade for their supervision throughout the thesis, as well as for the personal advice. I also want to thank my colleagues of the laboratory of Nanosensors for their continued support and collaboration.

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ANNEX (Supplementary information)

Immunoassay design and optimization: ELISA methodology

Table 1. Values of change in EMF (mV) in function of IgG concentration in µg/mL, Linear Range, and Sensitivity of detection. Direct ELISA and Sandwich ELISA sensors are compared.

[IgG] (µg/mL)	ΔEMF (mV)	
	Direct ELISA	**Sandwich ELISA**
0	-107,8±3,4	-92,3±3,6
0,1	-133,0±5,4	-103,6±10,6
0,5	-137,9±0,5	-105,2±2,9
1	-125,4±5,2	-107,9±6,5
Linear Range ([IgG] in µg/mL)	0 to 0,1	
Sensitivity in Linear Range (mV/µg·mL^{-1}):	-251,9	-112,8
ΔEMF in Linear Range (mv)	25,2	11,3

Immunoassay design and optimization: Blocking step

Table 2. Values of change in EMF (mV) in function of IgG concentration in µg/mL, Linear Range, and Sensitivity of detection. Direct ELISA sensors blocked with BSA 1%, skimmed milk 5% and Casein 0,1% are compared.

BSA 1%		**Skimmed milk 5%**		**Casein 0,1%**	
[IgG] (µg/mL)	ΔEMF (mV)	[IgG] (µg/mL)	ΔEMF (mV)	[IgG] (µg/mL)	ΔEMF (mV)
0	-107,8±3,4	0	-16,4±2,4	0	-24,7±8,7
0,1	-133,0±5,4	50	-96,1±0,7	1	-42,2±12,4
0,5	-137,9±0,5	100	-106,4±2,1	10	-53,5±9,5
1	-125,4±5,2	200	-104,5±0,7	100	-80,3±16,3
Linear Range ([IgG] in µg/mL)					
0 to 0,1		0 to 50		1 to 10	
Sensitivity in Linear Range (mV/µg·mL^{-1}):					
-251,9		-1,6		-2,2	
ΔEMF in Linear Range (mv)					
25,2		79,7		17,5	

Immunoassay design and optimization: Incubation period

Table 3. Values of change in EMF (mV) in function of IgG concentration in µg/mL, Linear Range, and Sensitivity of detection. Direct ELISA sensors incubated for 1,5 h and sensors incubated for 2 h are compared. Glucose concentration of 10^{-2}M (0,01M) was used during calibration.

[IgG] (µg/mL)	ΔEMF (mV)	
	1,5 h incubation	**2 h incubation**
0	-35,2±3,8	-22,0±1,3
0,1	-41,4±0,0	-35,3±0,7
0,5	-64,7±4,0	-40,9±1,1
1	-78,4±0,0	-46,8±3,0
Linear Range ([IgG] in µg/mL)	10 to 30	0 to 20
Sensitivity in Linear Range (mV/µg·mL^{-1}):	-1,9	-0,9
ΔEMF in Linear Range (mv)	37,0	18,9

Potentiometric measurements: Analytical performance

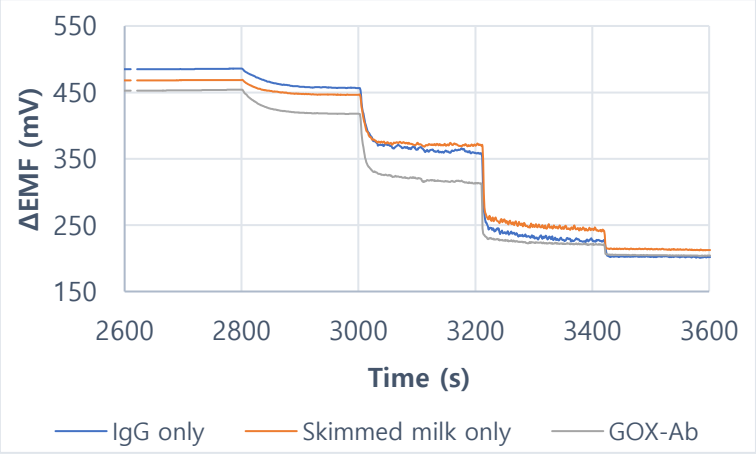

Figure 1. Time trace of calibration with hydrogen peroxide, of negative control sensors ([IgG] = 0µg/mL). Comparison of sensors stopped at IgG incubation, sensors stopped at skimmed milk incubation and sensors fully developed.

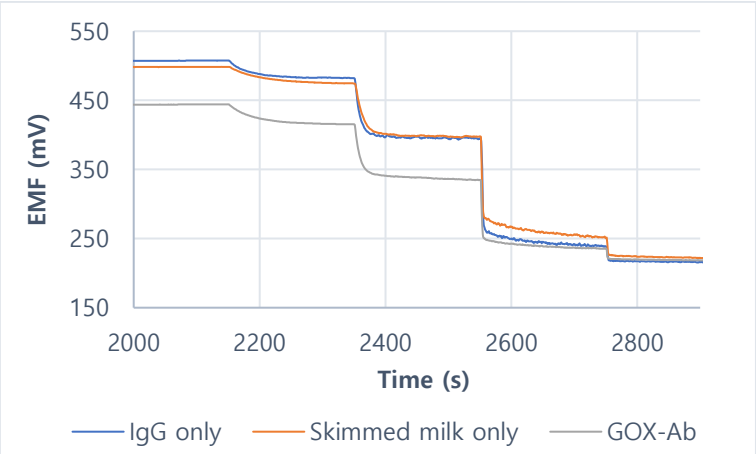

Figure 2. Time trace of calibration with hydrogen peroxide, of negative control sensors ([IgG] = 50µg/mL). Comparison of sensors stopped at IgG incubation, sensors stopped at skimmed milk incubation and sensors fully developed.