Characterization and validation of a platinum paper-based potentiometric sensor for glucose detection in saliva

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Abstract

This paper presents the characterization and validation of a platinum paper-based potentiometric electrode for the enzymatic detection of glucose in saliva. The analytical parameters obtained are suitable to determine high glucose concentrations in human saliva for diabetic patients. The linear range spans from 0.32 to 3.20 mM. The performance of the electrode was evaluated by measuring glucose in real human samples and comparing the values to the results of a commercial enzymatic colorimetric approach. The electrode is capable of determining glucose content in real saliva samples within an interval of glucose concentrations.

Keywords

Saliva, glucose detection, paper-based electrode, potentiometric, enzymatic sensor

1. Introduction

According to the International Diabetes Federation, the global prevalence of diabetes was estimated at 451 million cases in 2017, and following the continuous increasing trend over the last 40 years, it is expected to reach 693 million by 2045 [1]. Diabetes mellitus represents a group of metabolic disorders characterized by hyperglycemia. Uncontrolled blood glucose levels increase the risk of developing various serious vascular complications involving the heart, eyes, nerves and kidneys. Preventing these complications as well as improving

patients' quality of life are key factors in diabetes management. Monitoring of blood glucose levels can help determine the most appropriate treatment in terms of dietary uptake or insulin dosage adjustment.

Blood glucose concentration is currently monitored by means of blood draw or finger-prick testing as a self-monitoring practice. Nevertheless, these invasive methods are painful and can generate anxiety or fear in the patients, who have to repeat the process from three to six times per day. This may lead them to forego the monitoring process, resulting in the inadequate control of glucose levels. Moreover, exposure to blood-borne pathogens such as hepatitis and HIV [2,3] poses a risk of infection to both patients and medical professionals. Therefore, non-invasive methods to monitor glucose levels have been studied to mitigate patient pain and discomfort. The correlation of blood glucose levels and body fluid glucose levels has been the focus of many studies in recent decades in attempts to develop non-invasive sensors that could replace phlebotomic techniques. For instance, numerous sensors have been developed to determine glucose concentrations in urine, tears, sweat or saliva [4–6].

Saliva is considered as advantageous biological fluid for use in the early diagnosis of many different cardiovascular, infectious and autoimmune diseases [7]. Although water is the main component of saliva, the solid content is based on inorganic ions, such as Na^+, K^+, Mg^{2+} or Cl^- among others, and organic substances such as proteins, carbohydrates or lipids. In addition, saliva also contains exfoliated epithelial cells, bacteria and bacterial metabolites which confer an additional complexity to the matrix. These molecules can be used as biomarkers for the early detection of some physiological and pathological changes in the human body, and have already been used in the detection of different cancers, malaria, HIV and the diagnosis of diabetes [7,8].

Since saliva is constantly produced, collecting and storing samples is a simple and low-cost process that is painless and safe both for patients and for medical personnel. At the same time, saliva does not clot and is very stable. Therefore, salivary glucose determination provides a totally non-invasive and patient-friendly approach to monitoring glucose levels. However, some controversy remains regarding the correlation between glycaemia and salivary glucose [9–12] and some studies caution against using salivary glucose as diabetic diagnostic tool [13]. Although all studies confirm the fact that glucose concentration in saliva is higher in diabetic patient than in healthy ones, the differences on sample collection, glucose excretion rate and salivary flow hamper the correlation between salivary and blood glucose levels. These differences can be caused by multiple factors, such as medication, that can alter physiologic and metabolic regulation on diabetic patients. Nevertheless, many other studies have reported positive significant correlations between blood glucose levels and salivary glucose levels [14–19] with regression coefficient of 0.96, and thus, suggesting the determination of salivary glucose as reliable non-invasive method for predicting glucose concentrations in diabetics. The use of saliva as a diagnostic fluid requires highly sensitive sensors, since glucose concentrations in saliva are much lower than in blood (8 to 210 μM versus 3 to 30 mM, respectively). Many different techniques, such as liquid chromatography mass spectrometry, near and mid-infrared spectroscopy or fluorescence [20], for instance, have already been used to determine glucose in saliva matrices . Of all the techniques tested, electrochemical sensors [21] have been found to provide good sensitivity and selectivity, low operational costs and easy miniaturization and multiplexing for integration in portable devices. Within the electrochemical techniques, potentiometry has the advantages of simplicity of operation and instrumentation, low power consumption and the low-cost production of strips using, for instance, paper substrates, which facilitates miniaturization. Potentiometric devices can therefore be considered effective tools in the development of simple and affordable analytical platforms for use outside of the lab in keeping with the

increasing trend towards self-monitoring in the field of health care and management. The combination of such instrumentation with the advantages provided by the use of paper-based substrates, as the accessibility and affordability, has made potentiometric paper-based analytical devices very attractive in the sensing community for the last decade [22–24]. Paper-based potentiometric sensors have been developed to determine multiple electrolyte concentrations of K^+, NH_4^+ and pH, [25] or Cl^-, Ca^{2+}, K^+ and Na^+ [26] among others. Indeed, our group has recently developed a fully integrated wireless electrochemical potentiometric platform to determine glucose in serum and whole blood based on the interaction of the hydrogen peroxide (H_2O_2) generated during the enzymatic redox reaction (using glucose oxidase (GOx)) with the Nafion-coated platinum paper-based electrode [27]. The group has also reported on the use of the potentiometric enzyme-based electrode for the determination of glucose in fruit juices with high sensitivity and selectivity[28].

Taking advantage of the developed potentiometric electrodes and considering the advantages of using saliva as a means of non-invasive monitoring, this work aims to broaden the application of the paper-based potentiometric electrode with saliva determination as a new matrix of interest. Thus, the study presents the characterization and the analytical performance of the electrode for glucose detection in real human saliva, while maintaining the fabrication process and detection mechanism of the abovementioned electrodes. The results show good performance of the potentiometric electrode compared to a commercial enzymatic colorimetric assay, confirming the capability and versatility of the low-cost paper-based electrode to determine glucose levels in different human body fluids.

2. Materials and methods

2.1. Reagents

Whatman® Grade 5 qualitative filter paper was used for the fabrication of the working electrode. Nafion® perfluorinated resin solution (5 wt % in a mixture of lower aliphatic

alcohols and water, 45% water), glucose oxidase (GOx) from *Aspergillus niger* type X-S, lyophilized powder, 100,000-250,000 units per g solid, hydrogen peroxide solution 30% (w/w) (H_2O_2), and D-glucose were purchased from Sigma-Aldrich, Spain. In all cases, Nafion solution was used as received. Analytical grade salts of potassium chloride, sodium chloride, calcium chloride, disodium phosphate, potassium phosphate and sulfuric acid were purchased from Sigma-Aldrich, Spain. All solutions were prepared using 18.2 MΩ cm^{-1} double deionized water (Milli-Q water systems, Merck Millipore).

Phosphate buffered saline (PBS) was prepared 0.1 M at pH 7.4 (100 mM Na_2PO_4, 18 mM KH_2PO_4, 14 mM NaCl and 3 mM KCl) and used in all the experiments. Artificial saliva samples contained 10 mM KCl, 7.4 mM NaCl, 2 mM $CaCl_2$, 6.4 mM Na_2HPO_4, 2.5 mM $NaHCO_3$ at pH 7.4 [29].

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 on one side of a conventional filter paper to build the redox-sensitive electrode surface.

2.2. Electrochemical measurements

The electromotive force (EMF) was measured using a high input impedance (10^{15} Ω) EMF16 multichannel data acquisition device (Lawson Laboratories, Inc., Malvern, PA, USA). A double junction Ag/AgCl/KCl 3 M reference electrode (type 6.0726.100, Metrohm AG) containing 1 M LiAcO electrode bridge was used to study the working electrode. Laboratory measurements were taken using a 0.1 M PBS (pH 7.4) 4 mL cell at room temperature.

2.3. Fabrication of the enzymatic paper-based glucose sensor

The working electrode was built based on the procedure described in Cánovas et al. [27]. Briefly, the conducting platinum paper was cut into strips of 0.5 cm x 2.0 cm and then one strip was sandwiched between two 1.0 cm x 1.5 cm plastic masks (ARcare® 8565,

Adhesives Research Inc., Limerick, Ireland). The top mask had a 3 mm diameter circular window to expose the electroactive platinized paper to cast the biosensing membrane and functionalize the electrode (Fig. 1). A first layer of 7 µL Nafion solution was then drop cast and air-dried for at least 60 min at room temperature. Afterwards, 10 µL of a solution containing 20 mg mL^{-1} of glucose oxidase in distilled water was drop cast on top of the Nafion layer and left to dry for 24 h at 4 °C. Finally, a second 7 µL Nafion layer was drop cast on top in order to entrap the enzymatic layer and was also left to dry for 24 h at 4 °C. The electrodes (denoted as Pt/Nafion/GOx/Nafion) were kept at 4 °C when not in use.

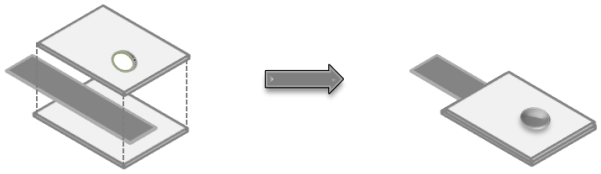

Fig.1 Schematic representation of the fabrication procedure of the working electrode.

2.4. Enzymatic assay

As a reference method, a commercial colorimetric glucose assay (glucose oxidase assay kit from Sigma-Aldrich) was used. Absorbance measurements were taken in an UV-Vis spectrophotometer (Agilent Technologies, Spain) with a 10 mm light path plastic cuvette (BRAND GMBH+CO KG, Germany). Real saliva was centrifuged and supernatant was collected to be used as a control test, without reagents, to avoid great turbid differences between the control and test samples.

2.5. Analysis of real samples

Real saliva volumes were provided by different non-diabetic volunteers directly by spitting with no previous stimulation, and used as received without any treatment. Highly viscous saliva samples were dismissed to ensure precision in volume measurements. To simulate diabetic salivary glucose levels, D-glucose was artificially added to the samples at different

concentrations (from 2 to 10 mM). The glucose oxidase colorimetric test was used as the standard method for the validation of the Pt/Nafion/GOx/Nafion potentiometric electrode.

3. Results and Discussion

3.1. Principle of detection and electrode response

Many approaches use the generation of H_2O_2 through glucose oxidase enzyme in order to quantify glucose concentration. The oxidation of D-glucose to gluconolactone uses oxygen as the electron acceptor and it is catalyzed by the enzyme glucose oxidase (GOx), generating hydrogen peroxide as a by-product of the reaction:

$$glucose + H_2O + O_2 \xrightarrow{GOx} gluconolactone + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (2)$$

Since there is a direct relation between glucose consumption and hydrogen peroxide production, the glucose concentration can be easily calculated from the change in redox potential generated by the hydrogen peroxide production. In previous works, our group has demonstrated the improved performance of H_2O_2 detection based on platinum electrodes by using a Nafion coating [30], where Nafion has proven to increase both sensitivity and selectivity parameters in potentiometric H_2O_2 sensors. A detailed description and characterization of the H_2O_2 detection through Nafion layers is described in Parrilla et al. [30,31]. Recently, our group has reported the use of polyelectrolytes, such as Nafion, as a way to control the mixed potential of the platinum based electrode [32]. The open circuit potential of the Pt electrode is indeed shown to work under kinetic control of the oxygen reduction reaction.

Thus, experiments were conducted by monitoring the change in the electrochemical potential generated with increasing glucose concentrations. At an initial stage, the EMF was

measured in a range from $10^{-4.5}$ to 10^{-2} M (0.03 to 10 mM) of H_2O_2 with sensors without enzyme (Pt/Nafion) to characterize the electrode response. Fig. 2 shows the calibration plot of Pt/Nafion electrodes in 0.1 M PBS pH 7.4 and in artificial saliva medium for comparison, where the electrode potential decreases upon the addition of H_2O_2.

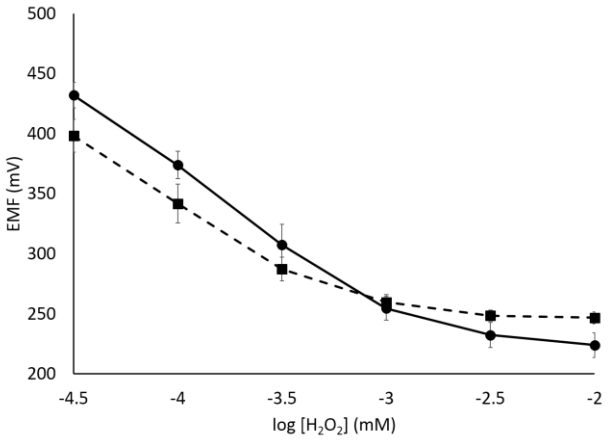

Fig.2 *Calibration plot of Pt/Nafion electrodes with H_2O_2 additions in ●) 0.1 M PBS and ▪) artificial saliva medium. The error bars correspond to the standard deviation of 5 independent electrodes on each medium*

The Pt/Nafion electrodes showed a sensitivity to direct H_2O_2 additions of -119.3 ± 5.9 mV/dec in 0.1 M PBS pH 7.4 with a regression coefficient of 0.998, within a linear range from $10^{-4.5}$ to 10^{-3}. H_2O_2 sensitivity in artificial saliva was -98.6 ± 2.3 mV/dec with a regression coefficient of 0.979 (same linear range). The difference in the electrode performances is related to the mixed potential theory. As first described by Parrilla et al. [30], the electrode response depends on the pH of the solution and on the total concentration of the supporting electrolyte. Although in both PBS and artificial saliva pH is 7.4, the composition and thus, total ion concentration are different and affect the potential between the electrode and solution, demonstrating the need for strictly controlling the measurement conditions.

In the case of the Pt/Nafion/GOx/Nafion electrodes, the decrease in the electrochemical potential after glucose additions followed the same trend as when H_2O_2 was added, and the

sensitivity to the H_2O_2 generated through the glucose oxidase reaction in 0.1 M PBS pH 7.4 was -93.2 ± 1.8 mV/dec with a regression coefficient of 0.985. The linear range in PBS measurements was from $10^{-3.5}$ to $10^{-2.5}$ M (0.3 to 3.2 mM), which is within the diabetic glucose saliva range values ($10^{-3.7}$ to $10^{-2.2}$ M or 0.2 to 6.3 mM) found in the literature [33–37]. Even though the thickness of the biosensing membrane is obviously higher in the Pt/Nafion/GOx/Nafion than in the bare Pt/Nafion electrode, the analytical performance is not compromised since the second layer of Nafion also helps in the immobilization of the enzyme by entrapment, as well as in the confinement of the produced H_2O_2 within the membrane, avoiding the leaching of both the enzyme and the by-product.

Taking into account that the diabetic salivary glucose range exceeds the linear range of our electrode and its complex matrix may influence analyte quantification, experiments with artificial saliva samples were done considering the dilution of the sample with 0.1 M PBS in order to, first, be able to detect the glucose in samples of saliva within the linear range of our potentiometric electrode, and second, study the matrix effect behavior of the final potential of the electrode. Artificial saliva containing 10 mM glucose was diluted with 0.1 M PBS pH 7.4 to different concentrations within the linear range of the potentiometric sensor ($10^{-3.5}$, 10^{-3} and $10^{-2.5}$ M or 0.32, 1 and 3.16 mM) in order to evaluate the analytical performance of Pt/Nafion/GOx/Nafion for glucose prediction in saliva matrix. An initial glucose calibration at 0.1 M PBS pH 7.4 was required to settle the calibration curve equation as the reference for further glucose predictions made with the artificial saliva samples (Fig. 3). Before the first glucose addition in Fig. 3 we made sure that the signal was stable and the EMF was constant. Henceforth, the other glucose additions were done every 300 s. Electrodes were rinsed with double deionized water between each artificial saliva glucose prediction in order to clean the electrode surface.

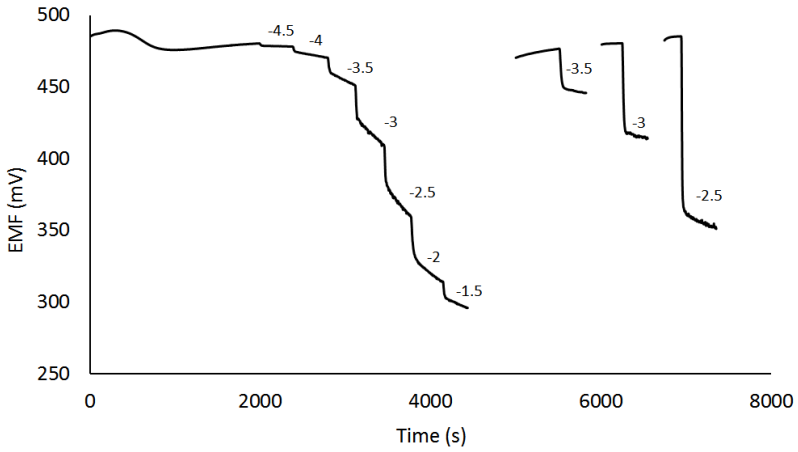

Fig.3 *Time trace of glucose calibration in 0.1 M PBS pH 7.4 and following glucose predictions in artificial saliva pH 7.4 at $10^{-3.5}$, 10^{-3} and $10^{-2.5}$ M glucose*

Table 1 shows the comparison between the theoretical and experimental values of glucose concentrations from predictions shown in Fig. 3, showing the recoveries and dilution factors needed in each case. Potentiometric experimental values are given as an average and their corresponding standard deviation from 23 different electrodes is also shown.

Table 1 *Comparison between theoretical and potentiometric values for 0.32, 1 and 3.16 mM glucose concentrations in artificial saliva (N=23)*

Dilution factor	Theoretical [glucose] (mM)	Experimental [glucose] (mM)	% recovery
1:20	0.32	0.36 ± 0.05	113
1:10	1.00	0.99 ± 0.29	98
1:2	3.16	5.22 ± 2.97	165

Although the electrode was already tested against possible interference substances, such as ascorbic acid, uric acid or fructose with no significant effect on glucose determination [31], these results confirm the influence of the matrix composition on the electrode potential. As the dilution factor decreases, the bias and deviation from the reference value increases due to the interference of other electroactive compounds from the matrix with the final potential of the system. In this case, the signal is enhanced, resulting in an erroneous final

glucose quantification that compromises both precision and selective detection. In contrast, potentiometric predictions were more precise and accurate the higher the dilution factor. Higher dilutions imply less matrix load in the cell, allowing a more homogeneous medium with control over experimental conditions, such as pH or ionic strength of the solution. They minimize the effect of the interfering compounds from the complex saliva matrix on the final electrode potential by stabilizing it with the PBS buffer.

An intrinsic advantage of diluting the samples is reflected in the reproducibility and repeatability of the measurements, where the useful life of the sensors can be prolonged due to the reduced number of interfering species interacting with the sensing electrode surface. This is reflected by the low relative standard deviation (RSD) of initial EMF (EMF_0) between calibrations, which were less than 1.7% in all the cases. However, this does not represent a disadvantage since the electrodes are built to be disposable, in keeping with the increasing trend of single-use low-cost point-of-care devices for self-monitoring and management [23,38].

In addition, repeatability and reproducibility among sensors on glucose calibrations were also evaluated in 0.1 M PBS pH 7.4 medium. Fig. 4a depicts the relative EMF in % of the different glucose additions compared to the logarithm of the glucose concentration, from three consecutive calibrations with four different sensors. Standard deviation is also represented and indicates the excellent repeatability of the measurements, and suggests the reusability of the electrodes for multiple measurements (at least three) while maintaining the same electrochemical response for each glucose concentration. Initial potential recoveries were 98.7% ± 1.2 for the second and 94.9% ± 1.5 for the third calibration compared to the original EMF_0 from the first calibration, resulting in an average RSD of 1.4%.

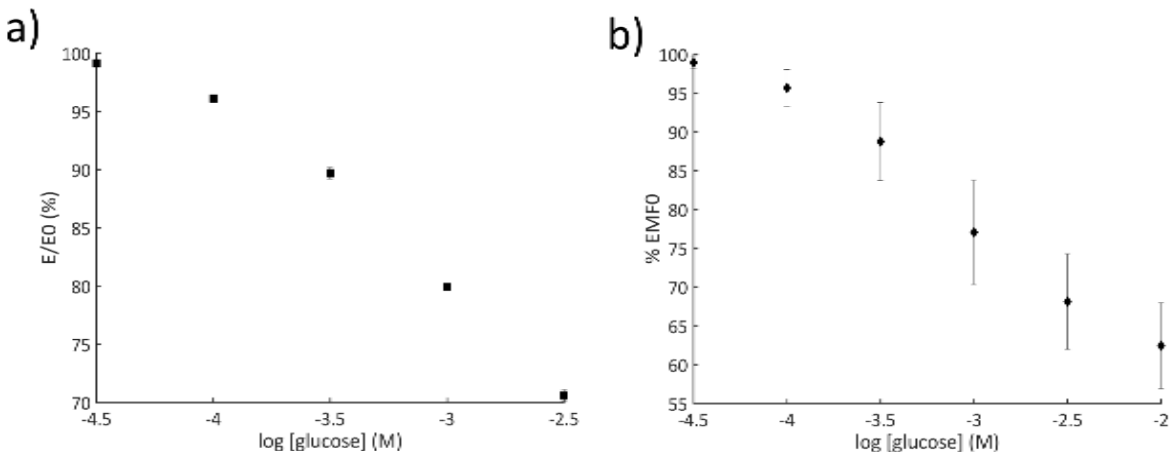

Fig.4 a) Measurement repeatability. Calibration plot of three glucose calibrations represented as % of the EMF_0. The error bars correspond to the standard deviation of 4 independent sensors **b)** Sensor precision at different days represented as the relative EMF compared to EMF_0 of each glucose concentrations from 80 different sensors

Moreover, Fig. 4b shows the corresponding average and standard deviation in % of the EMF at each glucose addition from glucose calibrations made with 80 individual sensors. The intermediate precision RSD from calibrations of 80 sensors on different days varies from an average of 2.9% for concentrations below $10^{-3.5}$ M to 8.8% for concentrations above $10^{-3.5}$ M.

Table 2 provides a comparison of the analytical performances of the potentiometric electrode described in this study with those of other recently reported electrochemical glucose sensors with different electrode configurations for glucose determination in real saliva matrices. Although the limit of detection is from two to three orders of magnitude higher than the other examples, including amperometric ones (which usually give lower limits of detection than potentiometric sensors), the Pt/Nafion/GOx/Nafion provides the highest upper limit of the linear range. In this way, the sensor fits the purpose of determining glucose concentrations of diabetic people, which tends to be higher than healthy individuals. In comparison, the Pt/Nafion/GOx/Nafion electrode also exhibits good sensitivity for glucose

detection in saliva and provides the intrinsic advantages of simplicity and low power consumption of the potentiometric devices.

Table 2 Comparison of analytical performances from different salivary glucose electrochemical based sensors

Working Electrode	**Technique**	**Sensitivity**	**Linear range** (µM)	**Limit of detection** (µM)	**Ref.**
Pt/PAA/SWCNT/CS/AuNPs/ GOx	amperometric	61.4 µA mM^{-1} cm^2	17 - 810	5.60	[17]
Tin bronze	amperometric	77 µA mM^{-1} cm^2	20 - 320	4.70	[39]
GCE/IrO_2@NiO/Nafion	amperometric	1439.4 µA mM^{-1} cm^2	0.5 - 2500	0.31	[40]
SPCE/AuNPs/pTBA/MIP	potentiometric	76.6 mV/dec	0.32 - 1000	0.19	[41]
Pt/Nafion/GOx/Nafion	potentiometric	-93.2 ± 1.8 mV/dec	316 - 3160	180.00	This work

Pt – Platinum // PAA – Poly (allylamine) // SWCNT – Single wall carbon nanotubes // CS – Chitosan // AuNPs – Gold nanoparticles// GOx – Glucose oxidase // GCE – Glassy carbon electrode // IrO_2 – iridium oxide // NiO – Nickel oxide // SPCE – Screen printed carbon electrodes // pTBA - poly (2,2' :5'5"-terthiophene-3' –*p*-benzoic acid) // MIP – molecular imprinted polymer.

3.2. Analysis of real samples

The Pt/Nafion/GOx/Nafion potentiometric electrode was validated by comparing its results with the results from a commercial enzymatic assay for glucose determination. Five different saliva samples were obtained from non-diabetic volunteers, with no restrictions on sample collection. Saliva collection was not induced, and neither fasting conditions nor differences in salivary gland production were considered for fluid extraction. Since non-diabetic people have low glucose concentrations in saliva, D-glucose had to be added to reproduce diabetic glucose levels (from 2 mM to 10 mM). In the potentiometric approach, a two-point calibration curve with glucose standards corresponding to both limits of the linear range of the sensor was used to determine the concentration of glucose. Saliva samples (2, 4, 6, 8 and 10 mM) were diluted 1:2, 1:4, 1:7, 1:10 and 1:13, respectively, to fit in an intermediate detectable concentration (1 mM) of the linear range of the potentiometric sensor. The same procedure

was carried out with the colorimetric approach, diluting each sample by factors of 1:8, 1:16, 1:24, 1:32 and 1:40, respectively, to reach a final glucose concentration of 0.25 mM which fit in the linear range of the commercial kit. Fig. 5 shows the comparison between the potentiometric and the commercial enzymatic assay results. As expected from previous results obtained with artificial saliva, the matrix effect is enhanced at lower dilution factors in the potentiometric electrodes leading to inaccurate concentration measurements where dilutions below 1:4 are required. Indeed, this effect was also evaluated by monitoring the pH and the conductivity of the solution during the potentiometric experiments in order to monitor possible changes in solution parameters that may affect the final potential read-out of the electrode. Since usual saliva pH ranges from 6 to 7.5 and the dilution buffer used was at pH 7.4, the pH of the solutions remained almost constant among all the different glucose concentrations tested (pH 7.40 ± 0.04). Meanwhile, the conductivity remained constant with a value of 23.4 ± 1.9 mS cm^{-1} in all cases except from dilution 1:2, which showed a decrease of 32% compared to the initial solution conductivity. It is not surprising then that changes in solution parameters due to the influence of the matrix compounds and characteristics may affect the charge distribution on the electrode membrane interfaces, resulting in an interfered change of potential, and thus, an erroneous glucose quantification.

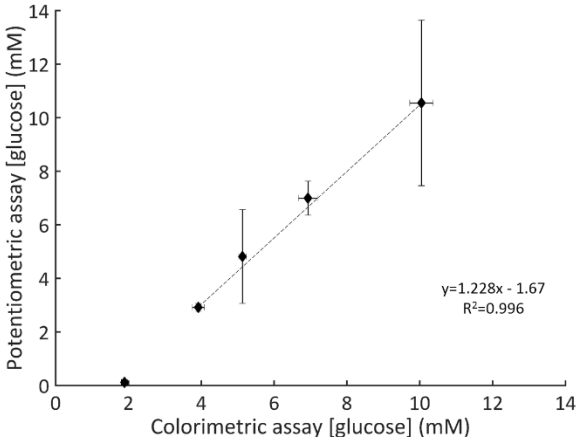

Fig.5 *Comparison of glucose determination in five real saliva samples determined by potentiometric sensor (mean ± S.D. N=10) vs. a commercial enzymatic assay (mean ± S.D. N=3). Linear regression corresponds to four values (from 4 to 10 mM).*

In contrast, the influence of saliva matrix was diminished when operating with dilutions of higher factors (above 1:4), which actually introduces fewer matrix components into the system. In these cases, neither pH nor conductivity changed significantly, and thus, glucose was properly quantified with the potentiometric Pt/Nafion/GOx/Nafion electrodes.

Our results show that the Pt/Nafion/GOx/Nafion electrode is able to accurately quantify glucose content in real saliva matrix with a dilution factor higher than 1:4. Changing the dilution buffer to one that could maintain optimum solution conditions without compromising the simulated physiological conditions may be one way to overcome issues in samples with low dilution factors. However, since salivary glucose levels in diabetic patients are usually high (reaching maximum concentrations of around 6.3 mM), the Pt/Nafion/GOx/Nafion electrode could be used to monitor glucose in saliva with the proper dilution factor without much inconvenience.

Therefore, and taking into account the results from 4 to 10 mM (corresponding to dilutions higher than 1:4), we performed a statistical study to validate our results. To check if the potentiometric and the commercial enzymatic results are comparable over the tested linear range (4 to 10 mM), one has to check if the coefficients of the regression line would be comparable to the coefficients of the theoretical regression line obtained if the results in comparison were identical (intercept=0 and slope=1). The joint confidence interval for the intercept and the slope of the regression line [42] consisting of verifying the presence of the theoretical point (0,1) within the limits of the joint confidence region of the experimental intercept and slope was used to compare the results of the two methods. As Fig. 6 shows, since the theoretical point (0,1) is within the limits of the joint confidence region for an α

significance value of 5% we can conclude that the potentiometric and the commercial enzymatic results are comparable for the interval tested (4 to 10 mM).

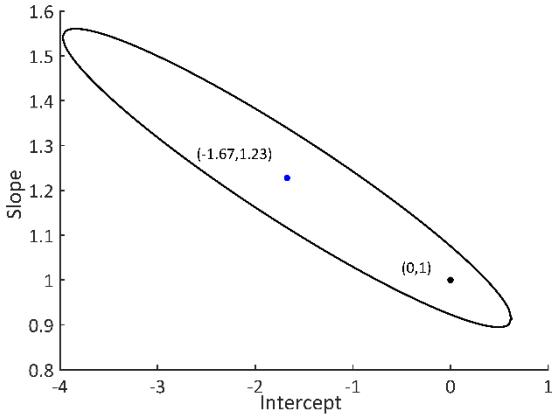

Fig.6 *Joint confidence region plot comparing the slope of the regression line from validation process with enzymatic and potentiometric methods against the theoretical one*

4. Conclusions

We have described the characterization and validation of a potentiometric enzyme-based electrode for the determination of glucose in human saliva. The combination of the potentiometric approach with a paper-based sensor, together with the use of Nafion to improve the analytical parameters, represents a simple and low-cost alternative for glucose detection in human saliva. Since saliva has been the focus of many studies into early diagnosis and glucose monitoring for decentralized and self-monitored health, the potentiometric sensor may be an effective alternative tool for that purpose. Results showed the potentiometric approach to be comparable to a conventional enzymatic commercial assay within an interval of glucose concentrations. The definition of this interval comes from the matrix effect that can somehow be modulated by diluting the sample. We have demonstrated accurate glucose quantification with dilutions higher than a factor of 1:4. Nevertheless, it is worth mentioning that real saliva samples were used as received without

any pretreatment, which may have helped to broaden the interval of operation by decreasing the matrix effect.

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6. Declaration of competing interest

The authors declare that they have no conflict of interest.

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