Julio César Zuaznabar Gardona

PAPER-BASED POTENTIOMETRIC SENSOR FOR MONITORING GALACTOSE LEVELS

MASTER'S DEGREE FINAL PROJECT

Supervised by Dr. Francisco J. Andrade

MASTER'S DEGREE IN NANOSCIENCE, MATERIALS AND PROCESSES: CHEMICAL TECHNOLOGY AT THE FRONTIER

UNIVERSITAT ROVIRA i VIRGILI

Tarragona

2016

Paper-based potentiometric sensor for monitoring galactose levels

Julio César Zuaznabar Gardona
Nanoscience, Materials and Processes: Chemical Technology at the Frontier Master Programme 2015-2016
juliocesar.zuaznabar@estudiants.urv.cat

Supervisor: Dr. Francisco J. Andrade
Department of Analytical Chemistry and Organic Chemistry. Universitat Rovira i Virgili
Campus Sescelades, c/ Marcel·lí Domingo, 1. Tarragona, 43007, Spain.

Abstract

Enzyme-based potentiometric sensors can become cost-effective platforms for the diagnosis of different diseases in a fast and reliable way. This work reports a paper-based potentiometric sensor as a new platform to evaluate galactose levels in blood samples for diagnosis and monitoring of galactosemia. The system works by detecting the hydrogen peroxide generated by the catalytic oxidation of galactose using the galactose oxidase enzyme immobilized on a substrate. Two approaches were employed to immobilize galactose oxidase, which is specific for galactose, on the surface of a piece of platinized paper: i) sandwiching it between two polymer layers and, ii) crosslinking it with an amine-rich polymer. An external oxidizing layer formed by MnO_2 particles is used to eliminate redox interferences. The system shows, sensitivity values of 23 and 41 mV/decade for sandwiching and crosslinking respectively, a limit of detection of $0.5*10^{-3}$ mol/L and a linear range from $1*10^{-3}$ to $1*10^{-2}$ mol/L, well within the clinically relevant range. This novel biosensor allows to perform determinations of galactose in few minutes instead of the typical hours or days required by the current lab-based approach.

Keywords: galactosemia, potentiometry, biosensor, galactose

Introduction

Galactosemia is a metabolic disorder where the body is not capable of transforming galactose into glucose.[1] It is caused by a deficiency of one or more of the three enzymes (viz. galactose-1-phosphate uridyl transferase, galactokinase and galactose-6-phosphate epimerase) intervening in the galactose metabolism through the Leloir pathway.[2] There are three types of galactosemia, referred to as classic galactosemia (galactosemia type I), galactokinase deficiency (galactosemia type II), or galactose epimerase deficiency (galactosemia type III).[3,4] Classic galactosemia is due to galactose-1-phosphate uridyl transferase deficiency and is the most common and sever form of the disease.[5] Newborns with either unrecognized or untreated classic galactosemia develop cataracts, liver diseases and kidney problems.[6] Furthermore, high galactose levels in blood (> 1 mmol/L)[7,8] can cause brain damage leading to newborn death in the first days of life.[9] Since early diagnosis of galactosemia can prevent developmental disorders or death in newborns, it is mandatory in many countries to perform diagnostic screening of all newborns.[10]

Most of the methods used for diagnosis of galactosemia are based on either the measurements of galactose or galactose-1-phosphate levels or evaluating the activity of 1-phosphate uridyl transferase in the blood of newborns.[3] Different methods to determine galactose levels and galactose-1-phosphate uridyl transferase activity have been reported. Such methods are classified as microbiological tests or enzymatic assays.[11] Among the microbiological test, the most representative are the Paigen test and the biological inhibition test.[12,13] Both methods use mutant Escherichia coli cultures which proliferate in the presence of galactose in dry blood spots. Thus, galactose concentrations in samples are determined by measuring the mutant Escherichia coli growth

zones around blood spots. Such tests possess several drawbacks, as for example long times needed to incubate bacteria culture (16−20 h), requirement for a special culture medium, difficulty to accurately measuring the zones of bacteria growth , sensitivity to antibiotic treatments either of newborns or their mothers and they are not suitable for automation.[9,10]

Enzymatic assays for diagnosis of galactosemia include the Beutler's test and the alkaline phosphatase-galactose dehydrogenase assay. The Beutler's test measures the activity of galactose-1-phosphate uridyl transferase by employing a coupled enzymatic assay involving phosphoglucomutase and glucose-6-phosphate dehydrogenase and measuring the intensity of fluorescence arising from formed nicotinamide adenine dinucleotide phosphate.[14] The alkaline phosphatase-galactose dehydrogenase assay quantifies total galactose (galactose + galactose-1-phosphate) in the blood sample.[15] Galactose-1-phosphate is converted to galactose by the addition of alkaline phosphatase. Galactose is then quantified by measuring the fluorescence produced by the reduction of nicotinamide adenine dinucleotide (NAD) to its reduced form, NADH by galactose dehydrogenase. These assays require special reagents with limited stability, involve multistep sample preparation procedures and are time-consuming (about 3 hours).

For these reasons, all current galactosemia diagnostic assays are performed on highly specialized laboratories by skilled personel.[9] Thus, developing an easy-to-use galactose sensor for diagnosis of galactosemia may have a significant impact on the control of this condition. Additionally, since the treatment of galactosemia consists on avoiding foods that contain galactose in the diet and controlling its blood levels,[11] it is also desirable to develop new, low-cost tools to control galactose level -either in blood or in food- outside of labs for dietary management of galactosemia patients.

Electrochemical biosensors are an interesting alternative to develop novel and easy-to-use tools for the outside lab diagnosis of different diseases.[16] They effectively combine a high selectivity, sensitivity and reliability with the simplicity, portability and low cost of the electrochemical instrumentation.[17] Two general approaches can be used to quantify galactose on samples by electrochemical measurements, i.e amperometric or potentiometric detection. In both cases, galactose oxidase (GALOx) is used as specific enzyme for galactose.[18,8] GALOx is a copper-containing oxidase enzyme with a molecular mass of 68 kDa which catalyzes the oxidation by oxygen of galactose to galactohexodialdose.[19] Hydrogen peroxide is also produced as a side-product of the enzymatic reaction.

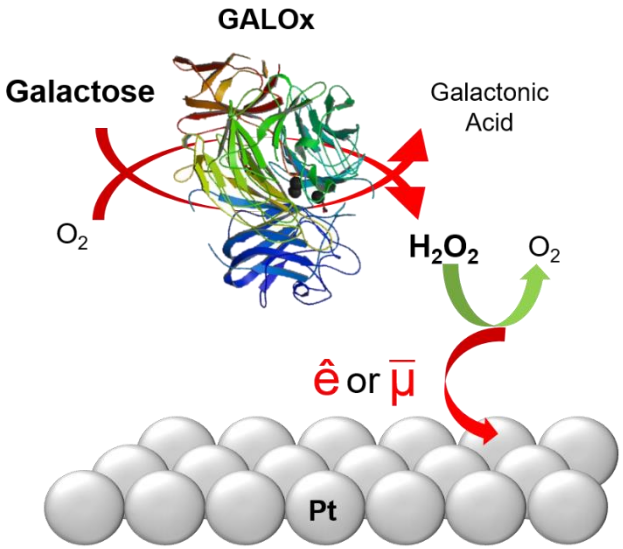

Figure 1. Scheme of electrochemical strategies for measurements of galactose.

All reported electrochemical methods to quantify galactose rely on the measurement of the amount of the hydrogen peroxide generated by such an enzymatic reaction (Fig. 1). In the case of amperometric detection, the current produced by the oxidation of hydrogen peroxide is measured and it is proportional to the amount of galactose in the sample. However, the results of this procedure are affected by different interferences (ascorbic acid, citrate and uric acid) which coexist on biological fluid sample. Different strategies

have been used to overcome this issue. For example the use of redox mediators to decouple the oxidation of the hydrogen peroxide and the interferences,[8] and the use of cianometalates with peroxidase-like activity which allow the detection of hydrogen peroxide at lower potentials.[20] Additionally, metallic nanoparticles and carbon nanostructures have been used to achieve the direct electron transfer between the active center of the enzyme and the electrode surface in order to get fast response time and to avoid intermediate reactions and interferences.[21,22]

On the other hand, the potentiometric approach is based on the measurement of the variations on the electrochemical potential of the system (i.e the electromotive force (EMF)) produced by a chemical reaction or variations on the density of charges at the electrode-solution interface.[23] Potentiometric biosensors to detect galactose are almost unexplored. To the best of our knowledge, there is only one report on potentiometric detection of galactose.[24] On this report, GALOx was immobilized on the surface of ZnO nanorods growth on gold coated glass electrodes. The authors claimed that EMF changes were produced by the increment of the amount of H_3O^+ ions around the electrode as a result of the enzymatic reaction between galactose and GALOx. These changes were correlated with the galactose concentrations. However, this potentiometric biosensor requires the thermal growing of ZnO nanorods which makes it unpractical for mass-production. On the other hand, redox molecules like H_2O_2 can be determined potentiometrically by using gold, palladium or platinum electrodes.[25] Hydrogen peroxide interacts with the electrode material to produce an electrical potential which is a function of its concentration in the solution. However, using noble-metals as bulk electrodes makes biosensors economically unaffordable. Another additional problem to address on potentiometric sensors using noble-metals as electrodes is their intrinsic sensitivity toward redox species from solution which causes interference on the measurement of the electrochemical potential.

In recent years, there has been a growing interest on the development of paper-based sensing platforms. This approach is attractive because it combines many of the inherent properties of paper, such as porosity, compatibility with biological entities and low-cost of manufacturing, among others.[16] Several reviews and previous works of our group demonstrated the feasibility and advantages of using paper as a substrate to fabricate low-cost electrochemical sensors.[16,26,17] Thus, depositing the noble metals as a thin layer on paper, instead of using them as bulk material could allow to create low-cost potentiometric sensors to determine galactose. However, the issue about redox interferences presents a challenge on the designing of the sensor. The aim of the present work is to present for the first time, the first steps towards the development of a low cost and disposable paper-based potentiometric sensor for diagnosis of galactosemia and control of the levels of galactose. GALOx was immobilized on the surface of platinized paper as H_2O_2 sensitive material. The potentiometric determination of hydrogen peroxide provided the possibility of quantifying the concentration of galactose. Different working parameters were optimized in order to get the best analytical performance of the sensors. Additionally, a Nafion® coating with MnO_2 nanoparticle was successful implemented as an approach to eliminate interferences. The optimized method shows very good analytical performance and outstanding simplicity of operation.

Experimental section

Reagents and materials

Nafion® 5% solution in mixture of lower aliphatic alcohols, polyethylenimine (PEI) (50% in water), glutaraldehyde and galactose oxidase (GALOx) from Dactylium dendroides (3000 U/g) were purchased from Sigma-Aldrich. Analytical grade salts, K_2HPO_4, NaH_2PO_4, manganese (II) acetate ($Mn(Ac)_2$), $KMnO_4$, sodium citrate (Na_3Cit),

sodium lactate (NaLac), ascorbic acid (HAsc) and uric acid (HUric) were purchased from Sigma-Aldrich. BioXtra grade D-(+)-galactose and D-(+)-glucose were also obtained from Sigma-Aldrich. All solutions were prepared with double distilled water (18,2 $M\Omega cm^{-1}$) obtained from a Milli-Q system (Millipore, Madrid, Spain).

Phosphate buffer (PB) 0.1 mol/L was prepared by mixing different volumes of 0.1 mol/L Na_2HPO_4 and 0.1 mol/L M KH_2PO_4 solutions. pH was adjusted with 1 mol/L NaOH or 1 mol/L HCl solutions. Artificial serum was prepared with 111 mmol/L NaCl, 29 mmol/L $NaHCO_3$, 2,2 mmol/L K_2HPO_4. pH was adjusted with 1 mol/L NaOH or 1 M HCl solutions.

Instrumentation and measurements

Platinum RF magnetron sputtering over filter paper was performed with an ATC Orion 8-HV from AJA International Inc. (MA, USA) at 150W.

Potentiometric measurements were carried out at room temperature (around 30ºC) with an electromotive force (EMF) high input impedance EMF16 multichannel data acquisition device from Lawson laboratories, Inc. (Malvern, USA). A double-junction Ag/AgCl/KCl electrode from Metrohm AG (Herisau, Switzerland) was used as reference electrode (RE). All the experiments were carried out in PB 0.1mol/L.

MnO_2 particles synthesis

MnO_2 particles were prepared by mixing diluted water solutions of $Mn(Ac)_2$ and $KMnO_4$ (eq. 1) as reported elsewhere.[27] Some modifications to this procedure are explained below.

$$3Mn(Ac)_{2(aq)} + 2KMnO_{4(aq)} + 2H_2O \rightarrow 5MnO_{2\,(s)} + 2KAc_{(aq)} + 4HAc_{(aq)} \quad \textbf{(Eq. 1)}$$

Briefly, 250 mL $KMnO_4$ 25 mmol/L were added dropwise (~1 drop/sec) to 250 mL $Mn(Ac)_2$ 37,5 mmol/L under vigorous stirring at room temperature to allow the reaction (Eq. 1) to take place. Then, the suspension was vacuum filtered using 934-AH glass microfiber filter Whatman™. The brown slurry was washed three times with 40 mL of milli-Q water and dried at 100 ^{0}C for 1 hour in an oven.

Base-electrodes fabrication

Base-electrodes were fabricated as follows: a qualitative number 5 filter paper Whatman™ was sputtered on one side with platinum to deposit a layer of a thickness of approximately 100 nm. Then, the platinized paper was cut in 2 cm x 0,5 cm strips and they were sandwiched and glued between two 1,5 cm x 1 cm polyester mask strips. One of the mask strips contained a circular window of 3 mm diameter to expose the platinized paper (Fig. 2a).

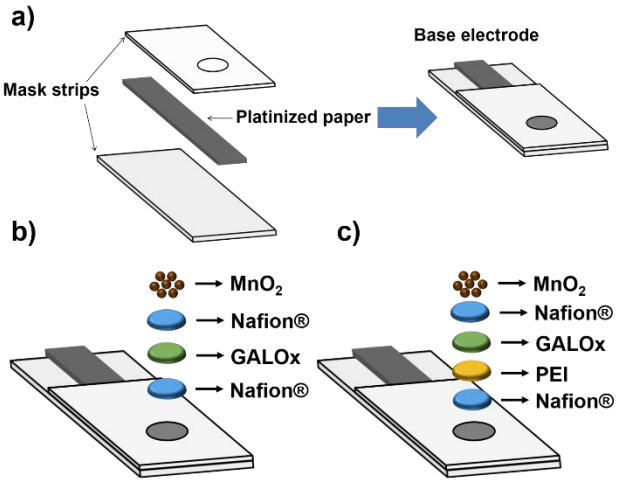

Figure 2. a) Scheme of the construction of the base-electrode and methods for GALOx immobilization, b) sandwiching and c) crosslinking.

Galactose biosensor

Two different methods for GALOx immobilization were used to deposit the enzyme on the electrode surface. To differentiate them they will be named sandwiching and crosslinking.

Sandwhiching

Seven microliters of Nafion® 0.25% solution in 2-propanol:H_2O mixture (1:1 v/v) were drop-cast on the base electrode. Then, the enzyme layer was formed by depositing appropriate amounts of GALOx dissolved in phosphate buffer 0.1 mol/L pH=6.6. Nafion® solution (3µL, 0.25%) was drop-cast on the top of the enzyme layer. To complete the preparation of sensors, an oxidizing layer was

subsequently deposited onto the Nafion® film (Fig. 2b).The oxidizing cocktail was prepared by dispersing the MnO_2 particles (50 mg/mL) in Nafion® 0.25% solution. Each step was carried out after drying the underlying film completely. The resulting galactose sensor was stored at 4 ºC in a desiccator during 24 h before use.

Crosslinking

Seven microliters of Nafion® 5% solution were drop-cast on the base electrode. Then, 5µL of PEI 1% were dispensed over the Nafion® layer. The enzyme layer was formed by depositing appropriate amounts of GALOx dissolved in phosphate buffer 0.1 mol/L pH=6.6. Glutaraldehyde 1% was used to crosslink the enzyme with the PEI layer. After that, Nafion® solution (2µL, 0.25%) was drop-cast on the top of the enzyme layer. To complete the preparation of sensors, MnO_2 layer was formed as was described in the previous session (Fig. 2c). Each step was carried out after drying the underlying film completely. The resulting galactose sensor was stored at 4 ºC in a desiccator during 24 h before use.

Results and discussion

Synthesis of MnO_2 nanoparticles

MnO_2 nanoparticles were synthesized by a redox reaction between $Mn(Ac)_2$ and $KMnO_4$ (Eq. 1). The reaction leads to formation of reddish brown solid corresponding to MnO_2 nanoparticles.

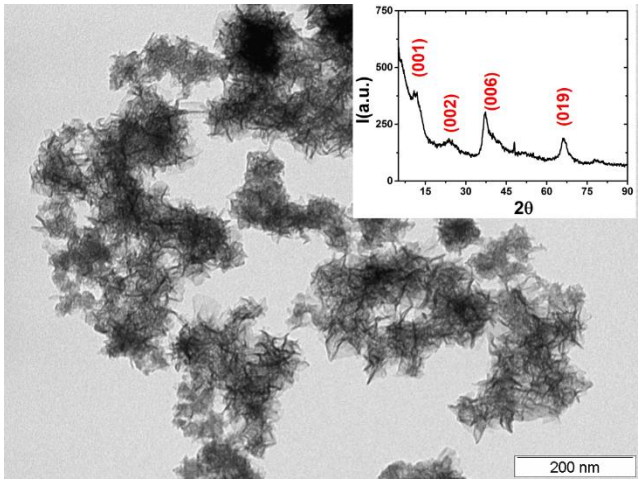

Figure 3. TEM image of MnO_2 nanoparticles. Inset: DRX pattern. The (*hkl*) planes are indicated.

TEM image shows 50 nm particles with wrinkled lamellar structure (Fig. 3).[27] The crystal phase of the MnO_2 was analyzed by powder X-ray diffraction. The diffraction peaks (see inset Fig. 3) were indexed to δ-MnO_2 in a poorly crystalline phase (JCPDS no. 18-0802).[28]

Galactose biosensor

Before studying the performance of the proposed potentiometric sensors, the first layer of Nafion® was optimized by changing the concentration and volumes of the Nafion® solutions drop-cast on the platinized paper electrodes. Nafion® was selected for two main reasons: i) it is a negatively charged polyelectrolyte, so GALOx which is positively charged is immobilized by electrostatic interactions, and ii) it allows to enhance the platinum sensitivity toward H_2O_2 by coupling its redox potential with those associated with the ions exchange (e.i Donnan potential).[29]

The working principle of the proposed galactose sensors is based on the potentiometric determination of the H_2O_2 generated as side-product of the oxidation of galactose by GALOx in presence of oxygen (Scheme 1).[19]

The analytical performance of the sensors constructed by sandwiching and crosslinking were studied in 0.1 mol/L phosphate buffer. Such sensors showed sensitivity values of -33 and -57 mV/decade for sandwiching and crosslinking respectively, a limit of detection (LOD) of $0.5*10^{-3}$ mol/L and a linear range from $1*10^{-3}$ to $1*10^{-2}$ mol/L as shown in Table 1.

Table 1. Analytical performance of the sensor

	Sandwiching	Crosslinking
Sensitivity (mV/dec)	-33±4	-57±3
LOD (mol/L)	$0.5*10^{-3}$	$0.5*10^{-3}$
Linear range (mol/L)	$1*10^{-3}$- $1*10^{-2}$	$1*10^{-3}$- $1*10^{-2}$
Time of response (min)	3	3

Time-trace potentiometric responses of sensors and the corresponding calibration plots are shown in Figure 4. Since the cutoff of galactose in blood for the diagnosis of galactosemia is 0.56-1.1*10^{-3} mol/L,[7,8] the proposed sensors could be used for galactosemia diagnosis.

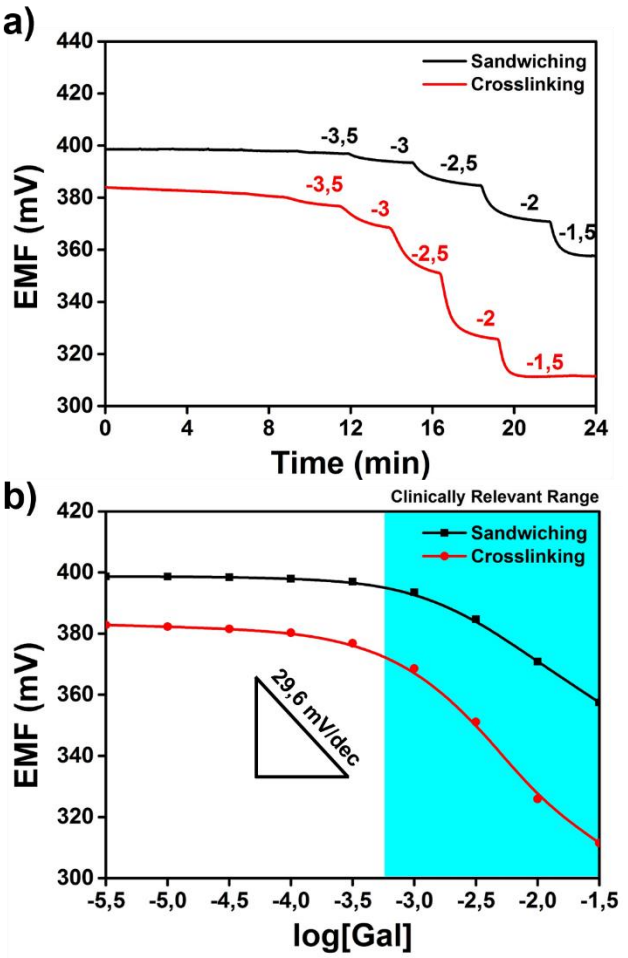

Figure 4. Potentiometric responses for sandwiching and crosslinking methods. a) The time-trace, and b) corresponding calibration plots upon increasing concentration of galactose.

The negative values of sensitivity suggest that H_2O_2 oxidation take place at the surface of platinized paper according to:

$H_2O_2 \rightarrow O_2 + 2H^+ + 2\hat{e}$ (**Eq. 2**)

By rearrangement of Nernst equation for this redox semi-equation, it is possible to demonstrate that ΔE is a linear function of the logarithm of H_2O_2 concentration with a negative slope (i.e. sensitivity). On the other hand, the difference on the obtained sensitivities respect to the expected according to Eq. 2 (= 29.6 mV/decade) could be explained by the coupling the redox potential with those associated with the ions exchange (e.i Donnan potential).[30]

Amount of enzyme loading

The effect of enzyme loading on the biosensors sensitivity was also assessed. Different biosensors were prepared with galactose oxidase loadings in the range of 0.02–0.36 U in the case of sandwiching, and 0.18-0.9 U for crosslinking. The potentiometric responses were determined for 1.9*10^{-4} - 2.3*10^{-3} mol/L galactose samples in PB 0.1 mol/L. Figure 5 shows that there is a clear influence of the enzyme loadings on the sensors sensitivity. In the case of sandwiching, the largest sensitivity value was achieved when 0.09 U of GALOx was deposited onto the electrode surface, while in the case of crosslinking was 0.54 U of enzyme. The use of higher enzyme loadings did not yield better results probably due to the lower availability of the active site on the enzyme. Also, diffusion of H_2O_2 and galactose is more hindered with thicker enzyme layers.

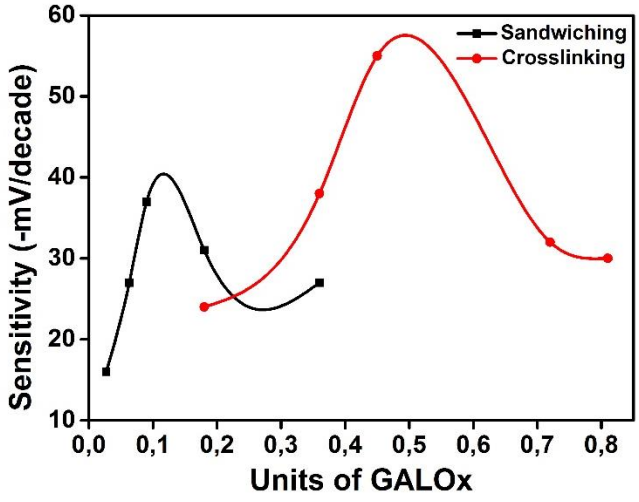

Figure 5. Variation of the sensor sensitivity with the enzyme loadings.

Working pH

The influence of pH on the potentiometric response for galactose was also evaluated using different 0.1 mol/L phosphate buffer solutions (Fig. 6) over the 5.0–8.0 range. In the case of the

sandwiching method, the biosensor sensitivity yielded a maximum response for a pH between 6.0 and 7.0, but for lower or higher values, the sensitivity decreases possibly due to the decrease on the enzyme activity. While in the case of the crosslinking method, a plateau is reached for pH values greater than 6.0 which could be due to a stabilization of the enzyme because of the covalent immobilization on the polymeric matrix. Thus, in order to get the highest sensitivity, pH 7.0 was selected as working pH.

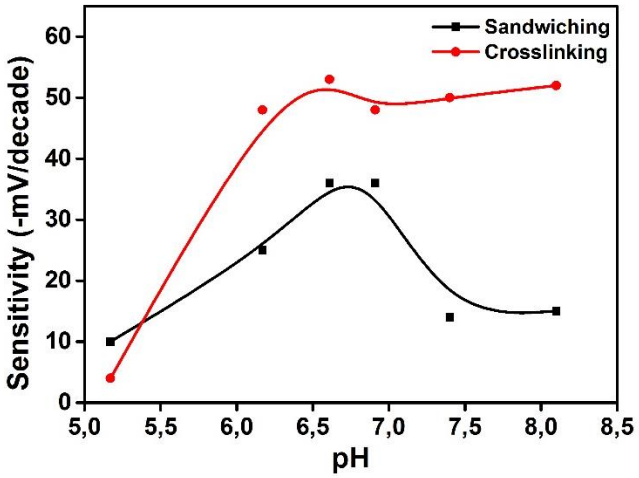

Figure 6. Influence of working pH on the sensitivity of the galactose sensors.

Interferences

Selectivity is a key parameter for optimum biosensor performance. There are different electroactive species in real serum samples which could affect the response of the electrochemical biosensors.[31] Thus, we examined the effect of common interferences presented in human serum such as glucose, citric acid, lactic acid, ascorbic acid and uric acid on the response of the proposed galactose biosensors. Figure 7 shows that ascorbic acid and uric acid cause strong interference (black and blue lines for sandwiching and crosslinking respectively) despite of the use of negative charge polyelectrolyte (Nafion®). Clearly, these redox-active molecules affect the potential read by the platinum electrode, and the permselectivity of the Nafion® membrane does not seem enough to avoid their interference. Thus, an approach where these redox anions can be eliminated was tested.

This approach consists on applying a mild oxidant –such as MnO_2- on the layer of membrane in contact with the solution. When a layer of MnO_2 particles is formed over a protective layer of Nafion® (red and green lines for sandwiching and crosslinking respectively in Fig. 7), the influence of all interferences was practically removed. Furthermore, such sensors exhibit response toward galactose.

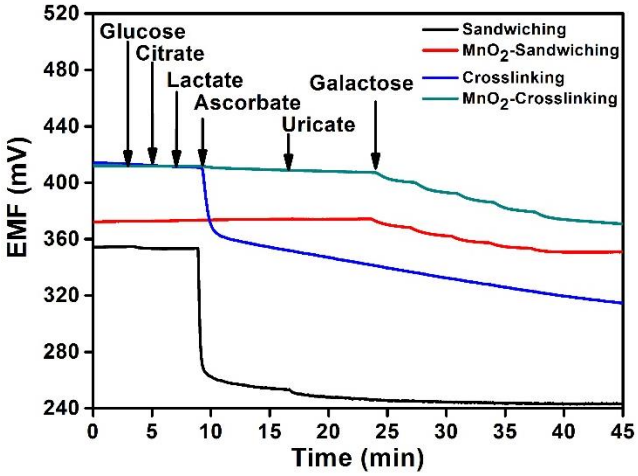

Figure 7. Response of the sensors without (black and blue lines) and with (red and green lines) MnO_2 layer to different interferences and galactose.

The elimination of the interferences is due to the mild oxidant power of MnO_2. Ascorbic and uric acid are easily oxidized to non-electroactive species by MnO_2 particles.[31] Thus, they are no able to change the electrochemical potential of the system, as a result there is not potentiometric response toward such substances.

The elimination of interferences with MnO_2 nanoparticles has a cost on the sensitivity of the sensors. Such sensors showed sensitivity values of -23 and -41 mV/decade for sandwiching and crosslinking respectively while their LOD, lineal range and response time were similar to those without using MnO_2. This reduction on the sensitivity could be explained by the fact that MnO_2 is also able to oxidize H_2O_2 as a result, less amounts of H_2O_2 reaches the platinum surface. Furthermore, the increase in the thickness of the

layers over the enzyme film could hindered the galactose diffusion toward the GALOx.

Artificial serum sample measurements

Galactose sensors fabricated by sandwiching and crosslinking were calibrated in artificial serum by adding galactose in the range between $1*10^{-3}$ to $3*10^{-3}$ mol/L. The galactose concentrations in five artificial samples were predicted using the calibration plot obtained and compared with the expected values. Results using the galactose sensors are shown in Table 2.

Table 2. Errors on predicting the concentration of galactose in artificial serum samples

	Sandwiching		Crosslinking	
$C(X)_{added}$ mol/L	$C(X)_{found}$ mol/L	% error	$C(X)_{found}$ mol/L	% error
$1.20*10^{-3}$	$1.27*10^{-3}$	6	$1.23*10^{-3}$	3
$1.80*10^{-3}$	$1.71*10^{-3}$	5	$1.74*10^{-3}$	3
$2.10*10^{-3}$	$2.0*10^{-3}$	5	$2.04*10^{-3}$	3
$2.40*10^{-3}$	$2.34*10^{-3}$	3	$2.36*10^{-3}$	2
$3.00*10^{-3}$	$3.17*10^{-3}$	6	$3.05*10^{-3}$	2

$\%=100*(|C_{added}-C_{found}|/C_{found})$

As can be observed in Table 2, errors on the prediction are smaller than 10% which is a standard value for margin of error for analytical determinations. However, further work is necessary to perform in order to test the sensor on real samples.

Conclusions

Paper-based enzymatic potentiometric galactose sensors based on platinum as transducer were prepared by immobilizing galactose oxidase through two different methods. MnO_2 particles were employed to remove the effect of common interferences presented in real samples. The potentiometric response of these sensors to varying galactose concentration showed linear response in the range of $1*10^{-3}$ to $1*10^{-2}$ mol/L with an average sensitivity of 23 and 41 mV/dec for sandwiching and crosslinking methods respectively. The response time was 3 min. These results suggest that proposed biosensor can be used for galactosemia diagnosis. Further work is needed to validate the approach and to optimize the design of the system to work in real decentralized settings.

Acknowledgments

Author wants to acknowledge Leonor Guadarrama, Pascal Blondeau, Francisco Javier Andrade and Francesc Xavier Rius for their advice, scientific guidance and help along the master course.

References

1. Isselbacher, K. J., Anderson, E. P., Kurahashi, K. & Kalcar, H. M. Congenital galactosemia, a single enzymatic block in galactose metabolism. *Science.* **123,** 635–636 (1956).

2. Frey, P. A. The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. *FASEB J.* **10,** 461–470 (1996).

3. Segal, S. Another aspect of the galactosemia enigma. *Mol. Genet. Metab.* **81,** 253–4 (2004).

4. Holden, H. M., Rayment, I. & Thoden, J. B. Structure and function of enzymes of the Leloir pathway for galactose metabolism. *J. Biol. Chem.* **278,** 43885–8 (2003).

5. Leslie, N. D. Insights into the pathogenesis of galactosemia. *Annu. Rev. Nutr.* **23,** 59–80 (2003).

6. Bosch, A. M. Classical galactosaemia revisited. *J. Inherit. Metab. Dis.* **29,** 516–25 (2006).

7. García, M., Alonso-Fernández, J. R. & Escarpa, A. Copper nanowires immobilized on the boards of microfluidic chips for the rapid and simultaneous diagnosis of galactosemia diseases in newborn urine samples. *Anal. Chem.* **85,** 9116–9125 (2013).

8. Kanyong, P., Pemberton, R. M., Jackson, S. K. & Hart, J. P. Development of an amperometric screen-printed galactose biosensor for serum analysis. *Anal. Biochem.* **435,** 114–119 (2013).

9. Jensen, U. G. *et al.* Neonatal screening for galactosemia by quantitative analysis of hexose monophosphates using tandem mass

spectrometry: a retrospective study. *Clin. Chem.* **47,** 1364–1372 (2001).

10. Woo, M.-A., Kim, M. Il, Cho, D. & Park, H. G. Cell-based galactosemia diagnosis system based on a galactose assay using a bioluminescent Escherichia coli array. *Anal. Chem.* **85,** 11083–9 (2013).

11. Beutler, E. Galactosemia: screening and diagnosis. *Clin. Biochem.* **24,** 293–300 (1991).

12. Paigen, K., Pacholec, F. & Levy, H. L. A new method of screening for inherited disorders of galactose metabolism. *J. Lab. Clin. Med.* **99,** 895–907 (1982).

13. Jinks, D. C., Vollmer, D., Orfanos, A. & Guthrie, R. An evaluation of a new test kit to screen for galactosemia. *Clin. Biochem.* **20,** 353–357 (1987).

14. Beutler, E. & Baluda, M. C. A simple spot screening test for galactosemia. *Transl. Res.* **68,** 137–141 (1966).

15. Orfanos, A. P., Jinks, D. C. & Guthrie, R. Microassay for estimation of galactose and galactose-1-phosphate in dried blood specimens. *Clin. Biochem.* **19,** 225–228 (1986).

16. Desmet, C., Marquette, C. A., Blum, L. J. & Doumèche, B. Paper electrodes for bioelectrochemistry: Biosensors and biofuel cells. *Biosens. Bioelectron.* **76,** 145–63 (2016).

17. Novell, M. *et al.* A paper-based potentiometric cell for decentralized monitoring of Li levels in whole blood. *Lab Chip* **14,** 1308 (2014).

18. Charmantray, F., Touisni, N., Hecquet, L. & Mousty, C. Amperometric biosensor based on galactose oxidase immobilized in clay matrix. *Electroanalysis* **25,** 630–635 (2013).

19. Ito, N. *et al.* Three-dimensional structure of galactose oxidase: an enzyme with a built-in secondary cofactor. *Faraday Discuss.* 75–84 (1992).

20. Charmantray, F., Touisni, N., Hecquet, L., Noguer, T. & Mousty, C. Galactose oxidase/Prussian Blue based biosensors. *Electroanalysis* **27,** 1341–1344 (2015).

21. Xie, J. *et al.* A galactose oxidase biosensor based on graphene composite film for the determination of galactose and dihydroxyacetone. *Electroanalysis* **28,** 183–188 (2016).

22. Wang, Y. *et al.* Direct electron transfer: electrochemical glucose biosensor based on hollow Pt nanosphere functionalized multiwall carbon nanotubes. *J. Mol. Catal. B Enzym.* **71,** 146–151 (2011).

23. Bakker, E., Bühlmann, P. & Pretsch, E. The phase-boundary potential model. *Talanta* **63,** 3–20 (2004).

24. Khun, K., Ibupoto, Z. H., Nur, O. & Willander, M. Development of galactose biosensor based on functionalized ZnO nanorods with galactose oxidase. *J. Sensors* **2012,** 1–7 (2012).

25. Schiller, J. G., Wingard, L. B. & Liu, C.-C. Potentiometric detection of hydrogen peroxide and apparatus therefor. US4340448 (1982).

26. Nery, E. W. & Kubota, L. T. Sensing approaches on paper-based devices: a review. *Anal. Bioanal. Chem.* **405,** 7573–95 (2013).

27. Dontsova, E. A. *et al.* Screen-printed carbon electrode for choline based on MnO_2 nanoparticles and choline oxidase/polyelectrolyte layers. *Sensors Actuators B Chem.* **159,** 261–270 (2011).

28. Devaraj, S. & Munichandraiah, N. Effect of crystallographic structure of MnO_2 on its electrochemical capacitance properties. *J. Phys. Chem. C* **112,** 4406–4417 (2008).

29. Zhang, J. *et al.* Donnan potential caused by polyelectrolyte monolayers. *Langmuir* **30,** 10127–32 (2014).

30. Parrilla, M., Cánovas, R. & Andrade, F. J. Enhanced potentiometric detection of hydrogen peroxide using a Nafion-coated platinum electrode. *Electroanalysis* (2016). doi:10.002/elan.201600403

31. Raicopol, M. D. *et al.* Organic layers via aryl diazonium electrochemistry: towards modifying platinum electrodes for interference free glucose biosensors. *Electrochim. Acta* (2016). doi:10.1016/j.electacta.2016.04.145