Preliminary study of a non-invasive portable device for continuous monitoring of blood alcohol concentration

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***Abstract*— Alcohol is one of the main constituents of alcoholic beverages and, surely, is the oldest and most abused drug among those currently known. This substance is one of the most common causes of traffic accidents and these in turn have become, over the years, a public health issue. The target audience of this study are users that want to consciously drink alcoholic beverages and particularly professional drivers. This is the target audience since despite having greater experience and responsibility, their profession is the most affected by the consumption of alcohol, especially due to the fact they spend more hours driving than average drivers, increasing the likelihood of traffic accidents. This paper describes a portable non-invasive device for continuous monitoring of the concentration of alcohol in the blood. This device consists essentially of two primary components: an amperometric biosensor, which promotes, collects and analyzes a sample of sweat, and the data acquisition and processing system, which makes the acquisition and analog/digital conversion of the bio-data and subsequent digital processing of the results. Sweat is promoted by applying the technique of iontophoresis. Measurements are performed every 5 minutes, allowing the user to know in real time and in any place, which is his alcohol level.**

Keywords*— *Alcohol, Amperometric sensors, Microcontrollers, Non-invasive, Monitoring, Portable.

I. Introduction

Ethanol (CH_3 CH_2OH or C_2H_6O), also called as ethyl alcohol and, in current language, alcohol, is an organic substance obtained from the fermentation of sugars, ethylene hydration or reduction of acetaldehyde. The ethanol is one of the main constituents of alcoholic beverages and, surely, is the oldest and most abused drug among those currently know. It is still considered one of the most widely used psychoactive substances by human societies and it is estimated that is one of the main factors to contribute to premature deaths and disabilities, as well as being a cause of violence, unemployment, among other consequences. For all these reasons, the consumption of alcohol is considered one of the most serious public health problems nowadays [1-2]. Currently, according to the estimates of the World Health Organization (WHO), it is estimated that there are 2 billion alcohol users in the world, which it reflected into a potential increase of diseases and economic costs [3]. Traffic accidents have become, over the years, a public health issue, as well as causing fatalities, leave sequelae of varying degrees between the survivors and adverse effects between the members of their families. Among the numerous factors triggering these consequences, driving under the influence of alcohol is highlighted [4-5]. The 2015 data showed that in developed countries, traffic accidents are the leading cause of death in the age group of 15 to 24 years. Consequently, for each young driver who dies in a car accident, on average, 1.3 other people lose their lives. Traffic accidents involving young drivers are often caused by the loss of control of the vehicle or by excessive speed and happen more frequently at night [5]. Driving under the influence of alcohol is a factor in approximately 25% of fatal accidents, having been responsible for 6.500 deaths in Europe in 2012. Both consumption of drugs as well as illegal drugs poses a problem in terms of road safety, either in isolation or in combination with alcohol [4]. In the other hand, there is an increasing education/instruction of the population about the serious consequences of the excessive consumption of alcohol. Nevertheless, one of the most consistent findings in recent nutrition research is that light and moderate alcohol consumption can improve health and lead to a longer life [6-11]. So, there are today informed and conscious alcohol drinkers, that appreciate alcoholic beverages but do not want to consume it excessively. Thus, the target audience of this study is this kind of users, and particularly professional drivers, because despite having greater experience and responsibility, their profession is the most affected by the consumption of alcohol, especially due to the fact they spend more hours driving than average drivers, increasing the likelihood of traffic accidents.

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Nowadays, there are some devices helping to monitor the alcohol in the blood to inform the driver that it is driving with an excessive concentration of alcohol in the blood or even prevent this from happening. A device developed for detection of alcohol content in human bodies is characterized by determining the concentration of alcohol through the detection of the sweat evaporated in the fingers. The sweat evaporated in the fingers is sampled and analyzed automatically by the device to determine the concentration of alcohol in the blood [12]. The detection device is capable of accurately and conveniently detecting alcohol contents in driver bodies, and has the advantages of simple and practical effects, stable performances and accurate detection data. A device for detection of the state of intoxication capable of detecting the concentration of alcohol contained in the sweat of an individual to a viable level and in a short time was developed by Osamu [13]. The sweat secreted from the hands of the driver's steering wheel is promoted by a heat generation system. This system raises the steering wheel temperature to 36ºC to increase the amount of alcohol component contained in the sweat that can be detected. Thus, the concentration of alcohol in sweat is sensed to a workable level in a short period by an alcohol sensor installed on the steering wheel of the vehicle. Jun & Hideo [14] developed a preventive device, able to detect alcohol in the driver of a vehicle, and to prevent driving under the influence of alcohol or drunk driving. Consists of an engine start switch of the vehicle that is provided with a source of infrared light with a wavelength that is absorbed by hemoglobin reduced in the blood and by an image pickup element that receives the infrared beam and captures an image corresponding to a single authentication for a pattern of blood vessels. The start switch is supplied as a sensor to detect the alcohol. The infrared beam is irradiated to the fingers of the driver, so that perspiration is promoted, and the alcohol in sweat can be detected. Cheng [15] developed a device for the detection of alcohol through sweat secreted from hands of driver in automobile steering wheel. The driver must hold the steering wheel with both hands (contact sensor). The alcohol content is converted into a voltage acquired by the microntroller that performs the value analysis. The microprocessor will be block the engine ignition circuit if the value is above the limit. Recent developments have been made on amperometry based alcohol detection methods. It can be highlighted the study conducted by Chinnadayyala *et al.* [16] for the development of a new and simple method for the synthesis of AuNPs conjugated AOx that provide an improved overall performance of the biosensor than previous reports. Cinti *et al.* [17] developed a novel ethanol biosensor fabricated onto office paper. Carbon black/Prussian blue nanoparticles enhance the hydrogen peroxide detection. The paper-based electrochemical device can be coupled with a portable potentiostat. Satisfactory test performances were achieved on the analysis of ethanol contained in four different types of beers. Bilgi & Ayranci [18] developed a screen printed carbon electrode modified with nanoparticles, polyneutral red and alcohol dehydrogenase. The biosensor properties of the modified electrode toward ethanol measurement was evaluated and tested on real alcoholic beverage samples. The results agreed with those certified by the suppliers. The ethanol biosensor has a promising application in ethanol analysis due to the simple, practical and disposable features of the device without requiring laborious sample pre-treatment producers. Gamella *et al.* [19] developed an electrochemical biosensing device for determining the blood's ethanol content (BAC). The prototype is based on bienzyme amperometric composite biosensors that are sensitive to the variation of ethanol concentration. Thus, the determination of BAC is performed by amperometric monitoring of ethanol in sweat. The BAC determination in single measurement or in continuous modes. The validation of the obtained results using the proposed device was performed by 40 volunteers. The obtained results were compared with results obtained by the gas chromatographic reference method. No significant differences between both methods was found. This paper describes a portable non-invasive device intended for real-time detection of alcohol in the blood. Although the mobile feature was part of the technical specifications of the above-mentioned devices, the portability feature is only common to the device developed by Gamella *et al.* [19]. After ingestion 80% of the alcohol is absorbed by the stomach and the intestine [20]. After being absorbed, is then released by the sweat, urine and breathe [21]. In this way, this device uses the sweat to monitor the ingested alcohol. This device causes sweating at the site of measurement and subsequently carries out the measurement of the amount of alcohol in sweat and relates it with the existing amount in the blood. The goal is that the user, as soon as it starts to ingest alcohol, turns on the device so that measurements are performed every 5 minutes, allowing to know in real time and in any place, which is the concentration of alcohol in the blood. The great contribution of the development of this device relies in providing a method to control the alcohol levels for drivers, and will focus primarily on professional drivers, such as light or heavy road transport, national or international, public or private transportation, etc., due to the number of traffic accidents per year that involve this type of vehicles and by the fact that they are directly related to the transportation of persons or goods. After performing the measurements on users, they will be fully aware that they can put themselves in risk, as well as other people, and thereby make the decision not to start or stop driving. The advantages of monitoring the alcohol level through non-invasive sweat analysis from the current project over the breath based systems are presented in Table 1.

Table 1. Advantages of monitoring the alcohol level through non-invasive sweat analysis from the current project over the breath based systems.

Sweat method	Breath method
Continuous monitoring	Discontinuous monitoring
Very practical and discreet	Little discrete
Non-invasive and autonomous	User intervention is required
Sweat monitoring is not limited by the individual's body condition.	Respiratory conditions of the user may interfere with the results.
Actual blood alcohol concentration results obtained only after 5 minutes after ingestion of alcohol.	Actual blood alcohol concentration results only 20 minutes after ingestion of alcohol.
No need to renew material at each	Replacement of nozzles at each

measurement.	test.
The programmed time for iontophoresis causes the enough amount of sweat for testing.	There is a need for a specific amount of expelled air to the correct performance of the test

II. Device Development

A. Biosensor

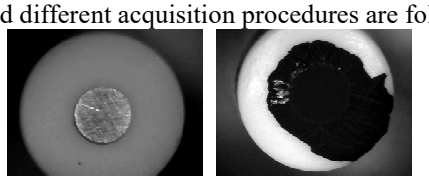
The biosensor developed for the non-invasive monitoring of the alcohol concentration in the blood is amperometric and enzymatic, following as reference the work developed by [22].

It was necessary to prepare three electrodes: a reference electrode, a working electrode and an auxiliary electrode for the development of the biosensor. In the case under study, an electrode of silver/silver chloride (Ag/AgCl) was chosen as the reference electrode. For its preparation, a silver wire has cleared with the 20% nitrate sulfate and curled in the shape of a spiral. The electrolyte was subsequently prepared: a solution of potassium chloride (KCl) of 0.1 M in a volume of 250 ml. The electrolyte is any substance that, dissociated or ionized, leads to positive ions (cathodes) and negative ions (anodes) by the addition of a solvent or heating [10]. Then, the silver wire was immersed in the electrolyte along with the auxiliary electrode (Platinum). The distance between the two electrodes must be at least 1 cm. A layer of silver chloride (AgCl) was deposited on the silver wire through electrochemistry, where the silver wire acts as anode. A positive electrical current was provided to promote the reaction to the silver electrode oxidase. For the oxidation to occur, there is a maximum energy level vacant in the electrode in order to receive electrons of the species in solution, which corresponds to a positive electric potential (in Volt) [23]. The reactions that occur are:

$$Ag(s) \rightarrow Ag^{+}(aq) + e^{-} \quad (1)$$

$$Ag^{+}(aq) + Cl^{-}(aq) \rightarrow AgCl(s) \quad (2)$$

Thus, as mentioned in [22], a pH meter is used and a voltage of 400 mV is applied between the electrodes. This was the optimal value to deposit a layer of silver chloride on the electrode. The process was completed when the electric power was reduced to zero (assurance that the electrode was prepared correctly). A modified platinum electrode was used as working electrode. The electrode surface was modified with polypyrrole by electroabsorption so that later enzymes could be fixed on it. So, first it was necessary to prepare a solution of 0.1 M of DBSS (Sodium dodecylbenzenesulfonates) in a volume of 25 ml. This solution was prepared with a solution of 0.1 M phosphate buffer for 1000 ml with a neutral pH (pH = 7), prepared beforehand [22-24]. The working electrode was immersed in 25 ml of the solution of DBSS electrolyte along with the auxiliary electrode and the reference electrode prepared previously. The electrochemical technique of linear sweep voltammetry was used to determine the electric current that arises in the working electrode by applying a voltage difference between the working electrode and the auxiliary electrode. The modification of the platinum electrode (working electrole) adding polypyrrole is shown in Figure 1. Comparing with the work developed by Gamella *et al.* [19], the biosensor described in this paper is modified using other enzyme and different acquisition procedures are followed.



(a) (b)

Fig. 1. Working electrole: (a) platinum electrode; (b) polypyrrole electrode.

The techniques and procedures described by [22-23] were used as reference on the experiments conducted. In the current study, a voltage difference of 1800 mV was considered. The experiment determined a 200 µA current on the electrode, as shown in Figure 2.

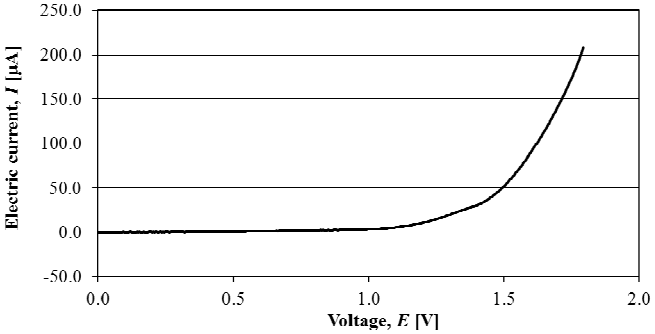


Fig. 2. Electrical current variation through linear sweep voltammetry of the platinum electrode in 25 ml of 0.1 M the DBSS at 1800 mV.

Then, 200 µl of pyrrole were added to the solution of DBSS and the linear sweep voltammetry technique was applied again with the same electric voltage difference. In this case, voltage difference of 800 mV is reached, with the passage of electric current corresponding approximately to 0.4 µA. Figure 3 shows the variation of the linear voltammetry with the addition of pyrrole. The voltammetry technique was used to determine the electric voltage potential needed to oxidise the enzyme.

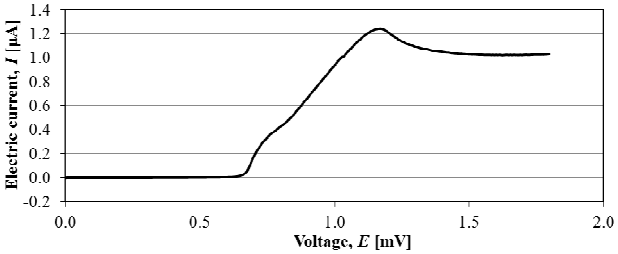


Fig. 3. Electrical current variation through linear sweep voltammetry with the platinum electrode in 25 ml 0.1 M DBSS solution with the addition of 200 µl of pyrrole at 1800 mV.

After, the polymerization of pyrrole was performed, that is, the surface of the electrode was covered with pyrrole, as indicated in [25-26]. This procedure used the technique of chronocoulometry until the electrode stayed covered with pyrrole. This condition corresponded to a 300 mC load

transfer, with a voltage difference of 800 mV for a DBSS electrolyte in a volume of 25 ml with a concentration of 0.1 M plus 200 µl of pyrrole. The addition of 200 µl of pyrrole is necessary to provide a coating of the electrode surface with pyrrole. If this procedure is not well performed, there may not be a good addition of the enzymes later. Figure 4 shows the load transfer by chronocoulometry with the platinum electrode with the addition of pyrrole. After modifying the electrode surface, the alcohol dehydrogenase enzyme was immobilized onto its surface using the covalent cross-linking technique [27-28].

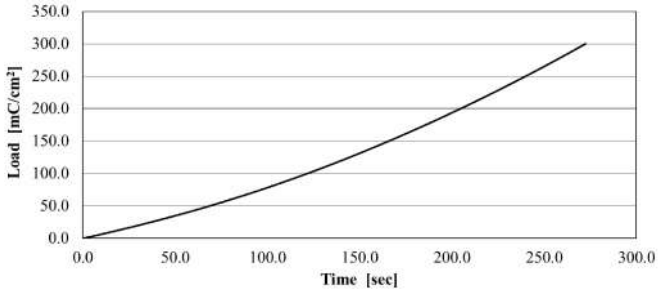


Fig. 4. Load transfer by chronocoulometry with the platinum electrode in 25 ml 0.1 M DBSS solution with the addition of 200 µl of pyrrole.

Later, it was necessary to verify the correct pyrrole synthesis. To this end, the cell was kept as it was and just traded away the DBSS electrolyte plus pyrrole by a solution of 0.1 M phosphate buffer. The technique of linear sweep voltammetry was used again. With the same electrochemical technique, the polypyrrole electrode was tested by comparing the results with the ones obtained with the platinum electrode. Figure 5 shows the overlap of both results using linear sweep voltammetry. As it can be seen, the results are quite different. The platinum electrode line increases exponentially because it oxidises the phosphate buffer solution more rapidly comparatively to a pyrrole electrode. This condition means that the pyrrole was polymerized correctly on surface of the platinum electrode. Then, 15 kU of enzyme alcohol dehydrogenase was diluted entirety in a buffered solution (0.1 M pH = 7) in a 25 ml volumetric flask. A solution of reduced Nicotinamide Adenine Dinucleotide (NADH) with a concentration of 0.0015 M in a volume of 25 ml was prepared. Both solutions have been deployed at a temperature of -20 °C for conservation. For the correct enzyme immobilization, it was necessary to prepare a Pork Serum Albumin (PSA) solution of 1 g, with 0.1 g of PSA and a buffer solution. Lastly, a glutaraldehyde (GA) solution of 10 g was prepared, with 0.25 g and buffer solution. After all the solutions were properly prepared, 5 µl of each were mixed up. Then, the working electrode with pyrrole was put into the mixture to stand at temperatures between 2 °C to 8°C during 1 hour. After this time, the electrode was placed to dry at the same temperature for 2 hours.

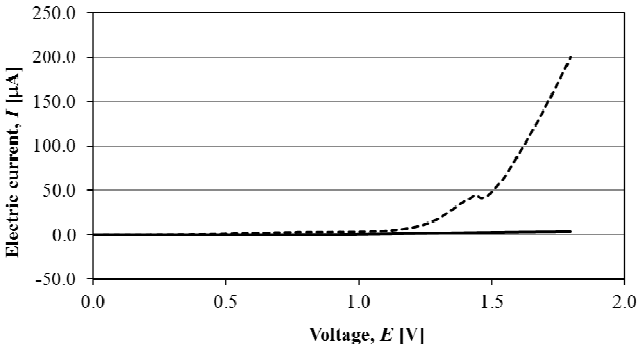


Fig. 5. Comparison of the current variation using the linear voltammetry with pyrrole electrode (—) and platinum electrode (- -) in 0.1 M phosphate buffer.

B. Signal acquisition and processing

A potenciostat was built for data acquisition. For the signal processing, the development board "MSP430FG4618/F2103 Experimenter Board" [29] from Texas Instruments which contains a MSP430gf4618 microcontroller was used. To program it, the IDE (Integrated Development Environment-IDE) Code Composer Studio using C language was used. In order to the enzyme alcohol dehydrogenase oxidize the ethanol, the electrochemical cell is kept at a constant electrical potential of 600 mV. The potenciostat was used to measure the electrical current generated between the working and the auxiliary electrodes. Then, it was necessary to convert the current to voltage, so that it could be read and converted by the analog-to-digital converter included in MSP430fg4618 microcontroller. Thus, a current/voltage converter was built using an operational amplifier and an electrical resistance of 1.1 MΩ. Figure 6 shows the sketch of the electric circuit of the potenciostat and the current/voltage converter built into the prototype of the data acquisition system.

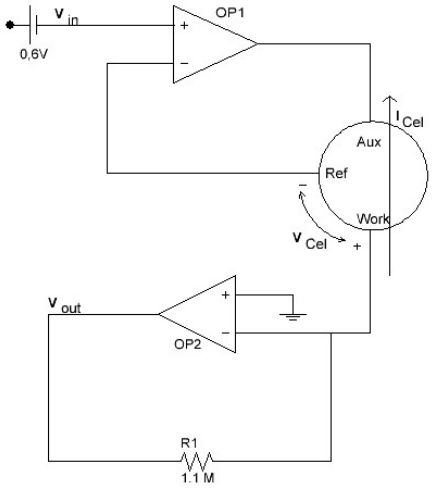


Fig. 6. Current/voltage converter and potenciostat.

To handle all the features of the device under study, it was necessary to add two more buttons to the microcontroller.

The iontophoresis process consists of a digital-to-analog

converter (DAC) that is activated for 5 minutes, providing an electric voltage of 1.5 V, converted into current and applied to the skin, this chain promotes the generation of sweat. There is also an On/Off button that only activates and deactivates the DAC. The Real Time Clock (RTC) is always on.

The analysis of the signal, that is, after the sweat is promoted by the DAC, it is necessary to realize the acquisition of the signal with the analog-digital converter (ADC). The microcontroller collects 50 samples, calculates an average value, converts it to an equivalent value of blood alcohol (g / l) and stores it in memory. The value is compared with the previous average value and if the difference between the values is equal to or greater than 0.1 g / l, the buzzer and the red LED will be activated. These alarms stop as soon as the user presses the button. Pressing this button also allows the user to see the alcohol value on the LCD.

III. Prototype Proposal

Fritzing version 0.9.3.0 software was used [30] to draw the entire assembly of the prototype proposal and the respective connection diagram, and in which all the components are designed. A voltage source, the biosensor, a breadboard, two operational amplifiers LM386, three resistors with values of 10 kΩ (2 resistors) and 1.1 MΩ, 2 buttons, MSP430FG4618 Experimenter Board F2013/development and connecting wires were used. Figure 7 shows the connections diagram of the prototype of the monitoring device. The amperometric biosensor, due to the modification of the surface of the electrode with the enzyme alcohol dehydrogenase, collects the sweat and reacts with the alcohol. For this reaction to occur, it is necessary to use a voltage source set to 0.6 V. The potenciostat assembly consisting of an operational amplifier that connects to the amperometric biosensor, collects the electrical current that is generated between the biosensor and the alcohol. A current/voltage converter consisting of an operational amplifier and a 1.1 MΩ resistance, converts the potenciostat current value to a voltage so that it can be acquired by the ADC. Lastly, the 2 buttons and the respective pull-up resistors are added to the assembly. An on/off button is used to reduce the power consumption of the device, ensuring a longer service life of the battery. The other button switches the display of hours to the value of concentration of alcohol (g/l) in the blood. This feature stems from the perspective of proposing a non-invasive portable device for monitoring the concentration of alcohol in the blood in the form and dimensions of a wristwatch. Thus, the final prototype of the monitoring device is portable and discreet in day-to-day operations as shown in Figure 8.

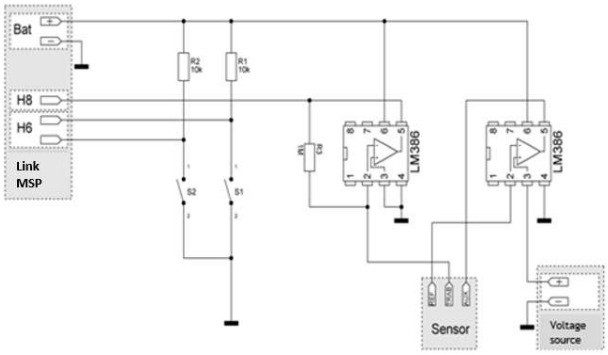


Fig. 7. Representative flowchart of the signal processing.

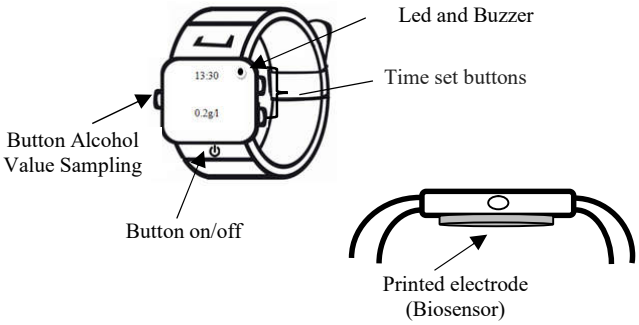


Fig. 8. Connection diagram of the prototype proposal of the monitoring device.

IV. DISCUSSION

Experimental tests were carried out to verify the correct immobilization of the alcohol dehydrogenase enzyme. The electrochemical cell was mounted with three electrodes and with 15 ml of buffer solution plus a potassium chloride (KCl) electrolyte. Previously, the voltammetry technique was used to determine the electric voltage potential required to the enzyme oxide. Figure 9 shows approximately a 600 mV voltage potential at which the enzyme oxidises.

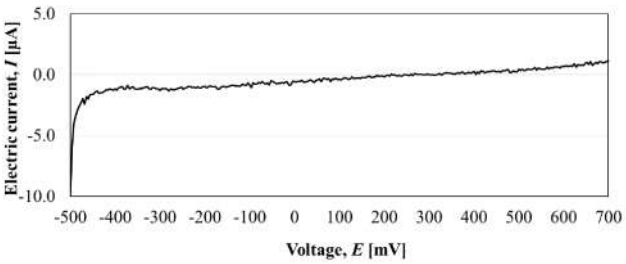


Fig. 9. Electrical current variation through linear sweep voltammetry of the work electrode with enzyme in 15 ml of 0.1 M phosphate buffer solution and pH = 7.

Subsequently, the technique of chronocoulometry was applied, and after a period of stabilization of about 3 min, 100 µl of 95% ethanol were added. The result is shown in Figure 10, where it can be verified when ethanol is added. A decrease of approximately -4 µA of the electric current is determined. The electric current decreases due to the oxidation of the substrate by the enzyme. A relative error of 37.5% was achieved for the set of experimental tests conducted.

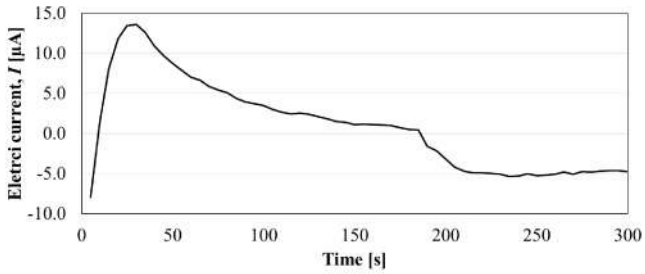


Fig. 10. Electrical current variation through chronocoulometry with addition of 100 µ l of ethanol 95% in 0.1 M phosphate buffer solution pH = 7 and of 15 ml.

To verify that was the enzyme that it was oxidizing the ethanol and not the ethanol oxidizing alone due to the high electrical potential that it was subjected to, the same electrochemical technique was repeated with the same potential and with the same addition of ethanol, but with a working electrode without the enzyme fixed on its surface. Figure 11 shows that the electric current does decrease when 100 µl of 95% ethanol was added, which means that the enzyme oxides the ethanol when subjected to a potential of 600 mV.

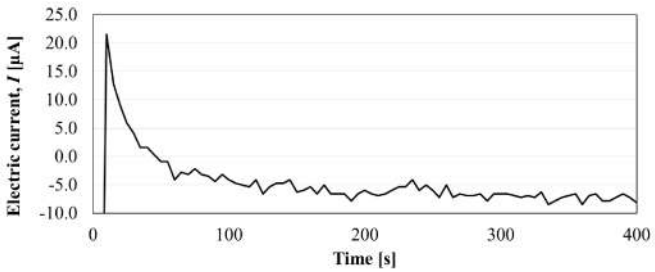


Fig. 11. Electrical current variation through chronocoulometry on the working electrode without enzyme electrode in a solution of 0.1 M phosphate buffer pH = 7 and adding 100 µl of 95% ethanol.

V. CALIBRATION PROCEDURE

Considering a 1 litre bottle of wine with 13% ethanol volume and knowing that the human body has approximately 5 litres of blood, drinking that beverage would correspond to a 20.6 g/l alcohol concentration in the blood. However, from the digested alcohol, only 13% is absorbed by the blood with the remaining 87% being filtered by the liver. Thus, the final concentration of alcohol in the blood is about 2.68 g/l, matching the values for a wine glass of 50 ml that is about 0.134 µg/l. Then, it was necessary to match these concentrations to the voltage values acquired by the ADC. As the solution becomes saturated by adding 100 µl of 95% ethanol, it is concluded that a concentration of alcohol of 4.96 g/l corresponds to the maximum voltage placed at the microcontroller ADC terminals, i.e. 3.3 V. Thus, if 1 litre of wine corresponds to a concentration of alcohol of 2.68 g/l, which corresponds to a voltage of 1.78 V, a concentration of alcohol of 0.13 g/l corresponds to an electrical voltage of 0.09 V. These results were also calculated for values of electrical current. Taking into account these values, 2 calibration lines were generated. One calibration line states the relation Voltage/Concentration and the other the Current/Concentration. Figure 12 shows the linear equation describing the relationship between the alcohol concentration and the electrical voltage.

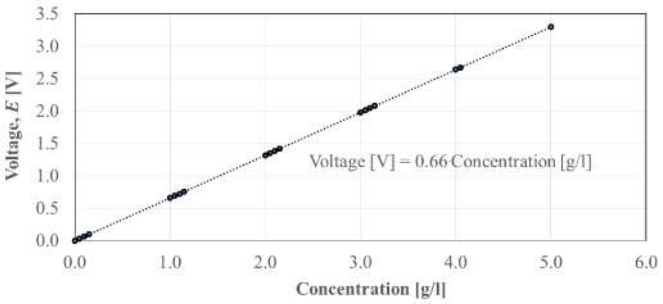


Fig. 12. Linear relation between electrical voltage and alcohol concentration.

The voltage increases linearly with the increase of the alcohol concentration. For any value of alcohol concentration in the blood, the voltage value must be divided by 0.66 arriving at equation (3).

$$\text{Concentration} = \text{Voltage} / 0.66 \quad (3)$$

Thus, the device detects an ethanol concentration from 0.0 g/l to about 5.0 g/l. Further tests are required to verify the accuracy of the ethanol concentration measurement.

VI. CONCLUSIONS

Traffic accidents have become, over the years, a public health issue, that besides causing fatalities, leave sequelae of varying degrees between the survivors and adverse effects between the members of their families. Among the numerous factors triggering this process, the alcohol consumption is highlighted, determining factor in approximately 25% of fatal accidents. In the other hand, nowadays the population is informed about the benefits to health of the light and moderate consumption of alcohol as well as the serious consequences of its excessive consumption of alcohol to the serious consequences of its excessive consumption. Part of this informed population are conscious alcohol drinkers that appreciate its light consumption. Therefore, this paper describes a prototype of a portable non-invasive device intended for real-time detection of alcohol concentration in the blood through sweat. The experimental results allow to conclude that it is necessary to use a voltage potential of approximately 0.6 V to the enzyme alcohol dehydrogenase oxidizes the alcohol. The reduction of current that exists when the alcohol is added to the buffer is significant, since it reaches an 8 µA reduction with only 100 µl. The biosensor after exposed to 0.6 V, takes about 4 minutes to stabilize the electrical current. Thus, in the final prototype, this time of stabilization has to exist before the measurements are made. After the bio-verification tests, the prototype of this monitoring device will be developed with microelectronics. To make the final, practical and portable device to be used on a daily basis, it is intended to achieve the design of a wristwatch. The entire device is intended and designed for the printed electrode to be placed at the bottom of the device so that it stays in contact with the skin of the user. All

electronic components (battery, potenciostat, amplifiers etc.) are designed to be inside the device.

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