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Author: M. Gamella S. Campuzano J. Manso G. González de Rivera F. López-Colino A.J. Reviejo J.M. Pingarrón<ce:footnote id="fn0005"><ce:note-para id="npar0005">[1]These authors have equally contributed to this work</ce:note-para></ce:footnote>

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A NOVEL NON-INVASIVE ELECTROCHEMICAL BIOSENSING DEVICE FOR IN SITU DETERMINATION OF THE ALCOHOL CONTENT IN BLOOD BY MONITORING ETHANOL IN SWEAT

M. Gamella,[†a] S. Campuzano,[†a] J. Manso,[a] G. González de Rivera,[b] F. López-Colino,[b] A. J. Reviejo,[a] J.M. Pingarrón[*a]

[†] These authors have equally contributed to this work

[a]*Dpto. Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain. E-mail: pingarro@quim.ucm.es. Phone number: 34913944315. Fax number: 34913944329.*

[b]*Escuela Politécnica Superior, Universidad Autónoma de Madrid. E-28049 Madrid, Spain.*

E-mails: mariagam@quim.ucm.es, susanacr@quim.ucm.es, javicomono@msn.com, guillermo.gdrivera@uam.es, fj.lopez@uam.es, reviejo@quim.ucm.es, pingarro@quim.ucm.es.

* To whom correspondence is to be addressed

Abstract

A non-invasive, passive and simple to use skin surface based sensing device for determining the blood's ethanol content (BAC) by monitoring transdermal alcohol concentration (TAC) is designed and developed. The proposed prototype is based on bienzyme amperometric composite biosensors that are sensitive to the variation of ethanol concentration. The prototype correlates, through previous calibration set-up, the amperometric signal generated from ethanol in sweat with its content in blood in a short period of time. The characteristics of this sensor device permit determination of the ethanol concentration in isolated and in continuous form, giving information of the BAC of a subject either in a given moment or its evolution during long periods of time (8 hours). Moreover, as the measurements are performed in a biological fluid, the evaluated individual is not able to alter the result of the analysis. The maximum limit of ethanol in blood allowed by legislation is included within the linear range of the device (0.0005-0.6 g L^{-1}). Moreover, the device shows higher sensitivity than the breathalysers marketed at the moment, allowing the monitoring of the ethanol content in blood to be obtained just 5 minutes after ingestion of the alcoholic drink. The comparison of the obtained results using the proposed device in the analysis of 40 volunteers with those provided by the gas chromatographic reference method for determination of BAC pointed out that there were no significant differences between both methods.

Keywords: portable device, amperometric bienzymatic biosensor, transdermal alcohol biosensor, blood alcohol concentration.

1. Introduction

The measurement of ethanol for clinical and safety purposes in fluids other than blood has become important because of major demands for non-invasive analysis [1]. In this context, sweat constitutes an attractive alternative because it is easier to get personal agreement for sampling, and its testing offers advantages over urine and blood regarding the easiness of collection under certain circumstances, such as the monitoring of drivers or individuals in safety-related work [1,2].

Besides enzymatic degradation, orally administered ethanol is also eliminated through skin perspiration [3–5]. In fact, some publications suggest using human perspiration, instead of blood, for estimating ethanol concentration [4, 6–8] or other clinically relevant analytes such as lactate [9]. In these works, concentration of ethanol in the sweat patch was measured by the aspiration of an equilibrated head-space sample into an electrochemical detector [6,7] or a gas chromatography system [4,8]. The amount of ethanol leaving the skin after single liquor consumption was determined using ion mobility spectrometry [5]. Kamei et al. proposed a novel instrumentation for the estimation of ethanol concentration in sweat consisting of a sampling probe attached directly to the skin surface, a sweat rate meter, a cold trap and capillary gas chromatography. The accuracy of the measurement of ethanol in blood was 0.1 mg mL^{-1} [1]. These authors demonstrated for the first time the existence of a clear correlation between ethanol concentrations in sweat and blood during its consumption in the human body. Later, Buono et al. concluded that ethanol concentration in sweat was approximately 19 % more than in whole blood. They measured ethanol concentrations using an enzymatic technique and the slope of the blood *vs* sweat ethanol concentration plot was 0.81 [10].

Currently BAC is estimated by measuring breath alcohol concentration (BrAC), the different generations of breath alcohol testing instruments used for BrAC detection since 1930 having

been reviewed by Wingmore et al. [11]. Breathalysers, at least as they are currently used in practice, are not calibrated to each individual subject. These instruments estimate BAC via an idealized linear stoichiometric calculation based on Henry´s law. However, the relationship between BAC and BrAC is not so simple and actually may vary from instrument to instrument and individual to individual [12].

Transdermal sensing systems are touch-sensors that can continuously monitor the analyte level in a person. In the particular case of BAC monitoring, traces of alcohol are present in the person's sweat when alcohol is consumed. Therefore, transdermal BAC devices measure BAC based on how much alcohol is present in perspiration. The only wearable transdermal alcohol sensor reported until now was the Giner WrisTAS V, developed primarily to monitor alcohol abstinence. Placed on the skin surface, the sensor oxidizes ethanol in a continuous manner, generating a current that is linearly related to the local alcohol concentration. It responds to 10–200 mg dL^{-1} BAC with a lag time of ~ 30 min to plateau [12]. Recently, Dumett et al. have developed a calibrated model for estimating (breath measured) blood alcohol concentration from measurements of transdermal alcohol produced by the Giner WrisTAS V alcohol sensor. However, a number of unknown subject and device specific physical and physiological parameters must be estimated before the model can be used [12].

In this work a prototype for in situ and real time determination of ethanol content in blood through measurement of the amperometric signal obtained from the monitoring of ethanol in sweat, collected following pilocarpine iontophoresis, is described. The prototype comprises a sensing system based on an electrochemical alcohol oxidase/horseradish peroxidase (AOD/HRP) biosensor, a miniaturised potentiostat and a microprocessor that transduces the analytical signal obtained in perspiration into the BAC. As the proposed method uses non-invasive sampling, it may be applicable to determine BAC instead of the gas

chromatography conventional method using blood sample. The goal for this prototype is to compete with the breathalysers that are currently commercialised, as measurement of ethanol is performed directly in a biological fluid (sweat) hardly altered by the user. The proposed instrumentation may be very useful in checking drivers´ ethanol consumption or to protect workers from risk due to the residual effects of ethanol digestion.

2. Experimental

2.1 Apparatus and electrodes

Composite bienzyme electrodes were fabricated in the form of cylindrical pellets as reported earlier [13]. Graphite (ultra F purity; Carbon of America, Bay City, USA), AOD (EC 1.1.3.13, from *Pichia Pastoris*, activity 1,430 U mL^{-1}, Sigma, St. Louis, MO, USA), HRP (EC 1.11.1.7, type II, activity 240 U mg^{-1} of solid, Sigma), ferrocene (Fluka, Buchs, Switzerland) as redox mediator, and Teflon powder (Aldrich) were used to construct the pellets. A final Teflon percentage of 70 % was employed. Several 3.0 mm diameter cylindrical portions of the pellet were bored, and each portion was press-fitted into the electrochemical cell designed specified below, making the electrical contact through a stainless-steel wire.

Commercial (BAS Model LC-4C connected to a Linseis Model L250 recorder) and homemade miniaturized amperometric detectors (see Section 2.8) were used for the measurements.

2.2 Reagents and solutions

Ethanol (96 % v/v, Scharlau) and acetonitrile (Panreac) were employed. The water used was obtained from a Millipore Milli-Q purification system. Stock solutions of ethanol (0.1 mol L^{-1}) were prepared in 0.05 mol L^{-1} phosphate buffer of pH 7.4. More dilute standards were prepared by suitable dilution with the same buffer solution. Polytetrafluoroethylene

(PTFE) TF-1000 membranes (pore size 1 μm, O.D. 37 mm, Supelco), hydrophobic but permeable to ethanol, were used for the preparation of the biodevices.

2.3 Biodevice design

After designing different electrochemical cells, the one depicted in Fig. 1a) was selected for building the biodevice. The cell is made up of the bienzyme composite graphite-Teflon electrode, an Ag/AgCl reference electrode and an auxiliary Pt electrode. The three electrodes are immersed in a working solution (phosphate buffer 0.05 M pH 7.4) separated from the skin by a PTFE membrane.

2.4 Sweat analysis

The sweat analysis implied firstly the sweat stimulation. This was accomplished by using the Macroduct 3700-SYS sampler unit based on active iontophoresis (IZASA). Briefly, two recessed stainless steel electrodes, covered with Pilogel® Iontophoretic Discs (pilocarpine nitrate-impregnated discs containing pilocarpine and 96 % water), were strapped on the volunteers forearm and a small electrical current (1.5 mA) was passed through the electrodes for 5 min by using a battery-powered device. The electrodes were then removed and the biodevice was strapped onto the same spot. Sweat production varied considerably from patient to patient, but the average individual produced between 70 to 85 μL of sweat during a 30–40 min collection interval. Ethanol monitoring in sweat with the biodevice was carried out by both continuous and single measurement modes:

- *Continuous mode:* Before the alcohol intake by the volunteer, sweat was stimulated with the iontophoresis unit. Then, the biodevice was placed on the skin, the electrodes connected to the miniaturized potentiostat and the current measured at an applied constant potential of 0.00 V (*vs* the Ag/AgCl reference electrode) was recorded and allowed stabilizing

147 for 5 min. Then, the volunteer started the intake of alcohol which was continuously monitored
148 by recording the current during the testing time (from 30 min to 2 h).

149 - *Single measurement mode:* Just after 15 min of alcohol ingestion by the volunteer,
150 sweat was stimulated. Then, the biodevice was placed on the volunteer forearm and the
151 membrane permeable to ethanol was separated from the skin by a PVC impermeable
152 membrane. Next, the electrodes were connected to the potentiostat. 30 min after the alcohol
153 intake, the PVC impermeable membrane was removed permitting the ethanol diffusion from
154 the skin to the biodevice across the PTFE membrane to be produced. The amperometric signal
155 at an applied constant potential of 0.00 V (*vs* the Ag/AgCl reference electrode) was measured
156 after a period of time of 5 min.

157

158 *2.5 Calibration graph construction*

159 The amperometric responses obtained with the biodevice were correlated with the
160 alcohol content in blood measured by means of a graphite-Teflon-AOD-HRP-ferrocene
161 composite electrode. In this way, a calibration graph was constructed to translate the current
162 signal measured with the biodevice (in μA) into the BAC (in g L^{-1}). Accordingly, BAC results
163 were obtained by interpolating the amperometric measurements made with the transdermal
164 electrochemical sensor developed both in the continuous and single measurement modes into
165 the corresponding regression equation.

166

167 *2.6 Blood analysis*

168 Blood was collected from prewarmed, free-flowing, fingertip punctures into
169 heparinised capillary tubes. Blood analyses were carried out either using a graphite-Teflon-
170 AOD-HRP-ferrocene composite electrode [13] or the official gas chromatography method at
171 the SENRA S.A. clinical analysis laboratory (Madrid).

172

173 *2.7 Clinical comparison*

174 A control group formed by 40 volunteers was constituted to validate the developed
175 methodology. The ages of all the volunteers were between 18 and 30, they weighed between
176 40 and 100 kg and half of them were women. Individual card files were filled out for each
177 volunteer who voluntarily signed their informed clinical study consent.

178 Any volunteer consumed alcohol for at least 24 h prior to testing, but no other dietary
179 controls were imposed. The dose of alcohol given was between 30 and 140 mL. The alcoholic
180 beverage they drank, in 5−10 min, was gin, rum or whisky mixed with a cola or juice soft
181 drink. Apart from the ethanol content, different blood parameters were determined with the
182 purpose of discarding those volunteers with some dysfunction affecting the BAC.

183 Once the perspiration was stimulated as described in *Section 2.4*, the biodevice was
184 placed on the skin and the ethanol in sweat was monitored. At the same time, blood was
185 extracted and analysed by the gas chromatography method just after carried out the single
186 measurement with the biodevice or 30 minutes after alcohol intake in the case of continuous
187 measurements.

188

189 *2.8 Miniaturized amperometric detector description*

190 Fig. 1S in in the Supporting Information shows the components and the working of the
191 miniaturized amperometric detector.

192

193 **3. Results and discussion**

194 The biochemical principle on which the biodevice behaviour relies is the measurement
195 of ethanol in sweat by means of a composite graphite-Teflon-AOD-HRP-ferrocene electrode.
196 This biosensor responded to ethanol which is catalytically oxidized by AOD in the presence

of oxygen, producing H_2O_2. The enzyme reaction with HRP mediated by ferrocene was employed to monitor the process at the low potential value of 0.00 V *vs* the Ag/AgCl reference electrode. The measured current corresponded to the electrochemical reduction of ferricinium (see Fig. 1b) [13].

3.1 Performance of the biodevice for ethanol in solution

Fig. 2 shows the amperometric recording obtained with the biodevice for a 5.0×10^{-4} mol L^{-1} ethanol solution in 0.05 mol L^{-1} phosphate buffer (pH 7.4). The times elapsed between the application of the detection potential to the electrodes and the baseline stabilization (t_1), for the signal appearance (t_2) and for reaching the steady state (t_3), were evaluated. The values of t_1 varied between approximately 2 and 6 minutes. This time cannot be considered as a serious operational drawback for the biodevice usefulness to measure ethanol in sweat, because a suitable time may be awaited before placing the biodevice on the individual. The time for the signal to appear (t_2) varied between approximately 2 and 5 seconds, whereas the time required to reach the steady state (t_3) varied between 2 and 4 minutes depending on the ethanol content.

The repeatability of measurements with a single biodevice was tested by measuring ten different 5.0×10^{-4} mol L^{-1} ethanol solutions. A RSD value of 9.1 % demonstrated an acceptable repeatability for successive measurements made with the same biodevice.

An important practical aspect of the biosensor design is that it provided reproducible amperometric signals with all the biodevices built from the same composite pellet or with different pellets. An overall RSD value of 10.5 % was obtained for the steady state current values measured for 5.0×10^{-4} mol L^{-1} ethanol with 10 different biodevices 5 of them fabricated from the same pellet and the other 5 fabricated from different pellets.

The stability of the biodevice with the storage time is another important variable to be evaluated for practical purposes. This was checked by storing 5 different biodevices in the 0.05 mol L^{-1} phosphate buffer solution of pH 7.4 and other 5 biodevices under dry conditions. Only 2 from the 5 biodevices stored in the buffer solution provided measurable responses during 30 days, and the measured current decreased gradually and noticeably with the storage time. On the contrary, all the dry stored biodevices provided measurable and reproducible responses during at least 2 months. Accordingly, all the biodevices were stored under dry conditions at 4 ºC until their use.

Considering that the biodevice must be placed in contact with the skin and, therefore, the working temperature should be near 36 ^{o}C, the dependence of the steady state current measured for an ethanol concentration of 5.0×10^{-4} mol L^{-1} with temperature was also tested. As can be seen in Fig. S2 in the Supporting Information, the amperometric signal increased with temperature and reached a maximum at 40 ^{o}C, which is attributed to a higher enzyme catalytic efficiency at this temperature and a better diffusion of ethanol through the membrane, allowing the steady state current to be reached in a shorter time. Above 40 ^{o}C, the the amperometric signal decreased probably due to partial ethanol evaporation.

3.2 Performance characteristics of the biodevice for the measurement of ethanol in sweat

We verified that all the tests carried out with the biodevice, either in the single measurement or in continuous modes, provided similar results independently of connecting it to the miniaturised or to a commercial potentiostat.

3.2.1 Continuous measurement mode

All tests were carried out by placing the biodevice on the skin after sweat generation following the protocol described in *Section 2.4* and the generated current was continuously

recorded versus time from 30 min to 2 h. In parallel, blood samples were collected every 10 minutes and subsequently analyzed. Fig. 3 (a–e) shows typical amperometric recordings for three volunteers after the ingestion of alcohol. The evolution of the ethanol content in sweat (expressed as current intensity values) and in blood (measured as ethanol concentration in g L^{-1}) for six different volunteers is displayed in Fig. S3a in the Supporting Information. A detailed comparison between both types of results obtained for all the volunteers demonstrated that the maximum ethanol signal was reached later in sweat than in blood. However, the decrease in the ethanol content observed after the maximum was smoother in sweat than in blood. It is important to remark that these results demonstrated the feasibility of continuous monitoring the ethanol content with the same biodevice during relatively long periods of time (6–8 hours).

3.2.2 Single measurement mode

Another goal of this work was the possibility of evaluating the BAC at a given time after the alcohol ingestion through the measurements in sweat. These studies were carried out following the methodology described in *Section 2.4* placing the biodevice in contact with the skin for five minutes every 15 minutes.

Fig. 3b shows the current-time recordings obtained for one volunteer. Moreover, the comparison with the corresponding BAC determined with the graphite-Teflon-AOD-HRP-ferrocene composite electrode for three different volunteers is depicted in Fig. S3 (d-f) in the Supporting Information. As it can be seen, the concentration of ethanol in blood reached the highest level after 30 min of ethanol ingestion. It is also interesting to remark that both curves exhibited a similar behaviour reaching the maximum at nearly the same period of time. Therefore, it was concluded that the ethanol concentration in sweat correlated well with the concentration in blood and that ethanol in blood can reach the skin surface very rapidly.

When comparing Fig. S3 (a–c) and (d–f), it appears that the ethanol content in blood is similar with a maximum concentration at approximately 30 minutes of alcohol ingestion. However, the profile of the measurements in sweat depended somewhat on the measurement mode. While in the continuous mode the ethanol measurement reached the highest value somewhat latter than in blood and then decreased slowly, in the single measurement mode the maximum response was reached at the same time and the subsequent decrease is similar in both biological fluids. Therefore, it can be concluded that some kind of ethanol accumulation occurred at the biodevice when operating in the continuous mode while that effect was not observed in the single measurement mode, so that single measurements seem to provide more reliable results for correlating with discontinuous blood analysis.

It is important to remark that after single or continuous operation mode, the same biodevice could be repeatedly used for at least 6 more times. A simple cleaning protocol consisting of immersion of the biodevice in 0.05 mol L^{-1} phosphate buffer solution of pH 7.4 in order to prevent the accumulation of ethanol in the PTFE membrane was only needed.

3.3 Correlation between the ethanol content in sweat and in blood by gas chromatography

The ethanol content was determined for 12 volunteers by gas chromatography both in sweat (collected in the device provided by the trading house) and blood (extracted 15 minutes after sweat stimulation). The obtained results showed that the BAC measured at a fixed time, and the average content of ethanol in sweat collected during 30–40 min were correlated through a linear relationship given by the equation:

$$\text{BAC (g L}^{-1}) = 0.71 \times \text{sweat ethanol concentration (g L}^{-1}) \quad (r = 0.912)$$

This suggested a good correlation between the ethanol content in blood and sweat at a fixed time indicating a rapid equilibrium of ethanol across the sweat gland epithelium [10]. According to the former equation, BAC averaged 71 % of that found in sweat. These results are consistent with the theoretical estimation of Nyman and Palmlov [14] establishing that the "real" concentration of ethanol in sweat should be about 15 % higher than that found in whole blood.

3.4 Correlation between BAC and the current measured in sweat with the biodevice

The parameter used to establish the legal content of ethanol in humans comes from its concentration (g L^{-1}) in blood. Therefore, it is necessary to find out a reliable relationship between the current measured with the biodevice in sweat and the corresponding BAC. To find this correlation we compared the amperometric signals obtained in sweat with the biodevice for 12 and 8 volunteers upon different alcohol ingestions using the continuous and single measurement modes, respectively, with the corresponding BACs determined with the graphite-Teflon-AOD-HRP-ferrocene composite electrode.

Fig. S4a in the Supporting Information displays the correlation found between the current measured with the developed biodevice in sweat in the continuous mode and the BAC for the volunteers tested. Moreover, the correlation between BAC and the current measured in sweat in the single measurement mode was also checked. Data obtained measuring the current at different times: 1, 2, 3 min, and at the steady state (t = 5 min) are summarized in Table 1 and the correlation found for 5 min is shown in Fig. S4b. As it can be clearly deduced from Table 1, the best correlation resulted with the current values measured at steady state and, therefore, 5 min was selected as the time at which the measurements were performed in the single mode. Also, Fig. S4c gathers in the same plot the data obtained in both measurement modes (with t = 5 min in the single measurement mode) with the corresponding BAC values.

The rather good correlation coefficient obtained (r = 0.934) in this plot demonstrated that this calibration graph might be employed to transform the current values provided by the biodevice in the corresponding BAC independently of the measurement mode used

The maximun limits of ethanol in blood permitted by legislation (between 0.01 and 0.15 % BAC, equivalent to 0.002–0.03 g L^{-1}, depending on the country for the operation of a vehicle) are included within the linear range provided with the device (0.0005–0.6 g L^{-1}) (see Fig. S4c in the Supporting Information). Moreover, this device shows a better sensitivity than the breathalysers marketed presently, and allows the ethanol content in blood to be obtained just 5 minutes after ingestion of the alcoholic drink. Apart from this excellent performance, the developed biodevice offers an interesting alternative to avoid false results (positive or negative by ingestion of some medicines or masking agents, respectively) occurring with the conventional breathalysers.

3.5 Clinical tests

The constructed biodevice was employed in the single measurement mode to monitor the BAC for 40 volunteers who drank different amounts of alcoholic drinks (*Section 2.7*). None of the volunteers were rejected for the test according to the results obtained in the determination of different blood parameters (not shown).

Fig. 4 shows the plot of the current values measured with the biodevice in the single measurement mode for the 40 volunteers (black points) as well as those plotted in Fig. S4b (red points) *v*s the corresponding BAC values obtained by the gas chromatography method. As it can be seen, 10 of the data, representing 23% of the total data, were outside the intervals of the calibration prediction lines. Most of these results are above the upper straight calibrating line which may be due to some people are able to eliminate more quickly ethanol from the human body when having a relatively high BAC, for example, by increasing ethanol

345 excretion through perspiration. Obviously, other sources of error in the measurements cannot
346 be discarded considering the complexity of the direct measurements in sweat.

347 Fig. 5 compares the BAC values obtained by interpolation of the biodevice current
348 values measured in the single measurement mode into the calibration plot shown in Fig. S4c
349 with the BAC values determined by gas chromatography for all the volunteers. A statistical
350 validation of the results obtained was performed.

351 As the determined coefficient of asymmetry (0.40) shared between –2 and + 2, it could
352 be assumed that the data had a normal distribution and, therefore, Student´s t method could be
353 applied. The experimental t value (1.74) was lower than the tabulated t value (2.02) and,
354 therefore, it could be concluded that there were no significant differences for a 0.05
355 significance level between the results provided by the gas chromatographic method and the
356 biodevice. These results enabled us to claim the absence of systematic errors in the proposed
357 method indicating that the biodevice is suitable to determine the BAC by means of
358 measurements in sweat.

359 A straight line regression for comparing both methods was constructed by plotting the
360 experimental data obtained for the method to be evaluated (normally in the Y axis) and the
361 reference method (normally in the X axis). Two methods do not have systematic errors if the
362 ordinate at origin and the least squares obtained slope is respectively 0 and 1. As this never
363 occurs in practice, it is considered that the two methods are not significantly different when
364 the confidence intervals of the ordinate at origin and the slope contain 0 and 1, respectively.
365 In addition, the coefficient of correlation (r) obtained gives an idea of the correctness of the
366 adjustment and, therefore, of the similarity of methods. Fig. 6 shows the straight line
367 regression obtained when plotting the BAC values obtained with the gas chromatography
368 reference method and with the biodevice measuring in sweat. As it can be seen, the
369 confidence intervals for the ordinate at origin and of the slope calculated by the least squares

method included 0 and 1, respectively, thus confirming that the proposed method had no systematic errors and that any deviation observed should be attributable to random errors. These results undoubtedly supported the suitability of the biodevice functioning by measuring ethanol in sweat to determining the BAC in a real time and non-invasive way.

4. Conclusions

A novel electrochemical biosensing device for determining the BAC by monitoring ethanol content in sweat has been developed. Very rapid and repeated analyses are possible by using the exclusive biodevice which permits BAC determination in single measurement or in continuous modes, giving information in real time and in a non-invasive way of the BAC for a subject in a particular moment or during long periods of time. The biodevice was successfully validated with 40 volunteers who drank different amounts of alcoholic drinks. The comparison of the BAC values obtained with those provided by a reference method pointed out the absence of significant differences between both methods, confirming the suitability of the developed biodevice for the determination of BAC by amperometric monitoring of ethanol in sweat.

On the other hand, as the measurements are performed in a biological fluid, it is not possible for the evaluated individual to alter the result of the analysis avoiding the false results to which the common breathalysers are exposed. As the present non-invasive and innovating sampling method is much more accurate than those currently on the market measuring by exhaling and very easily adapted to human beings, the proposed instrumentation may be useful in checking drivers´ ethanol consumption or to protect workers from risk due to the residual effects of ethanol digestion.

5. Acknowledgements

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425 **Table 1.** Correlation between the current values measured with the biodevice in sweat at
426 different times and BAC values for all volunteers tested.

t, min	Slope, μA g^{-1} L	Intercept value, μA	r
1	(1.7 ± 0.4)	(0.03 ± 0.07)	0.646
2	(1.5 ± 0.2)	(0.05 ± 0.04)	0.793
3	(1.6 ± 0.1)	(0.03 ± 0.03)	0.884
5	(1.6 ± 0.1)	(0.02 ± 0.02)	0.934

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Figure captions

Fig. 1. Schematic diagrams and real image displaying the biodevice (a); enzyme and electrode reactions involved in the response of graphite-Teflon-AOD-HRP-ferrocene composite electrodes to alcohol (b).

Fig. 2. Amperometric trace measured with the biodevice at 0.00 V depicted for a 5.0×10^{-4} mol L^{-1} ethanol solution.

Fig. 3. Current-time recordings obtained after alcohol ingestion by placing the biodevice on the skin: continuous mode recordings for three different volunteers (a) and single measurements carried out for one volunteer (b). E_{app} = 0.00 V.

Fig. 4. Correlation between the cathodic current values measured with the biodevice in the single measurement mode for the 40 volunteers (■) as well as those plotted in Figure S4b (○) vs. the BAC values determined by gas chromatography. Calibrating straight lines prediction intervals are shown in blue.

Fig. 5. Comparison of the BAC values obtained with the biodevice through sweat measurements and with the gas chromatography method.

Fig. 6. Least square straight line regression resulting by plotting the BAC values (in g L^{-1}) obtained with the biodevice measuring in sweat and the gas chromatography reference method.

Fig. 1

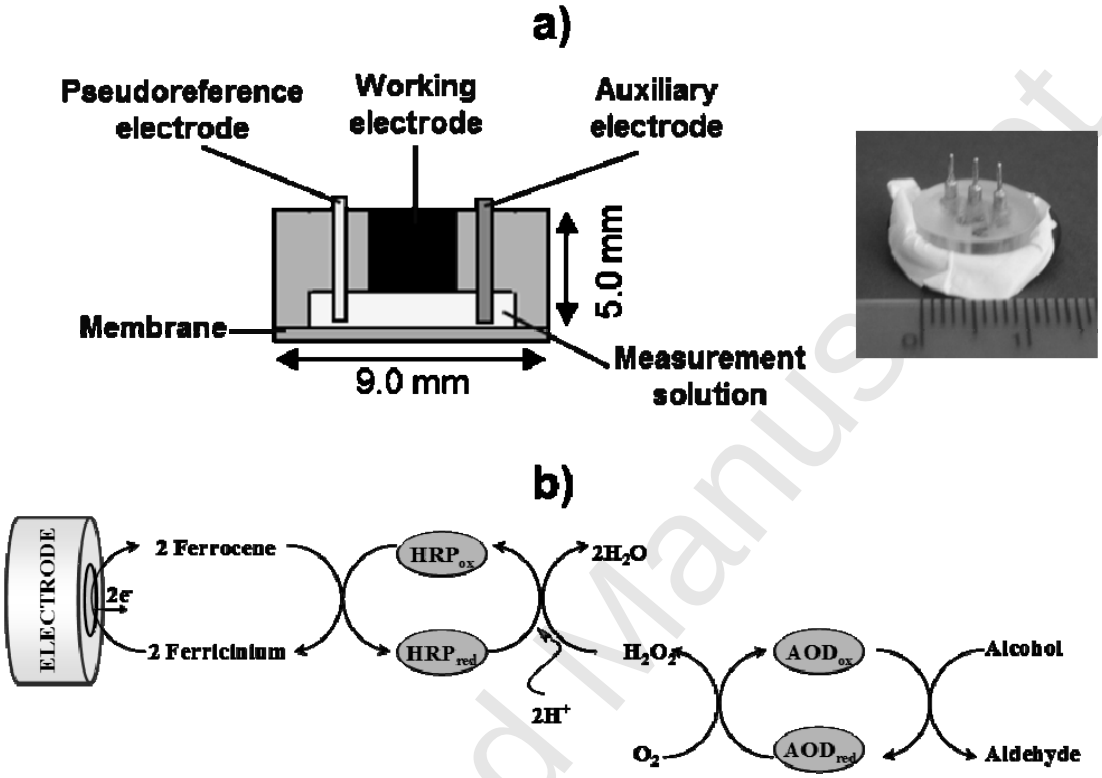


Fig. 2

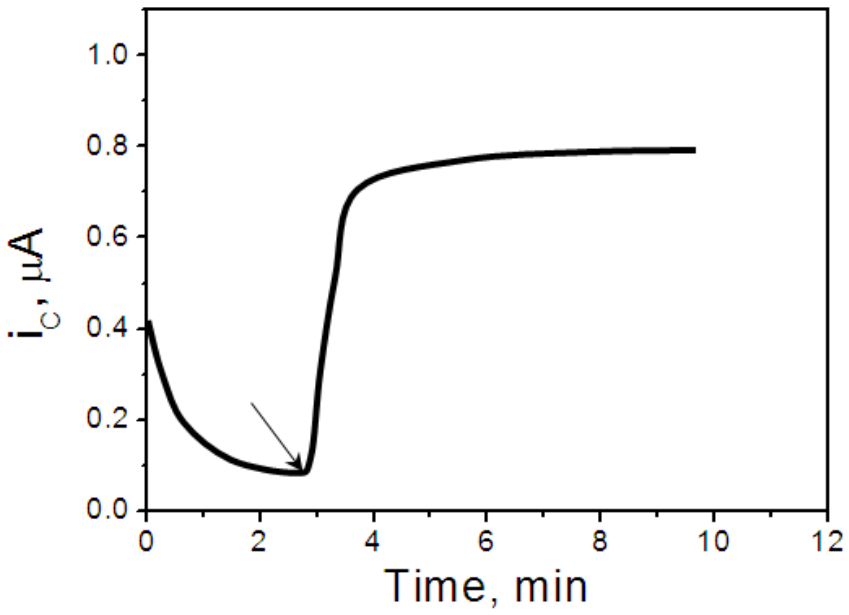


Fig. 3

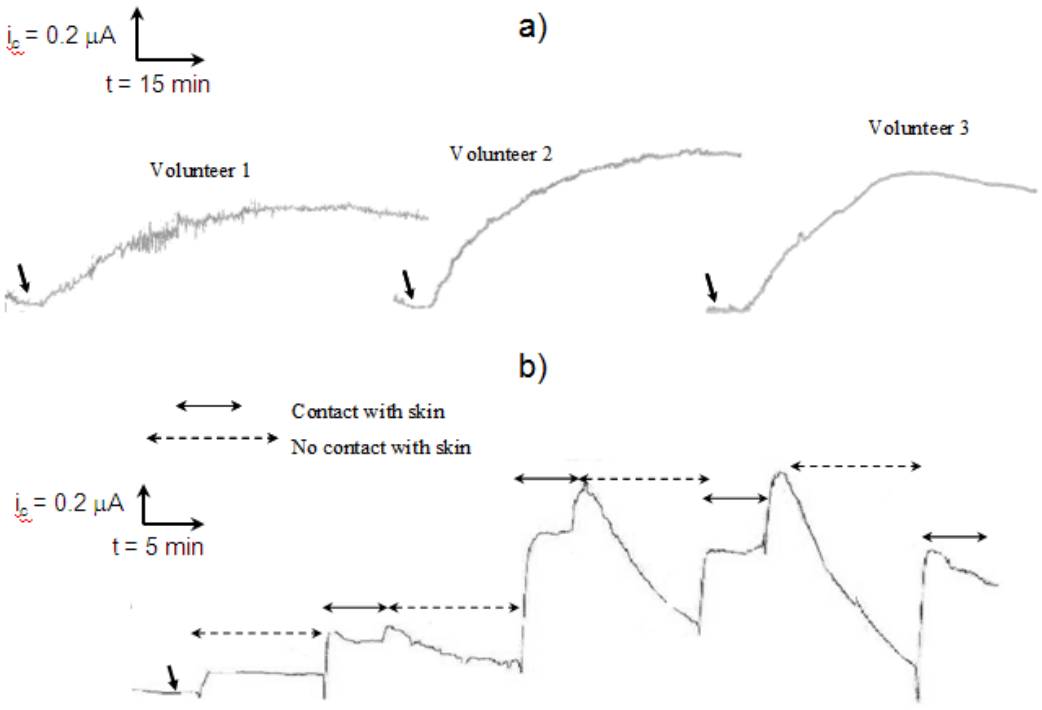


Fig. 4

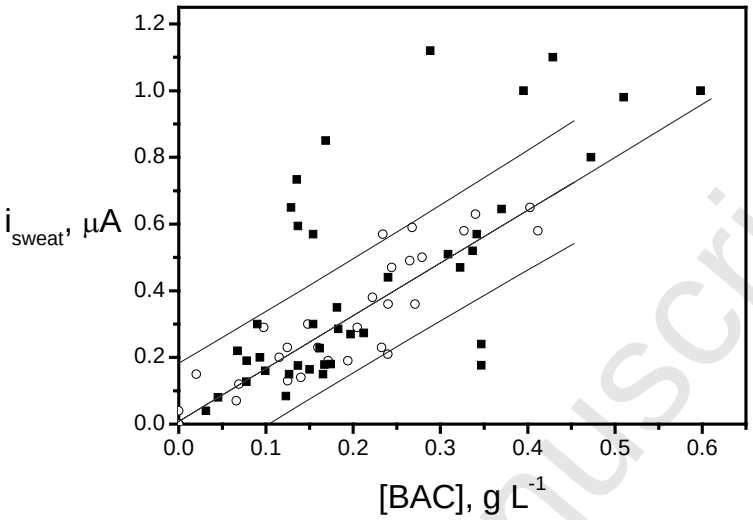


Fig. 5

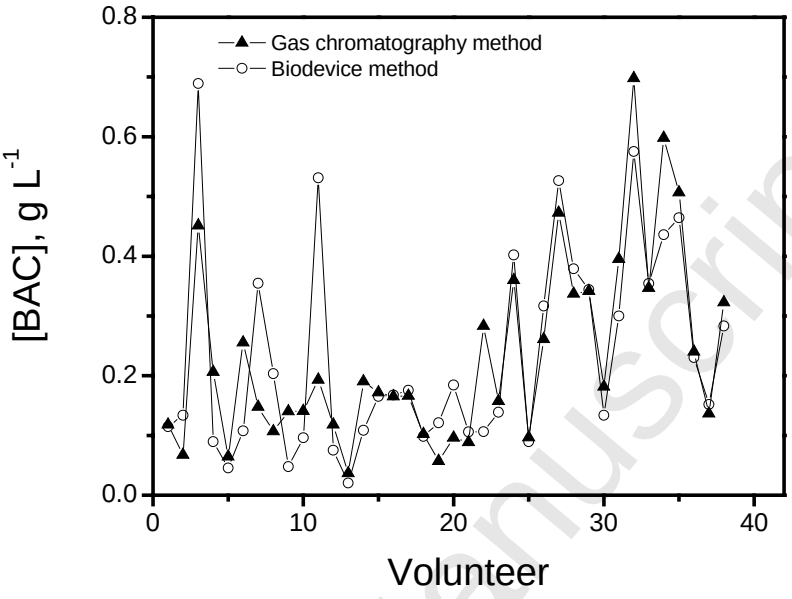
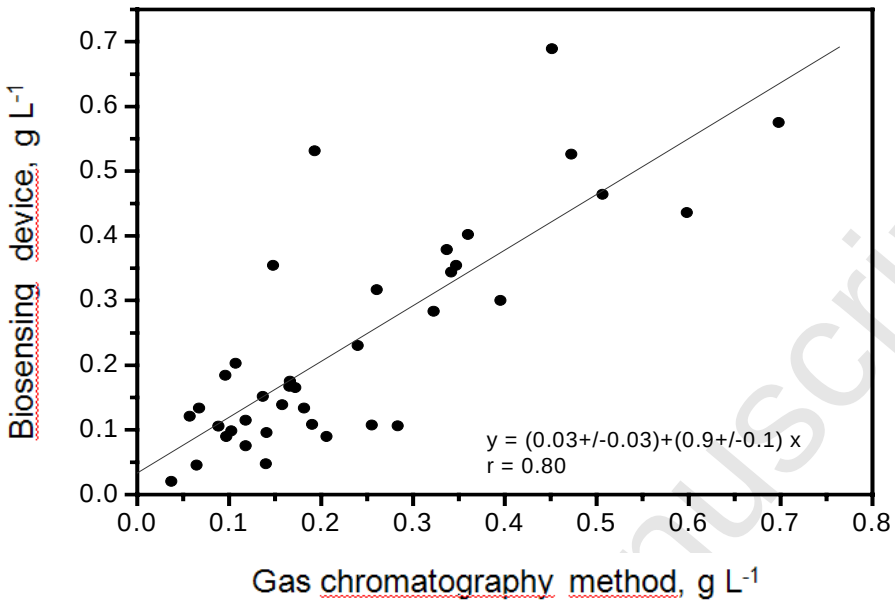


Fig. 6



Highlights:

- New electrochemical biosensing device for determining the blood's ethanol content (BAC).
- Prototype based on bienzyme amperometric composite biosensors.
- Determination of BAC by amperometric monitoring of ethanol in sweat.
- BAC determination in single measurement or in continuous modes.
- Successful validation with 40 volunteers.

Graphical Abstract