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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

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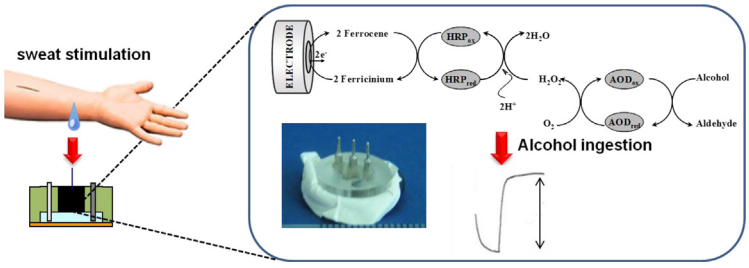
HIGHLIGHTS

- 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



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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 h). 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 breathalyzers marketed at the moment, allowing the monitoring of the ethanol content in blood to be obtained just 5 min 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.

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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 Wigmore et al. [11]. Breathalyzers, 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 miniaturized 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 breathalyzers that are currently commercialized, 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 1430 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. 1(a) 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 and 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: