Sweat as a Biological Fluid of Alcohol Detection

Subjects: Biochemical Research Methods

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The continued focus on improving the quality of human life has encouraged the development of increasingly efficient, durable, and cost-effective products in healthcare. Over the last decade, there has been substantial development in the field of technical and interactive textiles that combine expertise in electronics, biology, chemistry, and physics. Most recently, the creation of biosensors capable of quantifying biometric data in biological fluids to detect a specific disease or the physical condition of an individual is being studied. The ultimate goal is to provide access to medical diagnosis anytime and anywhere. Presently, alcohol is considered the most commonly used addictive substance worldwide, being one of the main causes of death in road accidents.

road accidents alcohol biomarkers biosensor biomimetic

1. Sweat Composition

Recently, there has been considerable interest in the diagnosis of sweat, that is, in the use of sweat as a non-invasive alternative to blood tests to provide data on physiology, health and human performance. Although blood remains the preferred standard in clinical, analyses of other common body fluids, such as sweat, are gaining relevance. The compositions of sweat and blood are osmotically related. Hence, similarly as with blood, the content of certain metabolites in sweat can directly reflect a disease or a physical condition. Most publications on sweat diagnosis are primarily focused on the development of skin-interfaced platforms capable of capturing and performing quantitative measurements of sweat chemistry, such as biosensors capable of detecting biomarkers like lactate [1], glucose [2], cortisol [3], adrenaline, dopamine, phenolic compounds [4][5], and electrolytes [6][7][8] in order to provide new tools for healthcare monitoring. Thus, it is essential to identify the known and unknown factors regarding the composition of sweat to generate hypotheses and guide future research in diagnosis.

Sweat is composed of 99% aqueous solution and a mixture of many chemicals in varying concentrations, including micronutrients (e.g., K^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and vitamins); metabolites (e.g., lactate, ammonia, urea, bicarbonate, amino acids, ethanol) as well as proteins and hormones such as cortisol. These compounds are mostly released through the eccrine glands. These glands are composed of a secretory spiral where sweat is generated, and a dermal duct, which is responsible for transporting sweat through the from the epidermis to the skin surface [9]. During this process, the referred chemicals are incorporated into sweat by passive diffusion and transdermal migration, as it shown in Figure 1.

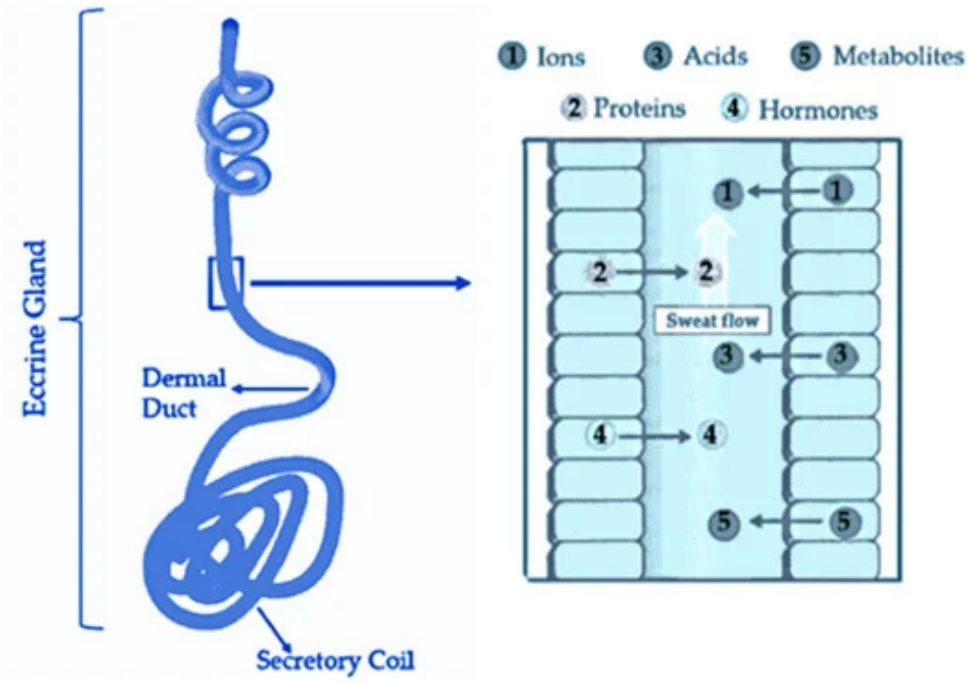

Figure 1. Eccrine sweat gland structure and biomarker partitioning [10].

It must be noticed that humans have between 2 and 5 million eccrine sweat glands throughout the body, which are more densely aggregated in certain regions [11]. In general, it is usually easier detect some substance in sweat in areas of the skin with higher amounts of eccrine glands like palms and sole of foot, as they allow the analysis of larger volumes.

The correlations of chemical molecular levels in blood and sweat have been reported, such as glucose [12], lactate [13], ethanol [14], ammonia and urea [15]. These studies concluded that, to some extent, blood analysis can be replaced by sweat analysis.

Moreover, the final composition of sweat is influenced by several factors such as extracellular concentrations solute, residual sweat, sweat flow rate, sweat gland metabolism in each area of skin, skin contamination and sample evaporation. Thus, despite all the non-invasive advantages, the diagnosis of human sweat on the skin surface of an individual may have some inconveniences such as low sample volumes, contamination of the skin and the possibility of evaporation. These challenges must be studied considering some factors such as:

- **Sweat Released Rate:** According to a study, the average sweat rate during physical activity is approximately 0.5 µL/min/cm^2 with a range of 0.17 to 1.21 µL/min/cm^2 [16]. When considering all areas of the skin, the resting sweat rate should be less than the sweat rate during exercise by 40% [16]. Thus, here can say that, on average, about 1.2 µL/min/cm^2 corresponds to the amount released by an individual at rest. However, it's important to note that the palm region has a skin with higher density of sweat glands, adding ease of collection [17]. Furthermore, in addition to liquid phase sweat detection (sensible sweat), there are some devices capable of measuring volatile organic compounds (VOCs) released through skin (insensible sweat), that have shown high correlation with blood alcohol levels [18][19].

- **Contamination:** Chemicals absorbed by the skin through different cosmetics can be released through sweat and interfere with detection capability. Thus, sweat should be quickly absorbed by the detection platform to avoid contamination from

the skin. A way to prevent different components from interfering with the intended analyte reading is, for example, the use of a semipermeable membrane, responsible for allowing only certain substances to pass through it by diffusion [9].

- **Sample Evaporation:** it is necessary to have a fast detection in order to obtain reliable results, as evaporation acts quickly on small volumes of exposed sweat, which may change the concentration of its constituents [9].

2. Alcohol in Sweat: Correlation between Sweat and Blood

Blood and urine are the most prevalent and used conventional biological matrices for carrying out alcohol consumption analyses. The use of these biological matrices requires the use of complex techniques such as gas chromatography [20]. Although this method produces extremely accurate blood acohol concdentration (BAC) measurements, it uses invasive data collection methods, and it cannot be determined on site. Later, the measurement of alcohol concentration from exhaled air was developed, as it demonstrated a good correlation to blood alcohol levels.

As an alternative to blood and breath tests, sweat analysis has the advantage of containing compounds capable of serving as biomarkers without the need for standard invasive testing methods. In addition, through the analysis of the alcohol content in sweat, the results cannot be altered in the same way as with the breathalyzer method, so there is a lower risk of tampering by interference with foods/drugs taken orally. Circulating in the blood stream, ethanol diffuses into surrounding tissue, including the skin, being the amount of direct excretion of unchanged alcohol in sweat approximately 1% [21]. This allows the concentration of ethanol in sweat to be used as an indicator of the presence of ethanol in blood. However, as alcohol does not diffuse through the skin instantly, there may be a slight delay in the corresponding values of its concentration in blood and sweat.

The development of alcohol detection devices through sweat and further comparative between ethanol concentrations obtained in sweat and blood has been already addressed in literature. One of the first studies was carried out by Buono [14], where it was possible to demonstrate that the ethanol concentration in sweat is highly correlated with blood ethanol concentration. In that study, sweat and blood samples were collected from ten volunteers 1, 2 and 3 h after the ingestion of approximately 13 mmol of ethanol diluted in a 15% fruit juice solution over a period of 30 min. These variables revealed a Pearson correlation of 0.98 indicating a linear variation [22] Furthermore, the slope obtained was 0.81, meaning that the ethanol concentration in blood corresponded to 81% of the ethanol concentration of ethanol in sweat. During this period, Kamei et.al [23] proposed a novel instrumentation capable of estimating and comparing the ethanol concentration in sweat and blood. This proposal consisted in a sampling probe attached directly on the surface of the skin, where it is possible to measure the rate of sweat released, as well as the ethanol concentration in sweat. Three volunteers participated in this study, where each drank 700 mL of beer with 5% ethanol in a period of 2 to 5 min. During this process, the concentration of ethanol in blood was simultaneously measured through of an authorized clinical method. From the obtained results it was possible to conclude that the concentrations of ethanol in blood and sweat reached the maximum peak at almost the same time, demonstrating a very similar profile after ingestion of alcohol by humans. Other scientific studies, carried out by Nyman and Palmlov have reported that the concentration of ethanol in sweat is about 15% higher than the concentration of ethanol in blood [24] The common conclusion in all these studies concerns the high correlation between these concentrations (Pearson correlation indexes very close to 1). Moreover, they also conclude that the concentration of ethanol in sweat is higher than found in blood by approximately 15% [14][23][24]

It is important to highlight that for a good evaluation there are some factors that can influence the action of alcohol such as body weight, dose of ethanol ingested and metabolic rate. Studies have shown that body weight has little effect on the time

interval between peak BAC and TAC (transdermal alcohol concentration) and for a specific dose, the peak alcohol increases as body weight decreases. Regarding the rate of metabolism, the lower the metabolic capacity, the higher the concentration of ethanol present in the body, resulting in a longer peak delay [21].

3. Flexible Biosensors to Detect Alcohol in Sweat

A biosensor is a device capable of providing biometric data, including the concentration of certain chemical substances present in biological fluids, such as alcohol in sweat.

The interest in wearable biosensors has increased recently, since biosensor's flexibility has begun to attract considerable attention to the scientific community. Transdermal biosensors have been designed in tattoos/skin patches, shirts, and other flexible substrates such as woven fabrics or polymer-based manufactured fibrous structures.

In the past decade, the field of wearable and flexible biosensors has seen substantial development, and most applications are health-related through the transduction of physical parameters. The development of biosensors has brought a new era of advances in science. Of the electrochemical devices under evaluation, only the SCRAM™ unit is commercially available at present, but the technology underlying the devices mentioned above are already commercially viable. The SCRAM™ is designed as an ankle bracelet with a sensor compartment and a digital signal processing compartment, which transmits the collected data to an in-home modem [25]. This platform has been implemented by law enforcement personnel to monitor individuals with alcohol-related offenses. The WrisTAS^{TM} corresponds to the first wrist bracelet designed for use in medical settings to control alcohol abstinence [26]. In 2015 a new generation wrist-worn bracelet for alcohol monitoring with came out, and connects via Bluethooth to an app on a user smartphone [27]. Since then, wearable sensing devices have been designed to detect alcohol detection through flexible platforms, such as temporary tattoos [28].

As previously mentioned, in recent years, some alcohol detection systems have been developed in the analysis of sweat, such as attempts to obtain non-invasive, continuous, and discrete alcohol sensors. Currently, flexible electrochemical biosensors are the most commonly used in biofluids analysis, where its principle is based on the reaction of the bioreceptor (enzymes, antibodies; etc.) with the target analyte in order to obtain an electric response (current, potential difference).

A wearable electrochemical biosensor capable of monitoring alcohol consumption by detecting and quantifying Ethyl Glucuronide (EtG), was developed in 2016 [29]. To this end, in this study, two coplanar sensors were developed with gold (Au) and zinc oxide (ZnO) integrated into polyimide (PI) by bonding. Up until then, it was possible to detect EtG in sweat using complex techniques such as gas chromatography associated with mass spectrophotometry, however they were deemed and inadequate processes for on-site diagnosis, not allowing real-time feedback from the person [20][30]. Thus, this project allowed to monitor alcohol consumption by detecting EtG in sweat through wearable biosensors, reacting with colour change through a LED in the presence of EtG in human sweat. Regarding to the results obtained, the ZnO sensor showed a detection capability in the concentration range of 0.001–100 µg/L up to 4 h. On the other hand, the Au sensor demonstrated an ability to detect EtG in the concentration range of 1–10,000 µg/L up to 9 h [29]. The authors concluded that the biosensor could detect ingestion of alcohol up to 11 standard drinks in the United States for a period of 4 to 9 h [29]. In 2019, the chemist Jan Halamék and his team at the University of Albany in New York aimed at developing a new non-invasive method to assess the level of alcohol content in the blood of an individual based on the presence of ethanol in sweat. To do so, 26 volunteers of different ages, genres and eating habits participated [16]. This detection system uses a polyethylene strip composed of two enzymes, alcohol oxidase, and horseradish peroxidase in order to relate blood ethanol concentrations with sweat ethanol

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Table 1. Flexible biosensors to detect alcohol in sweat.

Platform	Target Analyte/Bioreceptor	Measurement Technique	Linear Range	Ref.
Electrochemical biosensor: flexible co-planar Au or ZnO integrated in PI from bonding	EtG/EtG antibody	Electrochemical Impedance Spectroscopy (EIS)	2 × 10^{-6}– 2.17 mM	[24]
Optical biosensor: polyethylene strip composed of two enzymes	Ethanol/Alcohol Oxidase (AOx) and Horseradish peroxidase (HRP)	Chronoamperometry	0–54.23 mM	[31]
Electrochemical biosensor: hydrogel adhesive with screen printed electrodes	Ethanol/AOx	Chronoamperometry	3.0–36.0 mM	[32]

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