

Article

Wearable enzymatic alcohol biosensor

Bob Lansdorp*, William Ramsay, Rashad Hamid, and Evan Strenk

Milo Sensors, Inc.; California NanoSystems Institute (CNSI) Incubator, University of California Santa Barbara, CA 93106-6105, USA

* Correspondence: bob@milosensor.com

Abstract: Transdermal alcohol biosensors have the ability to detect the alcohol that emanates from the bloodstream and diffuses through the skin. However, they have suffered from long-term fouling of the sensor element and drift in the resulting sensor readings over time. Here, we report a disposable cartridge platform that solves the problem of sensor fouling, and an enzymatic detection pathway that minimizes baseline drift for sensitive detection. Laboratory characterization of the enzymatic alcohol sensor demonstrates a linear sensor range of between 0 and 50 mM. Further, we show continuous transdermal alcohol data recorded with a human subject over 48 hours.

Keywords: alcohol; biosensor; cartridge; disposable; transdermal; Alcohol Use Disorder

1. Introduction

Every year, excess alcohol consumption in the United States is responsible for one in ten deaths of working-age people [1] and leads to \$249 billion in economic costs, including \$12 billion in specialty care for abuse/dependence [2]. Of the 18 million Americans with an Alcohol Use Disorder (AUD), only 1 in 7 have received treatment, and those who did seek treatment have faced the daunting prospect of a relapse rate of between 20–80% [3]. Furthermore, all existing data on alcohol use in a treatment setting collected using self-report has been called into question by recent results [4], which demonstrated that 92% of patients drank during treatment, while fewer than half of patients reported drinking. Therefore, there is a need for new tools to better understand, diagnose, and treat AUD. A discreet and continuous wearable alcohol sensor would provide biophysical data that could be more reliable than self-reported consumption. Further, such a wearable technology could enable new treatments that connect patients in treatment for AUD to their clinicians and support network.

Continuous alcohol sensors have received growing interest from the research community [5–14], but commercial realizations thus far have involved trade-offs in performance [15,16]. The only two commercialized platforms (SCRAM and WristAS) use platinum fuel cells. Baseline drift over time requires post-processing of the data [17], and possible degradation of the sensing element within days reduces data reliability [11,18,19]. Platinum fuel cells are further known to be prone to humidity-induced fouling [20,21]. Despite these drawbacks, efforts to introduce the SCRAM offender-monitoring bracelet into alcohol addiction treatment were encouraging, with a large majority (81%) of wearers reporting the bracelet to be useful in helping them reduce drinking, although social discomfort and physical irritation was observed, related to the physical shape and size of the bracelet. [22,23].

In light of the limitations of existing wearable alcohol sensors and encouraging initial results in a treatment setting, what is desired is a non-invasive, continuous, and discreet alcohol sensor that overcomes the issue of sensor fouling and circumvents the limitations of self-reported alcohol consumption [8,24]. In this work, we describe a disposable cartridge system to overcome the problem of sensor fouling. We demonstrate how an enzymatic detection pathway enables a low detection limit. Finally, we miniaturize the entire system into a wearable device that can pair with a smartphone and demonstrate effective monitoring of a human subject's transdermal alcohol concentration over a multi-day period.

2. Materials and Methods

2.1. Chemical detection and catalysis

To convert alcohol that emanates from the skin into an electrical signal that can be digitized, we used the enzyme Alcohol Oxidase (AOD) (*Pichia pastoris*; Sigma Aldrich) as the detector and a screen-printed Prussian blue electrochemical sensor (DropSens) as the transducer. These key elements were contained within a custom designed and manufactured disposable cartridge biosensor (Figure 1). Importantly, the disposable cartridge had a diffusion-limiting membrane (12.5 μm polyethylene (PE) film) that interfaced with the skin and had a surface area of 49 mm^2 . In the cartridge reservoir, 1.3 units of AOD [25] in 25 μL of 1x Phosphate Buffered Saline (PBS) at pH 7.4 oxidized ethanol that diffused through the membrane to acetaldehyde with simultaneous formation of hydrogen peroxide. Hydrogen peroxide diffused to and was sensed by the electrode: a custom screen-printed Prussian Blue [26–29] working electrode (surface area 21 mm^2) and a Ag/AgCl quasi-reference electrode (surface area 6.0 mm^2).

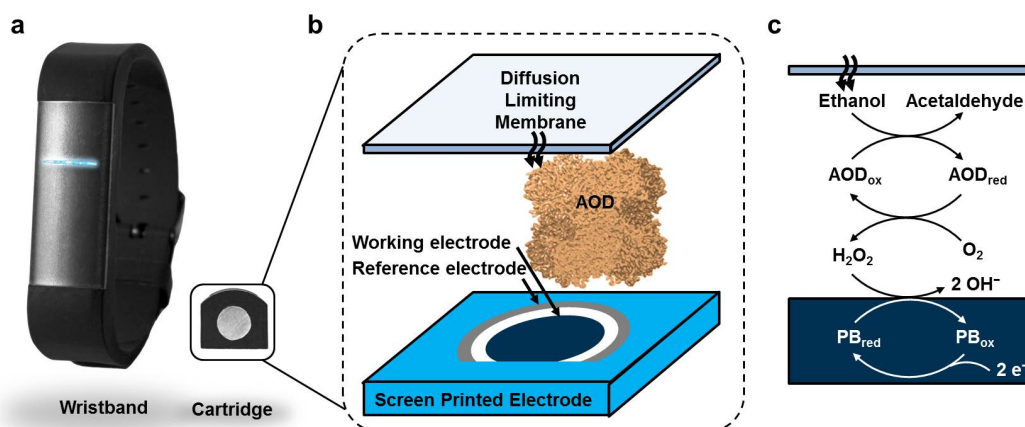


Figure 1. The primary functional components of the sensor: the wristband and disposable cartridge (a), a schematic depicting the primary functional components of the disposable enzymatic alcohol sensor cartridge (b), and the chemical pathway for detection within the sensor cartridge (c).

2.2. Laboratory Data Collection

In laboratory experiments, data was logged to a computer at 1 Hz with a NI-DAQ 6323 attached to a BNC-2090A (National Instruments), which accepted signals from a custom-built potentiostat configured to perform chronoamperometry at +93mV with respect to the Ag/AgCl quasi-reference electrode, to exploit the non-linear selectivity towards hydrogen peroxide over oxygen [28]. Sensors were contained within a temperature-controlled chamber at 30°C and measurements performed in triplicate. Syringe pumps were filled with either PBS solution or a PBS solution with ethanol of known concentration, which was refreshed at 0.49 mL/hr over the diffusion-limiting membrane to ensure a constant and known concentration of ethanol above the membrane.

2.3. Human subject data

For human subject measurements, a miniaturized potentiostat [30,31] in a wearable wristband recorded electrical currents at 0.2 Hz with a 12-bit ADC, and transmitted values to a corresponding smartphone application. The smartphone application sent data via cellular antenna to a web server, where timestamps and electrical current were logged digitally for subsequent analysis. The electronics were enclosed in an injection-molded silicone wristband (see Figure 2) which was powered by a



Figure 2. Photograph of the wearable alcohol sensor being worn in a real-world environment.

rechargeable lithium-polymer battery. The disposable cartridge inserted into the skin-touching side of the wristband and attached to the wristband via two gold-plated pogo pins on the wristband that make electrical contact with the internal circuitry. The wristband was attached to the human subject via a tang buckle and worn with the disposable cartridge in uniform contact with the dorsal side of the wrist.

The subject, author B.L., wore an alcohol-sensing wristband for two consecutive days on his left dorsal wrist. The subject carried one Apple iOS smartphone with a custom-designed app which connected to the wristband via Bluetooth. Data from the smartphone was relayed to a server, where it was logged continuously. At $t=8.9$ h (Event A), the subject consumed three 44 mL units of 35% alcohol in a period of 2 min and an additional 355 mL of 4.6% beer at $t=9.9$ h. At $t=13.9$ h, the subject went to sleep. Data was logged continuously up until an unexpected smartphone application disconnection was observed at $t=18.9$ h, at which time the app was restarted and data logging resumed. At $t=23.2$ h, the wristband was removed from the wrist (Event B), the disposable cartridge was discarded, and the wristband was connected to a 5V charger to recharge the lithium-polymer battery. At $t=25.7$ h, a second new cartridge was inserted into the wristband, and data logging was resumed. Between $t=31.5$ h and 31.8 h, the subject consumed three 44 mL units of 35% alcohol (Event C). At $t=40.6$ h, another unexpected app crash was observed, but the app was restarted and collection resumed at $t=44.1$ h. The study was concluded at $t=48$ h.

3. Results

3.1. In-situ laboratory results

A disposable cartridge was initially connected via pogo pins to the recording electronics housed in a temperature-controlled chamber at 30°C , and then a 1x PBS solution was flowed over the diffusion-limiting membrane (Figure 3a). A spike in current was initially recorded, which was observed to decay to less than 50 nA within 48 min. The baseline current was recorded over a 7 h period and a steady-state value of 6 ± 3 nA was measured ($t=3-7$ h). Then, a solution of 0.05 mol/L ethanol in 1x PBS was flowed over the diffusion-limiting membrane. Almost immediately, an exponential increase in current was observed, and a plateau current of 627 ± 7 nA ($t=12-15$ h) was recorded for over 8 h (representing a steady-state flux). We define the sensor response time as the time required for the current to reach 50 % of the maximal plateau current after addition of a known concentration of ethanol: thus we determined a response time of 35.8 ± 5.7 min with $n = 12$ sensors. The plateau currents were measured with different concentrations of ethanol, and we determined a linear sensor range of between 0 and 0.05 mol/L of ethanol (Figure 3b; error bars correspond to the standard deviation obtained from triplicate experiments at each concentration).

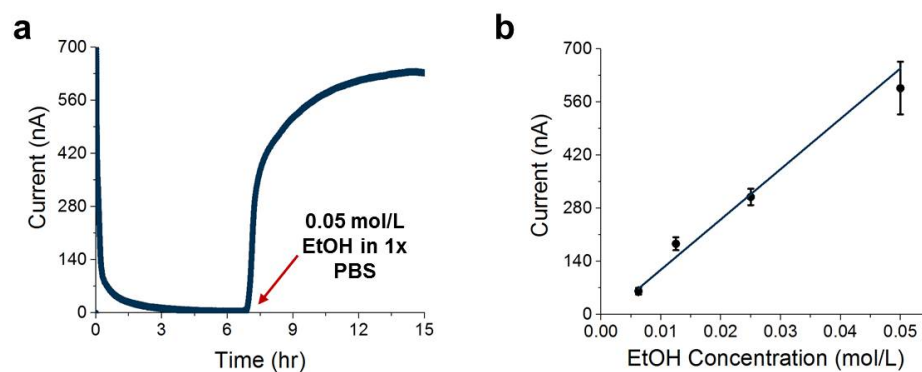


Figure 3. Laboratory characterization of the sensor response

3.2. In-vivo measurement results

Author B.L. wore a wristband for two consecutive days and the electrical current measured by two disposable sensors was plotted as a function of time (Figure 4). Data between $t=0$ h and $t=1$ h and between $t=25.6$ h and $t=26.6$ h correspond to "warm-up" periods when a new disposable cartridge is inserted, during which time current spikes occur. These are removed for clarity of presentation.

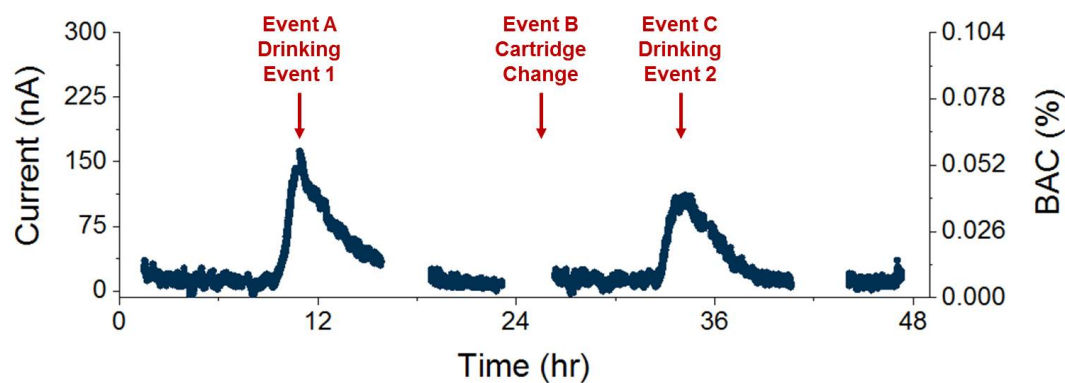


Figure 4. Blood Alcohol Concentration (BAC) versus time

4. Discussion

4.1. Transdermal alcohol

There are two primary mechanisms by which alcohol is excreted by the skin: passive diffusion (i.e., insensible perspiration), and active pumping through sweat glands (i.e., sensible perspiration) [32]. The physics of diffusion that govern the relationship between blood alcohol and insensible perspiration result in time-dependent kinetics [33,34]; transdermal alcohol is often described as being "delayed" with respect to blood alcohol concentration [35–38], although the relationship can be more accurately described as a convolution [17].

4.2. Membrane

Skin permeability to ethanol is known to fluctuate over time [39], and therefore a flux-type sensor placed directly on the skin will measure the product of blood alcohol concentration and skin permeability, resulting in signals that vary with time [16]. The introduction of a diffusion-limiting membrane with known permeability converts a flux-type sensor into a continuous concentration sensor [40–42]. To enable robust alcohol measurement across a variety of skin types and sweating

conditions, we introduced a membrane of well-defined permeability into our disposable cartridge design. The diffusion-limiting membrane limits the flux of ethanol to a membrane-limited rate when the wearer is sweating (sensible perspiration), while still enabling a detectable flux of alcohol when the wearer is not actively perspiring (insensible perspiration only).

Ethanol in the blood stream diffuses through an approximately 200 μm thick epidermis layer as well as a relatively impermeable, approximately 15 μm thick, stratum corneum [34]. The second-order differential equations that govern blood to transdermal alcohol have been described previously [33], and we simplify them here by combining solubility in both blood β_b and stratum corneum β_s , diffusion coefficient D_s , and thickness of the stratum corneum L_s into one permeability:

$$k_{\text{skin}} \approx \frac{\beta_s D_s}{\beta_b L_s} = 7.7 \times 10^{-7} \text{ cm/s} \quad (1)$$

We add a diffusion-limiting membrane of k_{membrane} , and write net flux into the sensor J as the product of blood concentration C_{Ethanol} and the reciprocal sum of permeabilities:

$$J = C_{\text{Ethanol}} (1/k_{\text{skin}} + 1/k_{\text{membrane}})^{-1} \quad (2)$$

The permeability k_{membrane} of the diffusion-limiting membrane is selected to be lower in permeability than the human skin k_{skin} , and thus whether an individual is sweating (sensible perspiration with $k_{\text{skin}} \gg k_{\text{membrane}}$) or not sweating (insensible perspiration only $k_{\text{skin}} > k_{\text{membrane}}$), the flux of alcohol into the sensor is always approximately:

$$J \approx C_{\text{Ethanol}} k_{\text{membrane}} \quad (3)$$

4.3. Laboratory measurements

When a disposable cartridge was first connected to the electronics, a spike in current was observed (Figure 3a), which we attributed to double-layer charging and cation diffusion through the Prussian Blue [43–45]. In the absence of alcohol, the current decays to a steady-state value of approximately $i_{\text{background}} = 6 \pm 3 \text{ nA}$, which we attribute to the non-zero reaction rate of Prussian Blue with dissolved oxygen [28].

Our sensor was operated in a regime where AOD and Prussian Blue were in excess. We therefore combined the flux calculation of Equation 3 together with the expected number of electrons generated per molecule of ethanol, to write the expected electrical current as:

$$i_{\text{measured}} = J \times F \times A \times N \times \eta + i_{\text{background}} = C_{\text{Ethanol}} \times k_{\text{membrane}} \times F \times A \times N \times \eta + i_{\text{background}} \quad (4)$$

For experimental values of $i_{\text{measured}} = 597 \pm 69 \text{ nA}$ at a concentration of $C_{\text{Ethanol}} = 0.05 \text{ mol/L}$, with a surface area of the membrane $A = 49 \text{ mm}^2$, $N = 2 \text{ electrons/(molecule of EtOH)}$, assuming $\eta = 100\%$, and with Faraday's constant in terms of number of electrons of $F = 9.6 \times 10^4 \text{ C/mol}$, solving yields $k_{\text{membrane}} = 1.3 \times 10^{-7} \text{ cm/s}$. As this result is less than the value of skin permeability to ethanol as previously published ($2.2 \times 10^{-7} \text{ cm/s}$) [46], and as calculated in Equation 1 ($7.7 \times 10^{-7} \text{ cm/s}$), we expect that Equation 3 is valid, and our sensor is in a regime where transdermal fluxes of ethanol should be reliably measurable.

The electrical current was linearly proportional to alcohol concentration in Equation 4, and we deduce that the constant of proportionality is linear with membrane permeability. Of note, the expected electrical current is independent of enzyme and catalytic activity, although it is dependent on enzyme and catalyst efficiency.

In laboratory measurements, the sensor was demonstrated to respond linearly to ethanol (see Figure 3), with a proportionality constant between steady-state current and ethanol concentration of:

$$m = \frac{231 \text{ nA}}{0.0174 \text{ mol/L}} \quad (5)$$

With a baseline current of less than 10 nA, a 231 nA signal represents a signal-to-noise ratio of over 20, without the use of any baseline current subtraction. The sensor has a characteristic response time of 35.8 min.

4.4. Transdermal measurements

Transdermal alcohol was measured continuously over two days (see Figure 4). We used m from Equation 5 to convert the electrical current to BAC, assuming that 0.08% is equivalent to $1.74 \times 10^{-2} \text{ mol L}^{-1}$, and that skin permeability is greater than our membrane as per Equation 3. On day one (0–24 h), the electrical current was observed to rise significantly above the baseline current ($>50 \text{ nA}$), at $t=10.10 \text{ h}$, or 70 min after alcohol was consumed. Based on the weight of the subject and the alcohol consumed, we expected a peak BAC of 0.07%, and measured a peak BAC of 0.056% (162 nA). On day two (24–48 h), the current was observed to cross the 50 nA threshold at $t=33.0 \text{ h}$, or 90 min after alcohol consumption. We recorded a peak BAC of 0.038% (110 nA), in good agreement with the calculated BAC of 0.05%. The response times are within the range of previously published values for transdermal alcohol [38].

Gaps in the data were caused by the smartphone app not being able to run as a background task on the smartphone, which we hope to overcome in future iterations. The gap caused by charging the wristband between study days could be easily overcome in clinical research by having two wristbands per participant, one charging while the other is being worn.

An assumption of this work is that the diffusion-limiting membrane is lower in permeability to ethanol than the skin. If this assumption is violated, the measured signal would be lower than the prediction of Equation 3. Future work to measure skin permeability k_{skin} in a wide range of environmental conditions would be of interest, particularly in a range of temperatures and skin sweating conditions. We speculate that the on-skin response time could be faster in the presence of sensible perspiration such as during physical exertion or elevated temperature.

5. Conclusions

We have demonstrated transdermal alcohol measurement over two consecutive days by using a miniaturized biosensor that mates with a discreet wristband, highlighting how a disposable cartridge can overcome the issue of sensor fouling that plagued previous transdermal alcohol sensors. Measurements with a human subject demonstrated the ability to capture real-world drinking events, with an increase in signal corresponding to alcohol consumption, and a decrease back to baseline corresponding to a return to sobriety while sleeping. The wearable described in this work has broad implications for the field of alcohol research, potentially enabling a new generation of researchers to perform non-invasive measurements in the field to improve on self-reported alcohol consumption. We expect that the transdermal alcohol sensing technology described above will find immediate application in research studies that seek to replace self-reported alcohol consumption with physiological data, and may find more broad commercial uses in the future.

6. Patents

We have patented the work in this manuscript: [47]

Supplementary Materials: The following are available online at <http://www.mdpi.com//xx/1/1/s1>, raw data files used to generate Figures 3 and 4

Author Contributions: Conceptualization, B.L., E.S., W.R.; Methodology, B.L., W.R., E.S., R.H.; Software, B.L.; Validation, R.H., W.R., B.L.; Formal Analysis, B.L., W.R.; Investigation, R.H., B.L., W.R.; Resources, E.S., B.L.; Data Curation, R.H., W.R., B.L.; Writing—Original Draft Preparation, B.L.; Writing—Review & Editing, W.R., B.L., R.H., E.S.; Visualization, W.R., E.S.; Supervision, B.L., E.S.; Project Administration, B.L., E.S.; Funding Acquisition, E.S., B.L.

Funding: Research reported in this publication was supported by the National Institute of Alcohol Abuse and Alcoholism of the National Institutes of Health under award number R43AA026125. Approximately 30% of the work was funded with NIH funding, the rest was non-governmental sources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Private funds were used to perform research with human subjects. We thank the angel investors that have backed us for their financial support.

Acknowledgments: Special thanks to Netz Arroyo for engaging in helpful discussions and sharing advice in electrochemistry. Thanks to Ammar Almousa, Thomas Nguyen, David Lunn, Shari Howard, Adam Chinn, and Mohammad Allamarah for helpful discussions. B.L. thanks Shubhaditya Majumdar, Jacob Lundbeck Serup, and William Wortman for engaging discussions on diffusion physics. Thanks to Roger Perez for help with obtaining the data from the backend server. Thanks to the UCSB CNSI and UCSB MRL for access to shared facilities.

Conflicts of Interest: The authors are employees, former employees, or shareholders of Milo Sensors, Inc, a Delaware C-corporation with a mission to develop non-invasive biosensors, including commercialization of the transdermal alcohol sensor technology described above. The fundamental technology demonstration contained in this article is not intended to be used as product claims.

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
AUD	Alcohol Use Disorder
AOD	Alcohol Oxidase
PB	Prussian Blue

References

1. Stahre, M.; Roeber, J.; Kanny, D.; Brewer, R.D.; Zhang, X. Peer reviewed: contribution of excessive alcohol consumption to deaths and years of potential life lost in the United States. *Preventing chronic disease* **2014**, *11*.
2. Sacks, J.J.; Gonzales, K.R.; Bouchery, E.E.; Tomedi, L.E.; Brewer, R.D. 2010 national and state costs of excessive alcohol consumption. *American journal of preventive medicine* **2015**, *49*, e73–e79.
3. Moos, R.H.; Moos, B.S. Rates and predictors of relapse after natural and treated remission from alcohol use disorders. *Addiction* **2006**, *101*, 212–222.
4. Alessi, S.; Petry, N.; Barnett, N. Objective Continuous Monitoring of Alcohol Consumption for Three Months Among Alcohol Use Disorder Treatment Outpatients. *Alcohol* **2019**. doi:10.1016/j.alcohol.2019.01.008.
5. Gamella, M.; Campuzano, S.; Manso, J.; de Rivera, G.G.; López-Colino, F.; Reviejo, A.; Pingarrón, J. A novel non-invasive electrochemical biosensing device for in situ determination of the alcohol content in blood by monitoring ethanol in sweat. *Analytica Chimica Acta* **2014**, *806*, 1–7. doi:10.1016/j.aca.2013.09.020.
6. Kim, J.; Jeerapan, I.; Imani, S.; Cho, T.N.; Bandodkar, A.; Cinti, S.; Mercier, P.P.; Wang, J. Noninvasive alcohol monitoring using a wearable tattoo-based iontophoretic-biosensing system. *Acs Sensors* **2016**, *1*, 1011–1019.
7. Mohan, A.V.; Windmiller, J.R.; Mishra, R.K.; Wang, J. Continuous minimally-invasive alcohol monitoring using microneedle sensor arrays. *Biosensors and Bioelectronics* **2017**, *91*, 574–579. doi:10.1016/j.bios.2017.01.016.
8. Conroy, D.E.; Pincus, A.L.; Ram, N.; al’Absi, M. Thirsting to understand the temporal dynamics of physical activity and alcohol use. *British Journal of Sports Medicine* **2017**, *52*, 1158–1159. doi:10.1136/bjsports-2017-097949.

9. Lawson, B.; Martini-Laithier, V.; Fiorido, T.; Annanouch, F.; Burtey, S.; Cassé-Perrot, C.; Audebert, C.; Bendahan, M.; Bouchakour, R.; Blin, O.; others. Transdermal Alcohol Measurements Using MOX Sensors in Clinical Trials. *Multidisciplinary Digital Publishing Institute Proceedings*, 2017, Vol. 1, p. 431.
10. Kim, J.; Sempionatto, J.R.; Imani, S.; Hartel, M.C.; Barfidokht, A.; Tang, G.; Campbell, A.S.; Mercier, P.P.; Wang, J. Simultaneous Monitoring of Sweat and Interstitial Fluid Using a Single Wearable Biosensor Platform. *Advanced Science* **2018**, p. 1800880. doi:10.1002/advs.201800880.
11. Campbell, A.S.; Kim, J.; Wang, J. Wearable electrochemical alcohol biosensors. *Current Opinion in Electrochemistry* **2018**, *10*, 126–135. doi:10.1016/j.coelec.2018.05.014.
12. Lawson, B.; Aguir, K.; Fiorido, T.; Martini-Laithier, V.; Bouchakour, R.; Burtey, S.; Reynard-Carette, C.; Bendahan, M. Skin alcohol perspiration measurements using MOX sensors. *Sensors and Actuators B: Chemical* **2019**, *280*, 306–312.
13. Kim, J.; Campbell, A.S.; de Ávila, B.E.F.; Wang, J. Wearable biosensors for healthcare monitoring. *Nature biotechnology* **2019**, p. 1.
14. Pandey, P.C.; Shukla, S.; Skoog, S.A.; Boehm, R.D.; Narayan, R.J. Current Advancements in Transdermal Biosensing and Targeted Drug Delivery. *Sensors* **2019**, *19*, 1028.
15. Rocchitta, G.; Spanu, A.; Babudieri, S.; Latte, G.; Madeddu, G.; Galleri, G.; Nuvoli, S.; Bagella, P.; Demartis, M.; Fiore, V.; Manetti, R.; Serra, P. Enzyme Biosensors for Biomedical Applications: Strategies for Safeguarding Analytical Performances in Biological Fluids. *Sensors* **2016**, *16*, 780. doi:10.3390/s16060780.
16. Wang, Y.; Fridberg, D.J.; Leeman, R.F.; Cook, R.L.; Porges, E.C. Wrist-Worn Alcohol Biosensors: Strengths, Limitations, and Future Directions. *Alcohol* **2018**. doi:10.1016/j.alcohol.2018.08.013.
17. Rosen, I.G.; Luczak, S.E.; Weiss, J. Blind deconvolution for distributed parameter systems with unbounded input and output and determining blood alcohol concentration from transdermal biosensor data. *Applied mathematics and computation* **2014**, *231*, 357–376.
18. Marques, P.R.; McKnight, A.S.; others. Evaluating transdermal alcohol measuring devices. Technical report, United States. National Highway Traffic Safety Administration, 2007.
19. Greenfield, T.K.; Bond, J.; Kerr, W.C. Biomonitoring for improving alcohol consumption surveys: the new gold standard? *Alcohol research: current reviews* **2014**, *36*, 39.
20. Roushey III, W.J.; Hawthorne, J.S.; Wojcik, M.H.; Collins, D.C.; McGee, S.K. Moisture control in a transdermal blood alcohol monitor, 2009. US Patent App. 12/013,931.
21. Allan, J.T.; Rahman, M.R.; Easton, E.B. The influence of relative humidity on the performance of fuel cell catalyst layers in ethanol sensors. *Sensors and Actuators B: Chemical* **2017**, *239*, 120–130.
22. Barnett, N.P.; Tidey, J.; Murphy, J.G.; Swift, R.; Colby, S.M. Contingency management for alcohol use reduction: a pilot study using a transdermal alcohol sensor. *Drug and alcohol dependence* **2011**, *118*, 391–399.
23. Alessi, S.M.; Barnett, N.P.; Petry, N.M. Experiences with SCRAMx alcohol monitoring technology in 100 alcohol treatment outpatients. *Drug and alcohol dependence* **2017**, *178*, 417–424.
24. White, A.M. Commentary on Patrick and Colleagues: High-Intensity Drinking Among Young Adults in the United States: Prevalence, Frequency, and Developmental Change. *Alcoholism: Clinical and Experimental Research* **2017**, *41*, 270–274. doi:10.1111/acer.13306.
25. Couderc, R.; Baratti, J. Oxidation of methanol by the yeast, *Pichia pastoris*. Purification and properties of the alcohol oxidase. *Agricultural and biological chemistry* **1980**, *44*, 2279–2289.
26. Karyakin, A.A.; Gitelmacher, O.V.; Karyakina, E.E. A high-sensitive glucose amperometric biosensor based on Prussian Blue modified electrodes. *Analytical Letters* **1994**, *27*, 2861–2869.
27. Neff, V.D. Electrochemical oxidation and reduction of thin films of Prussian Blue. *Journal of the Electrochemical Society* **1978**, *125*, 886–887.
28. Itaya, K.; Shoji, N.; Uchida, I. Catalysis of the reduction of molecular oxygen to water at Prussian blue modified electrodes. *Journal of the American Chemical Society* **1984**, *106*, 3423–3429. doi:10.1021/ja00324a007.
29. Karyakin, A.A.; Karyakina, E.E.; Gorton, L. Prussian-Blue-based amperometric biosensors in flow-injection analysis. *Talanta* **1996**, *43*, 1597–1606.
30. Fidler, J.; Penrose, W.; Bobis, J. A potentiostat based on a voltage-controlled current source for use with amperometric gas sensors. *IEEE Transactions on Instrumentation and Measurement* **1992**, *41*, 308–310. doi:10.1109/19.137366.
31. Umasankar, Y.; Jalal, A.H.; Gonzalez, P.J.; Chowdhury, M.; Alfonso, A.; Bhansali, S. Wearable alcohol monitoring device with auto-calibration ability for high chemical specificity. 2016 IEEE 13th International

- Conference on Wearable and Implantable Body Sensor Networks (BSN). IEEE, 2016, pp. 353–358. doi:10.1109/BSN.2016.7516287.
32. Swift, R. Direct measurement of alcohol and its metabolites. *Addiction* **2003**, *98*, 73–80. doi:10.1046/j.1359-6357.2003.00605.x.
 33. Anderson, J.C.; Hlastala, M.P. The kinetics of transdermal ethanol exchange. *Journal of Applied Physiology* **2006**, *100*, 649–655. doi:10.1152/japplphysiol.00927.2005.
 34. Webster, G.D.; Gabler, H.C. Feasibility of transdermal ethanol sensing for the detection of intoxicated drivers. Annual Proceedings/ Association for the Advancement of Automotive Medicine. Association for the Advancement of Automotive Medicine, 2007, Vol. 51, p. 449.
 35. Wagner, J.G.; Wilkinson, P.K.; Sedman, A.J.; Kay, D.R.; Weidler, D.J. Elimination of alcohol from human blood. *Journal of pharmaceutical sciences* **1976**, *65*, 152–154.
 36. Brown, D.J. A method for determining the excretion of volatile substances through skin. *Methods and findings in experimental and clinical pharmacology* **1985**, *7*, 269–274.
 37. Brown, D. The pharmacokinetics of alcohol excretion in human perspiration. *Methods and findings in experimental and clinical pharmacology* **1985**, *7*, 539–544.
 38. Karns-Wright, T.E.; Roache, J.D.; Hill-Kapturczak, N.; Liang, Y.; Mullen, J.; Dougherty, D.M. Time delays in transdermal alcohol concentrations relative to breath alcohol concentrations. *Alcohol and alcoholism* **2016**, *52*, 35–41.
 39. Arakawa, T.; Suzuki, T.; Tsujii, M.; Iitani, K.; Chien, P.J.; Ye, M.; Toma, K.; Iwasaki, Y.; Mitsubayashi, K. Real-time monitoring of skin ethanol gas by a high-sensitivity gas phase biosensor (bio-sniffer) for the non-invasive evaluation of volatile blood compounds. *Biosensors and Bioelectronics* **2018**.
 40. Clark, L.C.; Wolf, R.; Granger, D.; Taylor, Z. Continuous Recording of Blood Oxygen Tensions by Polarography. *Journal of Applied Physiology* **1953**, *6*, 189–193. doi:10.1152/jappl.1953.6.3.189.
 41. Swift, R.M.; Martin, C.S.; Swette, L.; Laconti, A.; Kackley, N. Studies on a wearable, electronic, transdermal alcohol sensor. *Alcoholism: Clinical and Experimental Research* **1992**, *16*, 721–725.
 42. Swette, L.L.; Griffith, A.E.; LaConti, A.B. Potential and diffusion controlled solid electrolyte sensor for continuous measurement of very low levels of transdermal alcohol, 1997. US Patent 5,944,661.
 43. Garcia-Jareno, J.; Sanmatias, A.; Vicente, F.; Gabrielli, C.; Keddam, M.; Perrot, H. Study of Prussian Blue (PB) films by ac-electrogravimetry: influence of PB morphology on ions movement. *Electrochimica Acta* **2000**, *45*, 3765–3776.
 44. Gabrielli, C.; García-Jareño, J.J.; Keddam, M.; Perrot, H.; Vicente, F. Ac-electrogravimetry study of electroactive thin films. I. Application to Prussian Blue. *The Journal of Physical Chemistry B* **2002**, *106*, 3182–3191.
 45. Ozeki, T.; Watanbe, I.; Ikeda, S. Study of the prussian blue/prussian white redox reaction by cyclic voltammetry. *Journal of electroanalytical chemistry and interfacial electrochemistry* **1987**, *236*, 209–218.
 46. Mitragotri, S. Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. *Journal of Controlled Release* **2003**, *86*, 69–92.
 47. Lansdorp, B.; Strenk, E.; Arroyo, N.; Imberman, D. Transdermal analyte sensing device, 2018. US Patent 9,855,000.