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A portable optical human sweat sensor

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We describe the use of HNQ (2-hydroxy-1,4-naphthoquinone or Lawsone) as a potential sweat sensor material to detect the hydration levels of human beings. We have conducted optical measurements using both artificial and human sweat to validate our approach. We have determined that the dominant compound that affects HNQ absorbance in artificial sweat is sodium. The presence of lactate decreases the reactivity of HNQ while urea promotes more interactions of sodium and potassium ions with HNQ. The interactions between the hydroxyl group of HNQ and the artificial sweat components (salts, lactic acid, and urea) were investigated comprehensively.

We have also proposed and developed a portable diode laser absorption sensor system that converts the absorbance at a particular wavelength range (at 455 ± 5 nm, where HNQ has an absorbance peak) into light intensity measurements via a photocell. The absorbance intensity values obtained from our portable sensor system agrees within 10.4% with measurements from a laboratory based ultraviolet-visible spectrometer. Findings of this research will provide significant information for researchers who are focusing on real-time, in-situ hydration level detection. © 2014 AIP Publishing LLC.

I. INTRODUCTION

As biosensors become ubiquitous and receive endorsement from the general public, research and development on biological sensing is experiencing a rapid rise.1,2 The persistent consumer demand for wearable and portable devices has shifted the research of biosensors toward more application-oriented processes. This particular shift has resulted in the development of biosensors that are non-invasive and have thus eclipsed the traditional blood analysis methods. To this point, the development of portable, non-invasive, and biocompatible sensors has focused mostly on temperature, relative humidity, electrolytes and minerals.13,14 Although contents in sweat can be considered the diluted version of blood in terms of constituents such as electrolytes and minerals,13,14 a good representation of sweat, however, can be standardized into four major constituents: sodium ions, potassium ions, lactate, and urea.15,16 Combinations of these compounds are used to create artificial sweat in order to investigate mainly corrosion and nanoparticle release effects on textiles and metal implants.16–20 In recent years, the electrolyte content of sweat has been used to determine the physiological status of a person, such as hydration or tiredness.8,21–23

In-situ sweat analysis has been centered mainly on pH level detection and sodium ion concentration monitoring. Diamond’s group, as a part of the BIOTEX project,24 developed textile sensors for sweat pH analysis using optical techniques.25,26 The analysis of the sodium ion concentration of sweat has been investigated using skin contact electrodes to detect the real-time electrolyte-level changes.27,28 In other research, the sweat lactate levels have been determined using electrochemical tattoos.29 Sweat ethanol levels have been monitored via an electrochemical transdermal alcohol concentration sensor.30

Besides electrochemical methods, the detection of optical property variation in response to changes in the concentration of sweat content has shown great promise. We have previously demonstrated that a natural dye known as HNQ can be used as a sweat sensing material based on color changes.31,32 Our previous work focused on the optical properties of HNQ under various hydration conditions where the optical absorbance is linearly related to the amount of ions that are present in a liquid media along with HNQ.32

In the current work, optical absorbance versus concentration of sweat components is analyzed. The effects of current sensor technology has made it possible to detect such changes. Sweat contains several hundred different chemicals.15,14 It has been well established that sweat can be considered the diluted version of blood in terms of constituents such as electrolytes and minerals.3,13,14 Although contents in sweat appear in lower concentration than in blood, the sensitivity of current sensor technology has made it possible to detect such changes. Sweat contains several hundred different chemicals.15,14 A good representation of sweat, however, can be standardized into four major constituents: sodium ions, potassium ions, lactate, and urea.15,16 Combinations of these compounds are used to create artificial sweat in order to investigate mainly corrosion and nanoparticle release effects on textiles and metal implants.16–20 In recent years, the electrolyte content of sweat has been used to determine the physiological status of a person, such as hydration or tiredness.8,21–23

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increasing the concentration of four primary chemical components of sweat both in aggregate as well as individually are presented and discussed. The sensitivity of the sensor technique is presented and shown to be sufficient for the proposed application. Finally, we have successfully implemented a portable diode laser absorption sensor (PDLAS) system that would eliminate the need for bulky laboratory instruments in testing the optical properties of sweat. This is a first step toward a deployable, wearable sweat sensor. Our investigation of sweat chemistry sensing is particularly groundbreaking as it will shed light on the effect of sweat content on the real-time physical condition of subjects such as athletes and soldiers.

II. ARTIFICIAL SWEAT

Artificial sweat is defined as a water solution with four primary dissolved compounds: sodium chloride (NaCl), potassium chloride (KCl), urea, and lactic acid. It is important to note that human sweat contains additional compounds in low concentrations besides the four compounds in artificial sweat. However, for many applications and for the purposes of this work, measuring these four compounds is sufficient. The artificial sweat used in this study was prepared according to the European Standard number EN1811: 2012 by dissolving the following compounds in 1 liter of ultra-purified deionized water (resistivity of 18.3 MΩ cm): 0.5% of NaCl, 0.1% of KCl, 0.1% of lactic acid, and 0.1% of urea (weight/volume ratio). NaCl, KCl, and lactic acid were acquired from VWR International, Inc. Urea was acquired from Fisher Scientific, Inc. The resulting constituent concentrations are 85 mM NaCl, 13 mM KCl, 17 mM lactic acid, and 16 mM urea. In this study, these concentrations of artificial sweat represent “normal” human levels. The target application of this work is dehydration sensing, which is manifested as an increase in the concentration of these 4 principal sweat components from their base values.

It has been reported that the HNQ dye has redox properties allowing the binding of cations at pH values between 5 and 7.33 For this reason, pH was carefully controlled in these experiments. The Eco Testr PHZ pH meter was used to determine the pH value for the samples; the pH of the artificial sweat solutions was adjusted to 5.4 ± 0.3 by drop-wise addition of 0.01 M ammonium hydroxide (NH₄OH, Fischer Scientific, Inc.).

III. SWEAT CHEMICAL ANALYSIS USING HNQ DYE

The main goal of this paper is to validate the use of HNQ as a sweat sensor material to quantify the hydration levels of human beings in-situ. One drop of real/artificial human sweat solution into 15 ml HNQ solution causes a significant change in the intensity of the absorbance peak centered at 455 nm as shown in Figure 1. We have previously shown that the sweat itself does not have an absorbance in the region of 400 nm and 500 nm.32 Sweat contains 98% water, and the remaining is mainly electrolytes, urea, and lactic acid. Figure 1 shows the UV-Vis spectrum of HNQ dye by itself, with artificial sweat as described in Sec. II and real human sweat collected from subjects (approved by Central Michigan University’s Institutional Review Board). The measurements were performed with a standard Varian Cary 1 Ultraviolet absorbance spectrometer with 1 nm resolution. The HNQ (Aldrich Chemical Company) is dissolved in a solution of 4:1 DI: Acetone (volumetric ratio).

In earlier research, we found that HNQ changes color when it is doped with sodium or potassium ions.34 It turns from pale orange to dark orange (see Figure 2(a) inset). The main reason behind the color change is that the hydroxyl group of HNQ (see Figure 1 inset) is deprotonated, and the negatively charged oxygen atom is attached to positively charged sodium or potassium ions, forming a doped HNQ structure.31 This effect changes the electron density of the

FIG. 1. UV-Vis spectrum of HNQ solutions with and without real/artificial sweat. Human/artificial sweat data were obtained by adding a drop of sweat into 15 ml of HNQ solution. Our focus is on the absorbance peak centered around 455 nm, which is not present in a pure sweat sample.32 The inset shows the chemical scheme of the HNQ molecule.

FIG. 2. (a) UV-Vis spectra at 5 representative artificial sweat constituent concentrations. Inset shows photograph of the 5 samples arranged from lowest concentration (left) to highest (right). (b) Average absorbance versus concentration at center wavelengths (λ<sub>avg</sub>) 455 nm, 555 nm, and 655 nm. The averaging was performed over a 10 nm bandwidth.
benzene, which enhances the absorption peak at 455 nm. As more blue/green light at 455 nm is absorbed, only the red/orange regions of the spectrum pass through the sample giving its characteristic color.

Figure 2(a) shows the optical absorption spectra measured by conventional UV-Vis spectroscopy for 5 representative artificial sweat + HNQ samples. Various concentrations of artificial sweat were prepared by increasing the concentrations of the 4 primary compounds while keeping the relative ratios the same. The goal is to demonstrate the ability to measure a wide range of hydration levels. The broad peak centered at 455 nm grows in amplitude with increasing artificial sweat concentration, whereas in the wavelength region 500 nm–800 nm, the absorbance is relatively unchanged.

Figure 2(a) inset illustrates that the effect of absorption due to large differences in concentration of artificial sweat is visible to the naked eye. To ensure that the optical absorption results from HNQ molecular absorption and not simply from increasing conductivity of the liquid due to a higher concentration of dissolved charged species, the average absorbance centered at 455 nm, 555 nm, and 655 nm is plotted in Figure 2(b). These are absorbance values averaged over a 10 nm region centered at the wavelength of interest. The wavelength value at which the maximum near 455 nm occurs can shift by as much as ±5 nm due mostly to the breadth of the peak and the limited spectrometer dynamic range. The 10 nm averaging procedure removes any ambiguity in concentration measurement due to small spectral shifting. In a deployed and/or wearable sweat sensor, a light source with a sufficiently broad spectrum will automatically perform this averaging procedure.

In Figure 2(b), it is clear that the strongest absorbance occurs at 455 nm, whereas the absorbance at 655 nm is barely above the measurement noise floor. The continuous lines are least squares fits of the formula $\text{ABS}_{\text{avg}} = a\sqrt{C} + b$, where $\text{ABS}_{\text{avg}}$ is the average absorbance, C is the concentration, and a and b are fit parameters. The values of the fit parameters are $(a, b) = (6.98, -0.57); (0.674, 0.059); (0.061, 0.005)$ for 455 nm, 555 nm, and 655 nm, respectively. The excellent agreement between the trend lines and the experimental data suggests that the dependence of $\text{ABS}_{\text{avg}}$ on C is indeed of the form $\text{ABS}_{\text{avg}} = a\sqrt{C} + b$. On the other hand, Beer’s law states that the absorbance is linearly related to the concentration of the media C, the attenuation coefficient or molar absorptivity $\varepsilon$, and the path length $l$:

$$\text{ABS} = \varepsilon lC. \quad (1)$$

However, the law is only valid for low concentration samples, particularly for concentrations below 10 mM. As the concentration increases, the spacing between ions or molecules in the medium are reduced so that neighboring particles contribute to the charge distribution, ultimately altering the absorption. In other words, solutions that contain electrolytes can alter the molar absorptivity due to electrostatic interactions. This effect is apparent in Figure 2(b) as the concentration range is from 10 mM to 250 mM. In fact, derivation of Beer’s law excludes the interaction between particles.

Here, sensitivity is defined in terms of the minimum measurable change in transmitted light intensity for a given change in component concentration $\Delta C_{\text{min}} = \frac{\Delta I}{I_0/C} \Delta I_{\text{min}}$, where $I = I_0 e^{-\alpha x}$ (Beer-Lambert law) and

$$\frac{dI}{dC} = I_0 e^{-\alpha x} \frac{d(-\alpha x)}{dC} = -2.3I_0 \frac{d(\text{ABS}_{\text{avg}})}{dC} = -2.3I_0 a/(2\sqrt{C}). \quad (2)$$

Here, $\Delta C_{\text{min}}$ and $\Delta I_{\text{min}}$ are defined as the minimum detectable concentration and light intensity changes, respectively. $I_0$ is the nominal intensity, $x$ is the absorption coefficient, and $a$ is the distance that the light travels in the medium (i.e., the size of the sample container). The factor 2.3 appears because $\text{ABS} = -\log_{10}(I/I_0)$ and $\alpha x = -\ln(I/I_0)$, and $2.3 \approx 1/\log_{10}e$ converts between natural log and log base 10.

If a 10% relative intensity change can be reliably measured, then $\Delta I_{\text{min}} = 0.1$ and $\Delta C_{\text{min}} = 0.2\sqrt{C}/(2.3a)$. Figure 3(a) shows $\Delta C_{\text{min}}$ calculated from the trend lines. Because of the sublinear increase in $\text{ABS}_{\text{avg}}$ with C, the minimum reliable sweat component concentration increases as C increases. If the dependence of $\text{ABS}_{\text{avg}}$ on C was linear or superlinear, then $\Delta C_{\text{min}}$ would remain constant or decrease, respectively. While the sensitivity trend depicted in Figure 3(a) is superior to a linear or superlinear dependence of $\text{ABS}_{\text{avg}}$ on C, it does not limit the performance of the sensor in the present application. Figure 3(b) shows the percent concentration uncertainty (100% $\times \Delta C_{\text{min}}/C$) assuming 10% confidence in light intensity changes. The uncertainty percentage decreases as concentration increases with the most uncertainty occurring at low sweat component concentrations. The uncertainty is well below 10% in the neighborhood of “normal” human sweat concentration of 0.1 M. From this analysis, we conclude that the proposed sensor technology is sufficiently accurate for reliable measurement of human sweat concentration.
TABLE I. The optical absorbance measured for different combination of artificial sweat while adjusting pH using NH4OH. The binary representation corresponds to the presence of the compound (represented as “1”).

<table>
<thead>
<tr>
<th>Sample number</th>
<th>NaCl (85 mM)</th>
<th>KCl (13 mM)</th>
<th>Urea (16 mM)</th>
<th>Lactic acid (17 mM)</th>
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<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.58</td>
</tr>
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</table>

IV. SWEAT CHEMISTRY

In order to understand the effect of individual compounds in sweat, different combinations of urea, lactic acid, NaCl, and KCl were added to HNQ and the resulting optical spectra were analyzed. All possible combinations of the four artificial sweat components were prepared as shown in Table I. Because normal human sweat has a pH in the range 5 to 7, the pH of all samples was set to 5.4 ± 0.3. The standardized artificial sweat concentration values were used. The data are arranged in a binary setting where “1” representing the existence of the compound in the solution, as shown in Table I. “0000” means there is no compound added to the HNQ.

The main reason of the increase in the absorbance peak at 455 nm is that sodium and potassium ions are attached to the deprotonated HNQ as illustrated in Figure 1. It is evident that 83% of HNQ will be in deprotonated (forming the HNQ ion, HNQ−), as shown in Figure 4) since the pKα value of HNQ is 4.0 (percentage of deprotonation can be calculated using the Henderson-Hasselbach equation: pH = pKα - log \( \frac{[\text{HNQ}]}{[\text{HNQ}^-]} \)) and the pH is 5.6. Positively charged sodium and potassium ions in the artificial sweat solution can then be bonded to HNQ−, forming either HNQ-Na or HNQ-K, which changes the electron density and thus energy of the ring, as detailed in Figure 4.

This molecular change is reflected in the optical absorbance spectrum and clearly observed in the experiments presented in Table I. In the cases where only NaCl and/or KCl (samples #4, #8, and #12) are present in the solution, ABS (maximum absorption intensity obtained from UV-Vis measurements in the region of 450 nm–460 nm) significantly increased compared to the HNQ sample alone (sample #0). As shown in Figure 5, adding KCl increases absorption by 1.5 times, adding NaCl increases the absorption by 2.6 times and adding both KCl and NaCl increase the absorption by 3.1 times.

On the other hand, the presence of lactic acid (lactate−) lowers the absorbance (sample #2 vs. #3, #6 vs. #7, #8 vs. #9, and #12 vs. #13) as shown in Figure 6(a). Lactate (Lactic acid) will be 96% dissociated (pKa = 3.86) to form negatively charged lactate ions (lactate− in Figure 4), which will prevent the process of HNQ protonation. Therefore, lower percentage of HNQ− will cause a reduced intensity absorbance. In two specific cases (sample #4 vs. 5 and #10 vs. #11), lactate has a different effect; we postulate that some of the Na+ and K+ ions are consumed by binding to lactate and thus are unavailable to bind to HNQ. As every negative ion needs a positive ion, and Na+ and K+ bind a carboxylate ion more strongly than NH4+, the results of sample #4 vs. 5 and #10 vs. #11 are actually expected.

Urea, on the other hand, has two NH2 groups, which are capable of accepting protons to form protonated urea, as illustrated in Figure 4. Due to its pKα (pKa = 0.2, i.e., pKα = 13.8), 98% of the available urea molecules can donate electrons to form ammonia ions, which can further deprotonate the remaining HNQ (17% from the initial solution) resulting in higher concentration of HNQ-Na and/or HNQ-K. This theory is well observed in Table I comparing samples #0 vs. #2, #1 vs. #3, #9 vs. #11, etc., as shown in Figure 6(b). Finally, the pH of the solutions was adjusted with NH4OH to 5.4 ± 0.3. This compound can react with the remaining unreacted HNQ and create further negatively

FIG. 4. HNQ reacts with sweat components resulting in doped HNQ (HNQ-Na and HNQ-K), charged urea (Urea−), and charged lactate (Lactate−). A by-product of ammonia ions is also generated due to the pH adjustment with NH4OH.
charged HNQ ions (HNQ\textsuperscript{−}), which ultimately results in advance reaction of sodium and potassium ions.

As a result, the main mechanism behind the interaction between sodium and potassium ions in sweat is the deprotonated HNQ. While urea promotes further deprotonation and thus doping, lactic acid prevents the reaction. Although this is the general trend, it must be noted that there are some cases where this theory was not supported. We believe this is mainly because some complex reactions we have yet to identify. A deeper study on the each compound’s interactions with HNQ is needed.

![Graph of HNQ, KCl, NaCl, KCl+NaCl absorbance](image)

FIG. 5. Bar chart representation of samples 0, 4, 8, and 12 from Table I. Adding salt into HNQ increases the absorbance significantly.

V. PORTABLE SWEAT SENSOR

With a view toward a wearable, commercially available diagnostic system, a size-scalable single wavelength optical absorption measurement system (PDLAS) was built using commercially available, off-the-shelf components. The light source is an OSRAM PL450B blue-violet laser diode (\(\lambda_0 = 450\text{ nm}\)). This laser wavelength targets the maximum of the HNQ absorbance peak. An Advanced Photonix PDV-P9003 photoconductive photocell was used for detection. A schematic diagram and a photograph of the actual setup are shown in Figure 7. A spherical lens with one-inch focal length is used to collimate the laser beam. The beam intensity is controlled by an optical attenuator that consists of a stationary linear polarizer and a rotatable linear polarizer. Intensity outputted from the attenuator is \(I_0 \cos^2(\theta)\), where \(\theta\) is the angle of rotation between the two linear polarizers and \(I_0\) is the nominal laser intensity. Standard current control circuitry was used as a buffer between the DC power supply (BK Precision 1651 A) and the laser diode. The resistance of the photocell was measured with a BK precision 5492B standard multimeter.

Characterization of the PDLAS is shown in Figure 8(a). The relationship between the intensity and resistance of a photocell is \(-\log_{10}(I/I_0) = \xi \log_{10}(R/R_0)\), where \(R_0\) is experimentally measured to be 1.9 kΩ. Figure 8(a) shows the experimental calibration used to determine \(\xi = 1.85\), which holds over a large dynamic range. Figure 8(b) shows the comparison between the absorption coefficient measured using a conventional UV-Vis spectrophotometer and the PDLAS system. The absorption coefficient is related to \(ABS\) via \(ABS = -\log_{10}(I/I_0) = \alpha x \log_{10} e\), so that \(x = 2.3 \times ABS/x\) where \(2.3 \approx 1/\log_{10} e\). The samples size is \(1.1\) cm. The PDLAS system is able to reproduce the UV-Vis measurements with an average disagreement of 10.4% (minimum and maximum errors are 1.2% and 25.2%, respectively). While further iterations of the PDLAS implementation can potentially improve the average measurement agreement to within 5%, even in its present form the PDLAS can be used for reliable sweat concentration monitoring.

VI. CONCLUSION

The effect of hydration on the performance of athletes has been studied over the years. There is a current trend where non-invasive hydration detection systems are of interest. Sweat analysis provides a viable alternative to blood analysis towards this goal. However, current techniques of sweat analysis require time consuming laboratory analysis (results being obtained even in a longer time than a blood analysis), hence are not applicable for frequent sampling.

This work has investigated HNQ dye and the role of four primary sweat components (sodium chloride, potassium chloride, lactic acid, and urea) in the ability to detect sweat component detection optically. The sensing mechanism is shown to be robust with sufficient accuracy for reliable detection. Furthermore, the HNQ optical sensing approach is scalable to a compact and potentially wearable form. As a step toward a deployable sensor device, we have successfully implemented a PDLAS that can be used to quantitatively
determine the hydration levels of human sweat in-situ by eliminating the need for a complicated laboratory based measurement setup. We believe that our approach will help researchers to investigate advanced materials including but not limited to natural dyes for their physical and chemical properties towards compact, wearable sweat analysis systems.

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