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Development of a microfluidic collection system to measure electrolyte variations in sweat during exercise

A.S.M. Steijlen, J. Bastemeijer, W.A. Groen, K.M.B. Jansen, P.J. French, A. Bossche

***Abstract*—A wide variety of electrochemical sweat sensors are recently being developed for real-time monitoring of biomarkers. However, from a physiological perspective, little is known about how sweat biomarkers change over time. This paper presents a method to collect and analyze sweat to identify inter and intraindividual variations of electrolytes during exercise. A new microfluidic sweat collection system is developed which consists of a patch covering the collection surface and a sequence of reservoirs. Na^+, Cl^- and K^+ are measured with ion chromatography afterwards. The measurements show that with the new collector, variations in these ion concentrations can be measured reliably over time.**

I. INTRODUCTION

Technological developments in wearable sweat sensing receive a lot of attention in current literature, because sweat sensing offers opportunities for non-invasive real-time monitoring of biomarkers. A wide range of articles present successful demonstrations of sweat sensing by electrochemical measurement. Most studies particularly focus on the development of the sensor itself. For example, Gao et al. [1] developed a flexible sensor array which contains amperometric lactate and glucose sensors and ion sensitive electrodes for sodium and potassium monitoring. Zörner et al. created a screen-printed potentiometric sensor for ammonia sensing [2]. Bellando et al. [3] use ion sensitive field effect transistors to detect sodium and potassium. Only a few examples are mentioned above. A more complete overview about developments in electrochemical sweat sensing can be found in several recently published review papers [4-6].

However, when looking at physiological literature, it can be concluded that for most sweat analytes it is not known yet, which inter and intraindividual variations in sweat analyte concentrations can be expected [7]. So, there is still not much known about how these biomarkers can be used in diagnostics or in determining the physical status of a subject.

Since it is difficult to validate a sensor on human subjects, when it is not known which variations in analytes can be expected, this article proposes another approach. Instead of directly developing a sensor, this research starts from lab measurements to identify challenges of sweat measurement in practice and to collect data about sweat analyte variations during exercise that can be used as a reference for testing new electrochemical sensors.

The research focusses on real-time sweat measurement of electrolytes to determine internal training load of athletes. In literature, several biomarkers of interest were found.
Changes in Na^+ and Cl^- concentrations in sweat can indicate changes in sweat rate [8], dehydration/overhydration and electrolyte loss [9]. Some researchers claim that NH_4^+ concentrations in sweat are related to lactate concentrations in blood. However, data in different articles show contradictory results. Zörner et al. and Ament et al. measured a decrease of absolute ammonium concentration over time, while Guinovart et al. measured an increase during exercise [2, 10, 11]. K^+ concentrations in arterial plasma increase with increased muscle activity [12, 13]. For K^+ in sweat Holmes et al. measured higher K^+ levels at higher exercise intensities as well. However, in that study, sweat samples were just collected after exercises of different intensities [14].

In short, there are still many unknown aspects of how these sweat electrolytes change during exercise. Therefore, this research focuses on reliable measurement of sweat analytes during exercise. In this case sodium, chloride, and potassium levels are monitored.

II. METHOD

A. Design of the Sweat Collector

Absorbent patches and the Macroduct sweat collector (EliTECH Group, France) are examples of commonly used sweat collection systems. This study started with pilot experiments with Macroduct collectors to collect sweat during exercise. The collector is a disposable device that contains a concave undersurface which is attached to the skin. Via a small orifice in the middle of the collector, sweat is collected into a coiled tube (diameter: 0.6 mm, capacity: 85 µl).

From experiments, it was found that with this collector, we were not able to collect enough sweat to reliably interpret variations in ions over a one-hour cycling period. Proper

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A.S.M. Steijlen, J. Bastemeijer, P.J. French and A. Bossche are with Delft University of Technology, Faculty of Electrical Engineering, Mathematics & Computer Science, Mekelweg 4, 2628 CD, Delft, The Netherlands, Department of Electronic Instrumentation. (corresponding author: phone: +31 (0)15 2781819; email:a.s.m.steijlen@tudelft.nl) (j.bastemeijer@tudelft.nl; p.j.french@tudelft.nl; a.bossche@tudelft.nl)

K.M.B. Jansen is with Delft University of Technology, Faculty Industrial Design Engineering, Landbergstraat, 15, 2628 CE, Delft, The Netherlands, Emerging Materials group (email: k.m.b.jansen@tudelft.nl).

W.A. Groen is with Delft University of Technology, Faculty of Aerospace Engineering, Kluyverweg 1, 2629 HS Delft, The Netherlands, Department of Aerospace Structures & Materials (email: w.a.groen@tudelft.nl)

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attachment at other places than the arm with higher sweat rates appeared difficult and time-consuming. Therefore, it is concluded that a new sweat collection system needs to be designed to perform this study. The most important criteria of the new system are the following:

- The collector collects sweat over a defined surface
- Sample volumes are at least 80 µl
- There is minimal contamination between new sweat and old sweat
- Sweat rate measurement is possible

To enable rapid prototyping and improving the design iteratively, it was chosen to create the collection system from a polyester film with a hydrophilic coating and a double-sided adhesive tape as a spacer material. Table I shows important specifications of the materials. A CO_2 laser (Merlin Lasers, Lion Laser Systems, The Netherlands) was used to cut the materials.

TABLE I. SPECIFICATION OF THE MATERIALS

Materials	**Specification**		
	Carrier	***Coating***	***Contact angle (º)***
Hydrophilic film (Visgard 275 [15])	Polyethylene-tereftalate	Polyurethane matrix (surfactant is washed off, to prevent ion contamination of samples)	50.5*
Double sided adhesive (3M 1522 [16])	Polyethylene	Acrylate	92.2*

* Contact angles were measured with optical tensiometry (KSV Instruments Ltd)

A single reservoir collector, containing a single sample, is developed and tested first. Thereafter, a multiple reservoir collection system is created, which can collect five consecutive samples. The designs are shown in the results chapter.

CFD simulations are performed using COMSOL Multiphysics software, to test the operation of the collectors. For the simulations, a level set method is used to calculate the displacement of the fluid interface and a laminar flow study is executed to describe mass and momentum transport.

Lastly, the collectors are fabricated, and the sweat collection capability is tested using a syringe pump (KDScientific 200, USA), which is set at a rate of 48 µl/min. This is based on a sweat rate at the back of 1.2 mg/cm^2/min [17] times a collection surface of 40 cm^2, which is the size of the patch. The syringe pump is placed at the inlet of the collector and the filling process is filmed with a camera.

B. *Physiological experiment*

To test the collectors in practice, a series of collectors is fabricated for a physiological experiment. Approval for sweat collection and analysis during exercise was obtained from the Human Research Ethics Committee of the TU Delft. In this experiment, a participant is asked to cycle for 60 minutes at an average heartrate of 160 bpm and a cadence of 90 rpm. A Garmin Edge 820 (Garmin, USA) including a cadence sensor and a heart rate sensor and a power meter, the Garmin Vector 3s, are used to keep track of this. The participant (male, age: 27 years, recreational racing cyclist) gave informed consent and did not drink during the exercise.

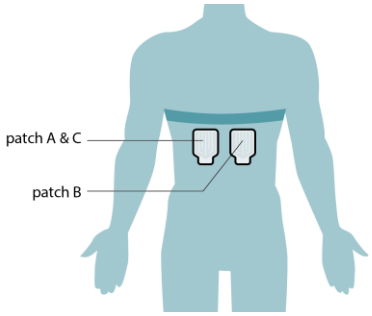

Figure 1. Anatomical location of patch placement at the back. Patch A is placed first. After 15 min, patch B is placed. When patch A was filled, it was replaced by C

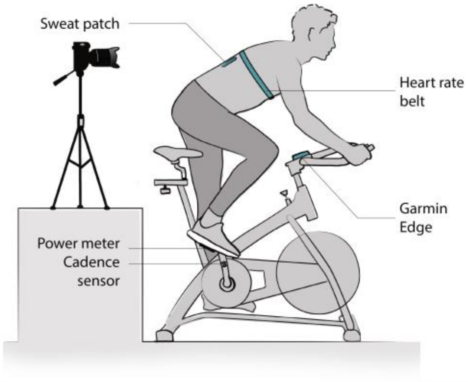

Figure 2. Setup of the physiological experiment

During the experiment, sweat patches are placed and replaced at the back. The back is chosen, because of high sweat rates and the presence of eccrine sweat glands. In Figure 1, the location and sequence of the patches can be found. A blue colorant is placed in the collector and the filling speed is filmed with a camera. The complete set-up is shown in figure 2. Before placement of each patch, the skin is cleaned with gauze pads and demineralized water.

C. *Chemical Analysis*

Samples were analyzed using a ion chromatography/high-performance liquid chromatography (IC/HPLC) method [18]. The IC system consists of a Metrohm 881 Anion system and an 883 Basic IC plus system (Metrohm, Switzerland) The anion system uses a Metrosep A supp 5 - 150/4.0 column and the cation system uses a Metrosep C6 - 150/4.0 column for ion separation. Calibration is performed with standard solutions of chloride, sodium and potassium (ranging from 0.1-100 ppm) The sweat samples of around 100 µl are diluted with 3 ml of ultrapure water.

III. RESULTS

A. *Sweat collector*

Figure 3 shows the design of the single reservoir collector that was developed first on the left. The final design of the multiple reservoir system is shown on the right. This design includes a series of reservoirs which are designed to fill

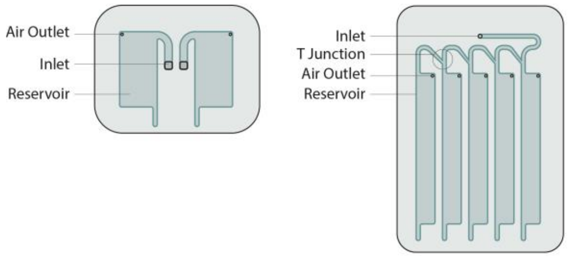

Figure 3. Designs of the sweat reservoirs

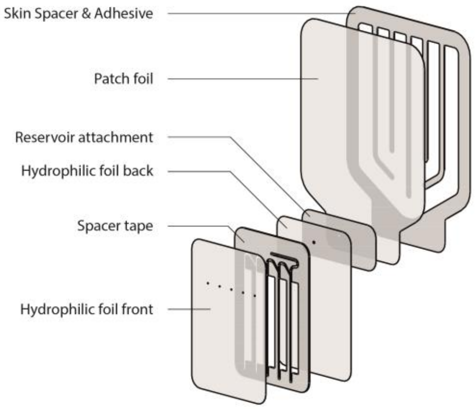

Figure 4. Exploded view of the sweat collection system (main dimensions of the patch: 74 * 144 mm)

consecutively. The volume of each reservoir is nearly 140 mm^3. Figure 4 shows a 3D drawing of the whole patch. The reservoirs are made from two layers of hydrophilic foil with a spacer tape in between. Via the larger patch with a funnel-shaped spacer to the skin, the sweat is directed to the inlet of the reservoir. Gravitational effects are used to make sure that the sweat drops down. Once at the inlet, the sweat flows into the reservoir by capillary effects. Air outlets are placed at the top of the reservoirs, to prevent entrapment of air.

A still from the experiment with a syringe pump that is set at a constant speed of 48 µl/min is shown in figure 5, and in figure 6 the results from the experiment can be found. The volume that is filled over time is calculated by counting the pixels from stills of the movie (every minute) and converting this to the surface dimensions. The graph shows that the volume flow rate of the collector compares well with that delivered by the syringe pump. The systematic difference of 50 µl (corresponding to 50 seconds) can be interpreted as a time delay needed for filling up the delivery channel system.

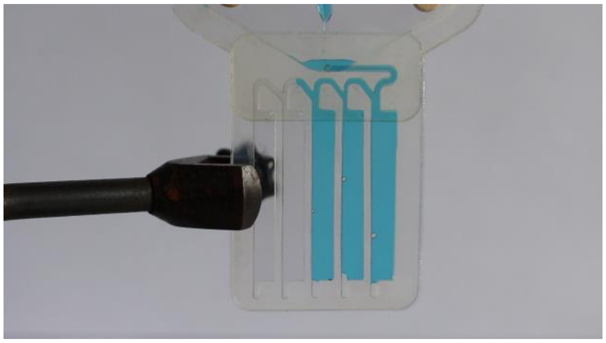

Figure 5. Still from syringe pump experiment

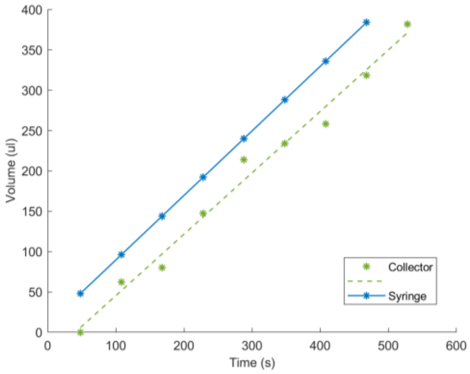

Figure 6. Volume flow rate of the syringe pump vs. the collector

B. *Physiological experiment*

The physiological experimental protocol was improved iteratively. Three test runs, including fabricating collectors and chemical analysis, with multiple participants were performed to exclude measurement artefacts. Problems encountered and solved included:

- Sodium contamination by the surfactants used in the hydrophilic foil.
- Cleaning the skin without demineralized water before placement of the patch introduced an increase of sodium and chloride in the first reservoirs
- Variations in anatomical placement of the patches introduced differences in concentration levels

Figure 7. shows a typical image of the exerted power, and heart rate during cycling. Heart rate increases slightly over time. The Cl^-, Na^+ and K^+ levels that were measured are shown in figure 8**.** In this case it took 855 seconds before the first sweat passed the inlet of the collector. From the graphs can be seen that Cl^- and Na^+ increase during the exercise. In the beginning of the exercise, the increase is larger than in the end of the exercise. Na^+ concentrations are on average 18 mM higher than Cl^- concentrations. It is expected that this difference is due to the other negative ions like $C_3H_5O_3^-$ and HCO_3^- in sweat. K^+ levels seem to decrease slightly. However, this decrease was not measured in all experiments.

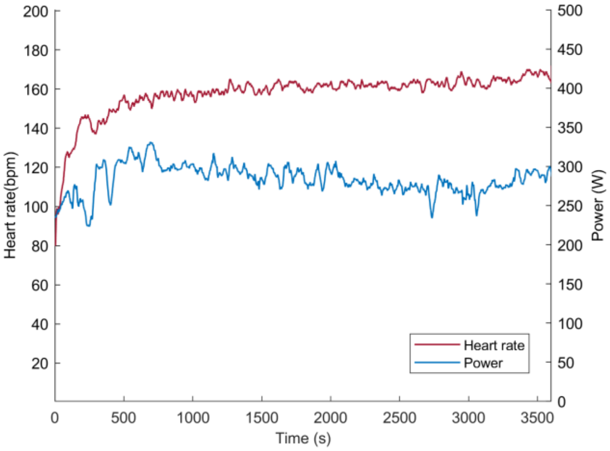

Figure 7. Measured power (mov. avg.) and heartrate during the exercise

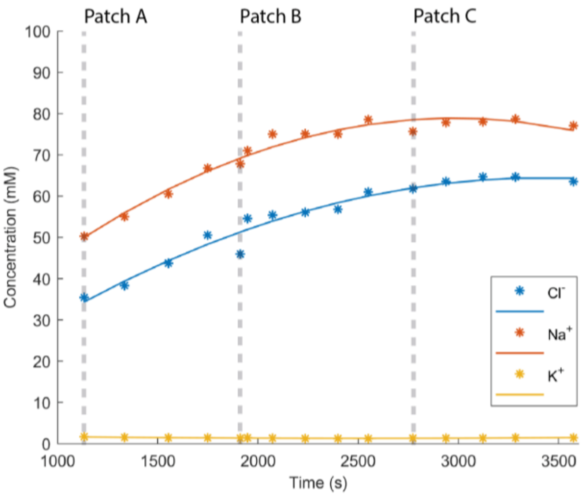

Figure 8. Cl$^-$, Na$^+$ and K$^+$ concentrations during the exercise (the concentrations of each reservoir are plotted at the end time of reservoir filling). Trend lines are a 2nd order polynomial fit. The dotted lines show the onset of filling of the first reservoir of a patch.

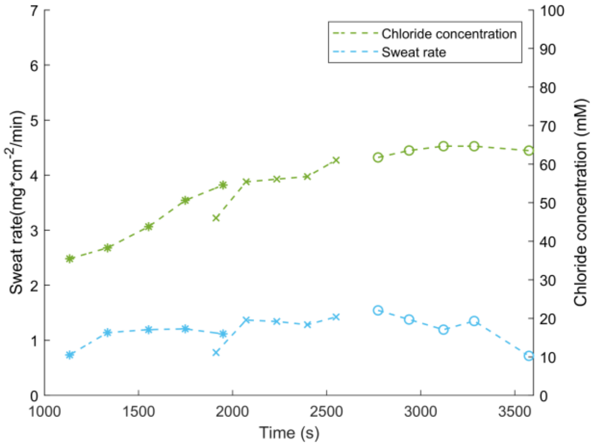

Figure 9. Sweat rate (left vert. axis) and chloride concentrations (right vert. axis) over time. Patch 1 is marked by '*', patch 2 by 'x', patch 3 by 'o'

Sweat rate was derived from video recordings (Figure 9). The average sweat collection rate was 1.2 mg/cm^2/min. The graph shows that the sweat rate varies between 0.7 and 1.5 mg/cm^2/min in the measurement. Body movement, entrapment of air and the patch itself may cause variations in measured sweat collection rate.

IV. CONCLUSION

In this study, a novel sweat collection system is presented. The new system enables reliable analysis of sweat analytes, such as Cl$^-$, Na$^+$ and K$^+$. It is shown that it is possible to obtain fifteen samples per hour. In this way, new possibilities arise to research how sweat analytes change over time and how they are related to the physical status of the athlete. The system provides technological opportunities for validating electrochemical sensors too. The system allows integration of sensors in the patch directly, so that output can be measured during the exercise and the sweat can be analyzed in a laboratory afterwards. The results can be compared to validate that the new sensor system works properly.

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