# Screen-printed Biochemical Sensors for Detection of Ammonia Levels in Sweat – Towards Integration with Vital Parameter Monitoring Sports Gear

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Abstract: The fabrication of fully screen-printed biochemical sensors employing planar integrated solid state electrodes is described. The sensors are developed to fit wearable devices and target the monitoring of ammonia respectively ammonium levels in sweat. Increased ammonium levels in sweat correlate to physical overstrain of muscles, indicated by the breakdown of proteins in muscle cells. The sensor on flexible foil uses an ion-selective working electrode and a reference electrode for potentiometric measurements of the electromotoric force, EMF. For the ammonium ion-selective electrode a cocktail of nonactin was deposited. The printed sensors were calibrated with ammonium standard solutions at a working range between $10^{-5}$ M to 0.1 M which corresponds to the range of physiological levels of ammonium in sweat before and during physical strain. The potentiometric characterization of the ion-selective sensor shows a linear behaviour of the EMF versus pC values with a Nernstian slope of 59.3 mV $\pm$ 11.2 mV. The combination of low-cost printed sensors, potentiometric sensing, and the integration with textiles represents a very attractive approach for non-invasive monitoring of individual sports performance to prevent overload during physical training.

## 1 INTRODUCTION

The monitoring of fitness status for identification of the ideal workout conditions or the prevention of muscular overstress is usually performed by logging vital data during physical exercise and sequential externally analysis by medical labs.

Direct analysis of body metabolism during physical strain can be performed in sports medicine laboratories using body fluids, e.g. blood and, more and more common, also sweat. Generally, blood is used to analyze the workout condition in combination with the registration of vital parameters like heart rate and respiratory gases. Analysis of blood withdrawals during physical strain are invasive, very much time-consuming, and costly and can only be achieved in cooperation with a sports medicine laboratory

Mobile fitness and health related data acquisition becomes more and more common and is the first success story for a range of wearable technologies. Motion trackers that count the steps and estimate the energy/calories consumption of the user have been in the market for years. Vital parameters such as respiration effort as well as heart rate and heart rate variabilities derived from the electrocardiogram (ECG), deliver more reliable information sources for assessing training success and cardiovascular health. Thus those systems are under research for integration in future wearable systems (Tantinger et al., 2012; Tobola et al., 2015). An even more detailed picture of health and fitness status can be derived with simultaneous monitoring of metabolic parameters. Non-invasive approaches, e.g. detection of relevant electrolyte concentrations are preferred against blood analysis. The appropriate sensing units along with challenging textile sensor adaptation and biosignal processing methods that can cope with motion artefacts have to be developed.

Generally, the body fluid sweat is only used for medical analysis for diseases or for analysis for drug

levels inside the body (Heikenfeld, 2014).

Sweat is an electrolyte solution that mostly consists of water with sodium, potassium, and bicarbonate. Additional inorganic compounds such as urea, pyruvate, lactate, as well as proteins, peptides, amines, amino acids, and ammonia are contained in sweat. Furthermore, metal ions, inhibitors, antigens, antibodies, and a variety of xenobiotics such as drugs, cosmetics, and ethanol have been found in sweat (Sato et al., 1989; Mena-Bravo and Luque de Castro 2014).

Next to these physical species, ammonia content in sweat is known for some decades to be a key parameter for sports performance diagnostics (Ament et al., 1997). The correlation of muscular overstrain to production of ammonia is known since last century. Ammonia and ammonium in blood are mostly a result of metabolic degradation of proteins (Schulz and Heck, 2001).

Czarnowski et al., (1992) published the mechanism of the ammonia transport from muscle cells into sweat. As ammonia generally is a cytotoxin, the human body tends to minimize the ammonia content (Czarnowski et al., 1992). The difference between the pH value of sweat (4.0-6.8) and blood (7.35-7.45) forms a pH gradient. Ammonia diffuses from a higher to a lower pH value, i.e. from blood to sweat. The size of ammonia molecules is similar to water molecules. As such they are permeable through cell membrane.

Some 20 years ago, investigations verified the mechanism published by Czarnowski et al., (1992) and therefore the correlation between physical strain and sweat ammonia. Ament et al., (1997) published the evaluation of ammonia in blood and sweat in correlation to physical strain. This study was performed utilizing an incremental cycle ergometer exercise. During physical strain the content of ammonia in sweat is in the range of millimol whereas it is only in the μmol range in blood at the same time.

Further publications from Alvear-Ordenes et al., (2005) and Meyer et al., (2007) show a direct correlation between muscle overload of rugby players during physical workout and also a gender effect of ammonia content in sweat. All ammonia diagnostics were done by analyzing sweat collected with gauze pads (Alvear-Ordenes et al., 2005; Meyer et al., 2007). One of the major challenges in monitoring ion concentrations in sweat is the sample recording. Extraction from human skin is time-consuming, complex, and subject to errors, e.g. in analyte concentration due to evaporation of solvent or contamination of the sweat sample. This explains

also the low number of publications on sports related sweat diagnostics.

For these reasons, direct monitoring of sweat composition on the skin before and during physical strain is a very attractive alternative. Bandodkar et al. (2013), Wang et al., (2010), and Guinovart et al., (2013) developed skin applicable microfluidic devices for sweat extraction and integrated pH monitoring as well as flexible screen-printed electrodes for application to skin-wearable devices. Rose et al., (2013) published a sodium-selective sensor in combination with a RFID chip.

Ion-selective electrodes are the most fundamental features of sensors for analysis of activity of ions in physiological fluids. In the last century they mostly were electrochemical glass electrodes an automated apparatus (Schulz and Heck, 2001; Czarnowski et al., 1992; Ament et al., 1997; Alvear-Ordenes et al., 2005; Meyer et al., 2007).

Koncki et al., (1999) and Tymecki et al., (2006) published screen-printed and planar electrodes designed from printed silver, carbon and insulating inks on flexible substrates. The potentiometric measurements were taken against commercially acquired reference electrodes.

In the last years, several potentiometric sweat electrolyte measurements were published using planar ion-selective sensors directly on skin (Yang et al., 2010; Guinovart et al., 2013; Rose et al., 2014). Koncki et al., (1999); Tymecki et al., (2006) and Guinovart et al. (2013) developed ammonium-selective electrodes by means of screen printing on flexible foils.

Guinovart et al. (2013) developed a potentiometric tattoo style ammonium sensor including the reference electrode that can be directly stuck to the skin.

Our present work combines the previously reported sensor designs of Koncki et al., (1999) and Guinovart et al., (2013). A flexible screen-printed sensor is prepared combining an ammonium-selective electrode based on nonactin ionophore with an integrated reference electrode. Screen-printing technology enables the deposition of thick layers, short fabrication time, and precise sensor patterns with a resolution of few hundreds of microns leading to the fabrication of cheap and disposable electrochemical sensors. The ion-selective biomedical sensor published in this paper shows a promising approach. The fully-printed biomedical sensor shows a sensitivity or Nernstian slope of over 2 orders of magnitude (0.001 M to 0.1 M). Furthermore, this work presents an outlook towards

the sensor integration in textiles to extent a functional sport shirt with low-power appliance for various electrolyte monitoring tasks (Tantinger et al., 2012; Tobola et al., 2015).

## 2 EXPERIMENTAL

### 2.1 Materials and Reagents

Silver-based (125-13), silver-silver chloride with a ratio of 65:35 (125-21) and carbon filled (120-24) pastes for screen printing were acquired from Creative Materials (Ayer, MA, USA).

The pastes were printed on flexible polyester (Hewlett Packard ink jet foil, 125µm) and polyimide (Kapton HN, 125µm, Müller GmbH) foils.

Analytical grade salts of ammonium chloride and sodium chloride were purchased from Bernd Kraft for standard calibration solutions.

Sodium chloride (NaCl, $\geq$ 99.5%, BioXtra), methanol 99.8% anhydrous) and polyvinyl butyral (PVB, Butvar® B-98) were purchased from Sigma Aldrich and used for an insulating layer on top of the reference electrode (Guinovart et al., 2013).

Ammonium ionophore (nonactin in Cocktail A, Fluka) was obtained from Sigma Aldrich for fabrication of the ion-selective electrode. Cocktail A consisted of Nonactin (6.9 wt%), 2-Nitrophenyl octyl ether (92.40 wt% NPOE) as plasticizer and potassium tetrakis(4-chlorophenyl)borate (0.7 wt% KTClPB).

For the insulating layer, a Barium titanate (PE-BT 101) paste from Conductive Compounds was used. All screen printing pastes offered annealing temperatures below 200°C. This is essential for sensor application on flexible foil substrates.

### 2.2 Equipment

An automated screen- and pattern printer from Ekra (series X1) was utilized to produce the printed electrodes. Polyester screen (110 µm mesh - 34 µm wire thickness x 22.5° cover angle, 10 µm -15 µm emulsion over mesh, EOM) was used for silver-silver chloride paste and a stainless steel screen (VA 270-0.036x22.5°, 5-10µm EOM) for silver- and carbon-filled paste.

The printed layers on foils were annealed at 150°C for different times (5 minutes up to 15 minutes) on a hot plate (PZ 28-2 EZ, Harry Gestigkeit). For potentiometric measurements a 2636B Sourcemeter from Keithley instruments (Cleveland, OH, USA) was used.

### 2.3 Screen-printing for Sensor Fabrication

The sensor design was adopted from two sensor layouts published by Koncki et al., (1999) and Guinovart et al., (2013). The schematic and a picture of the screen-printed sensor electrodes are shown in Figure 1. The layout of the sensor involves several steps and consists of a bielectrode system that combines silver working electrodes with silver-silver chloride electrodes as the reference electrode.

At first the conductive layer with silver-based paste (84% silver) was printed and afterwards the silver-silver chloride electrode. Koncki et al., (1999) proposed an additional carbon layer as a chemically inert layer between the ion-selective layer and the silver electrode. Guided by the same considerations we choose to realize the reference electrode both by a carbon/silver-based system as well as a pure silver-silver chloride electrode.

Finally, printing the insulating paste with contact openings for electrical contacts and opening at the active area with ion-selective membrane was done as fourth and last screen printing step.

The squeegee speeds during screen-printing of pastes were between 50 mm/s and 80 mm/s. After each printing step the flexible foil substrates were annealed at 150°C on a hotplate with annealing times between 5 minutes and 15 minutes. At the end of the printing process, the sensors were cut for further individual use.

For preparation of the measurements the reference electrode was covered with a mixture of PVB, methanol, and NaCl as was published by Guinovart et al., (2013). This process was done by dispensing the liquid by hand and letting the layer dry for a minimum of 12 hours. The final preparation step was the insertion of the ion-selective membrane. The cocktail of ammonium ionophore was drop-cast onto the inner circle (insulator opening, layer 4 in Figure 1a) with amounts of 20 µl and also dried overnight. The finished ammonium-selective biomedical sensor is shown in Figure 1b.

The additional silver electrodes shown left and right next to the sensor in Figure 1a can be used for conductivity measurements. The line gap was 100 µm (right) and 200 µm (left). The first generation of screen-printed ammonium-selective sensors had a dimension of 20 mm in width and 40 mm in length. The resolution pattern of the ink allows for shrinking the sensor size in future layouts.
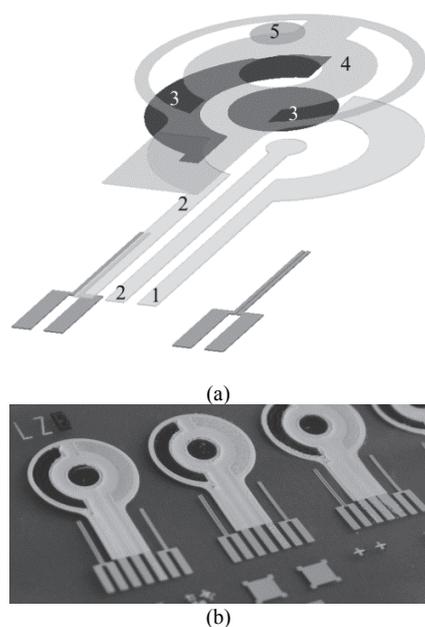
(a)



(b)

Figure 1: (a) Schematic and (b) photograph of screen-printed ammonium selective potentiometric sensor (1) silver-silver chloride reference electrode, (2) silver electrodes, (3) carbon-filled electrode, (4) insulating layer and (5) ion-selective area with ammonium ionophore.

## 2.4 Measurements

Calibration curves were obtained by standard solutions with different activities of the analyst ion. Potentiometric measurements were recorded using the high-impedance voltage measurement mode of the 2636B sourcemeter that delivers $10^{14}$ ohms of internal resistance, i.e. the measurement is current-less minimizing the feedback on the built-up potential difference at the electrodes. Data points were acquired with time intervals of 200 milliseconds (ms) or 500 ms respectively in a continuous data collection cycles of up to 1 hour in duration. The measurement schematic is depicted in Figure 2. The connection between flexible sensor and measurement equipment was realized via a flexible flat cable (FFC) connector with a 0.1 in. pitch.
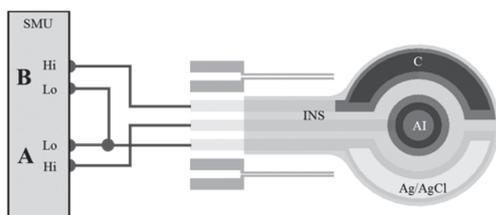


Figure 2: Schematic of cabling of potentiometric ion-sensitive sensor for measurement ammonium levels.

The ammonium ionophore, Nonactin, extracts its preferred cation, $NH_4^+$, out of the solution into the membrane. The potential difference measured at the device inputs is the sum of the potentials built-up at all interfaces: solid-solid, solid-liquid and liquid-liquid. In ideal case, all potentials except the potential at the ionophore membrane can be considered to be constant. The electromotive force, EMF is forming across the membrane when both reference and working electrodes are in contact with the solution. This potential over the ammonium-selective membrane depends on the $NH_4^+$ ion activity (Spichiger, 1998).

Potassium, $K^+$, is a critical interfered ion due to the same ionic diameter as of ammonium. The sensor selectivity will be tested in further work.

## 3 RESULTS AND DISCUSSION

### 3.1 Characterization of the Sensor

The potentiometric measurements of the ammonium-selective sensor were conducted by recording the voltage response upon modification of activity of the ammonium by exchanging standard solutions of concentrations from 0.1 mol/l (pC=1) up to 0.0001 mol/l (pC=4). The negative of the logarithm of base 10 of the ion activity ($a_{NH4+}$) is pC. A calibration of the sensor was realized by recording the potential deviation (electromotive force, EMF) versus the time and changing the activity of ammonium ions by adding drops of solutions on top of the sensor electrodes. After a delay time of 5 minutes, the first standard solution with pC=4 was added and measured for further 5 minutes. Afterwards the sensor was purged with deionized water and dried using nitrogen flow. This procedure was repeated for all standard solutions from pC=4 up to pC=1.

The range of the calibration concentrations from 0.00001 mol/l up to 0.1 mol/l covers the typical ammonium level in sweat with and without physical strain (Czarnowski et al., 1992; Guinovart et al., 2013). The curve progression of lower concentrations (pC values of 5 to 3 in Figure 3) is nonlinear. Between pC values of 3 and 1 the sensor shows a linear calibration function, that correlates with published calibration graphs of other groups (Koncki et al., 1999). The Nernstian slope in the linear range from pC values of 3 (0.001 M) to 1 (0.1 M) is 59.3 mV/pC ± 11.2 mV/pC.
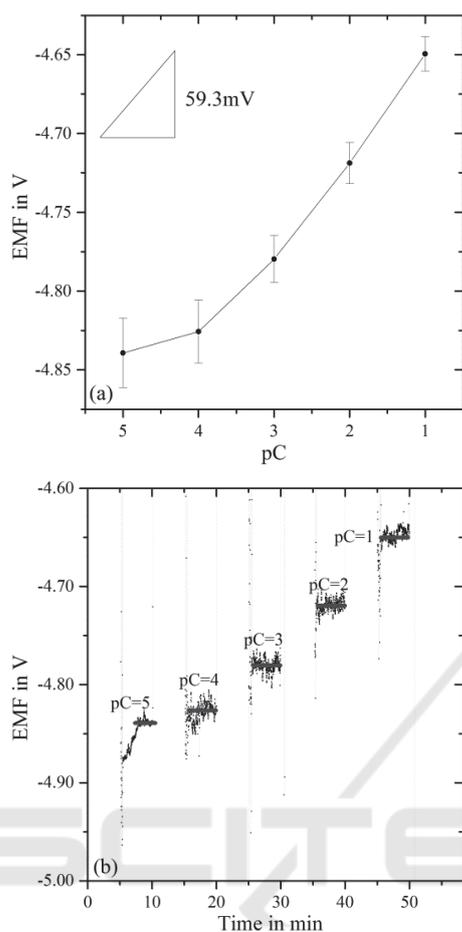
Figure 3: (a) Calibration graph and (b) potentiometric measurement (potential deviation, EMF in mV vs. changes of ion activity, $-\log(a_{NH4+})$) of screen-printed ammonium-selective biosensor with integrated screen-printed reference electrode.

The slope of an ideal electrode for the selectively-measured ion is given by the Nernstian equation:

$$E = E_0 + s \cdot \log(a_i) \tag{1}$$

$$s = 2.303 \frac{RT}{Fz_i} = \frac{59.16}{Fz_i} \, [mV] \text{ at } 25°C \tag{2}$$

with $E_0$ being the reference potential, s being the Nernstian slope, $a_i$ the activity of the ion i, R the universal gas constant, T the temperature, F the Faraday constant, and $z_i$ the valency of the ion i.

The performance of the sensor was achieved after considerable efford towards optimization of the integrity of the ion-selective membrane on the working electrode. Frequent defects include delamination, cracks or insufficient drying behavior and lead to dramatically changed sensing characteristics. Guinovart et al., (2013) fabricated an ammonium-selective sensor in a style of a skin tattoo with integrated PVB-based reference electrode. The range of the calibration concentrations from 0.00001 mol/l up to 0.1 mol/l is also used and the calculated Nernstian slope was 59.2 mV. Our approach of integrating the nonactin ionophor in a polymeric matrix lowers dramatically the consumption of the ionophor which is by far the most expensive ingredient of the sensor system. However we could show that the performance is kept at the same level.

In comparison to this work and the sensors published by Guinovart et al., (2013), Koncki et al. (1999) fabricated a screen-printed sensor which is measured against an external calomel reference electrode. The Nernstian slope of 53mV/pC was calculated in a range of the calibration concentrations from 0.00001 mol/l up to 0.01 mol/l. However the non-planar approach is less flexible with respect to sensor processing and system integration.

Our fastly and cheaply fabricated ammonium-selective screen-printed sensors show a high potential for analysis of physiological electrolytes as sweat in sports applications. In further work, the layout of the sensor will be shrinked. The resolution of the screen-printed silver pastes was better than 200 μm. The size minimization will on the one hand increase the amount of sensors yielded by one fabrication cycle as well as the spatial options for integration with functional sport textiles and will also reduce the materials and fabrication costs per sensor.

## 3.2 Integration with LokVitalTag

The printed sensor circuit will be attached to a multifunctional electronics box. The so called LokVitalTag combines various electronic modules that acquire data from the physiological sensor front ends (ECG-electrodes, respiration-band, ammonia-sensitive sensor front-end) and also tracks movement information from an inertial sensor. The gathered data will be processed and stored locally. Further on, the calculated values can be transmitted wirelessly. In addition the LokVitalTag carries electronics for the real-time-localization technology RedFIR 2.0, so localization information and vital parameters can be analysed in combination.

Under real conditions, in sweat, also interfering ions interact with the ionophore membrane. The effect on the overall potential can be related to the concentration of the analyte by applying the modified Nernstian equation, the so-called Nicolsky

equation. In further work, the selectivity factor will be calculated by this evaluation. The experimental selectivity coefficients in sweat are dependent on the method of determination, e.g. separate solution (SSM) or fixed interference method (FIM).

# 4 CONCLUSIONS

A facile route to the fabrication of planar, solid-state, ion-selective sensors for ammonium ions using screen-printing technology is presented. The performance of the fully-printed ion-selective sensor allows for the detection of ammonium ion concentration in the physiological levels of human sweat.

The results shown in this paper give the base for further research into especially the potentiometric analysis, the optimization of the sensor layout and area consumption, and the integration with textiles for wearable functional sport clothing.

# ACKNOWLEDGEMENTS

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