A wearable electrochemical sensor for the real-time measurement of sweat sodium concentration

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Abstract

We report a new easier method for the quantitative analysis of sodium in human sweat. To the best of our knowledge this is the first time this has been done successfully in a real-time manner. We consolidate sweat stimulation, collection and analysis functions into a single method. This temporal data opens up new possibilities in the study of human physiology, broadly applicable from assessing athletic performance and hydration levels to monitoring Cystic Fibrosis (CF) sufferers. Our compact Sodium Sensor Belt (SSB) consists of a sodium selective Ion Selective Electrode (ISE) integrated into a platform that can be interfaced with the human body during exercise. No skin cleaning regime or sweat storage technology is required as samples is continually wicked from skin to a sensing surface and on to a waste terminal via a fabric pump. After an initial equilibration period, a sodium plateau concentration was reached and monitored continuously. Atomic Absorption Spectroscopy (AAS) was used as a reference method, confirming accuracy. The plateau concentrations observed fell within expected literature ranges, further confirming accuracy. Daily calibration

repeatability (n=4) was ±3.0% RSD and over a three month period reproducibility was ±12.1% RSD (n=56). As a further application, we attempted to monitor the sweat of Cystic Fibrosis (CF) sufferers using the same device. We observed high sodium concentrations symptomatic of CF (~60mM Na^+) for 2 CF patients, with no conclusive results for the remaining patients due to their limited exercising capability. The real-time monitoring of hydration levels during physical exercise for health and performance purposes is a particularly promising application for the SSB at present.

Keywords

Sweat analysis, sodium in sweat, ion selective electrodes, cystic fibrosis, exercise, fluid replacement, dehydration.

Introduction

On a timescale of minutes and hours, the monitoring of sweat electrolyte concentrations can yield much information on the physical and chemical state of the human body. Blood plasma and sweat electrolyte concentrations are related. Low plasma water content (dehydration) and low sodium concentrations (hyponatremia) can result if these are not replaced effectively. These conditions can be detrimental to human health and lead to reduced physical and mental performance. Dehydration for example can manifest itself as an increased sodium concentrations in sweat during exercise, so the possibility of monitoring this quantitatively in real-time is an appealing prospect[1]. Currently, for studies involving athletic performance, instead of directly analysing specific electrolyte concentrations, available fluid volumes are usually inferred only from body weight loss and urine output volumes over a set period of exercise, with electrolyte concentrations only measured separately and retrospectively[2-5]. Measuring ion concentrations directly in bodily fluids such as sweat, inherently gives

information on both electrolyte volume and concentrations in plasma, and serves to indicate if these are in order. This approach is limited largely due to the unavailability of in-situ and/or real-time electrolyte measurement devices and methods. Such devices could be analogous to common heart rate monitors that are widely in use by amateurs and professionals alike. The formulation of specialist sports drinks (e.g. isotonic drinks) and their optimal use in terms of timing and quantity consumed around exercise, ties in with this area of research. Such drinks are generally based on water, carbohydrates and sodium (Na^+). Carbohydrates give energy but result in water loss whereas sodium facilitates the uptake of water[6, 7]. Sweat monitoring and analysis in a convenient and in situ manner could play a major role in this research.

Electrolyte levels in sweat including sodium (Na^+) levels vary considerably depending on the point of the body where they are measured. For example the forehead range was previously found to be as high as 56.7±28.9mmol/l Na^+ whereas the lower back typically gives half these values[8]. Additionally, sodium concentration varies widely depending on genetic predisposition, diet and heat acclimatization rate. Conversely, sex and aging do not appear to have a large effect on sweat electrolyte concentrations[1]. Interestingly, dietary salt intake and sweat rate do not specifically relate to sweat electrolyte concentrations[9]. Duplicate sentence: Furthermore there is no definite correlation between Sex, maturation and aging and sweat electrolyte concentrations[10, 11]. These factors underline the individual nature of absolute sweat electrolyte concentrations and coupled with exercise induced changes over a narrow timescale, make sweat a complex sample to analyse. We believe there remains a need for convenient devices for individualised use, yielding high density real-time data.

Clinical interest in sweat electrolyte analysis includes the diagnosis of Cystic Fibrosis (CF), indicated by abnormally high sodium levels in sweat[12]. In CF diagnosis, specific sweat electrolyte concentrations (usually Na^+ or Cl^-) are determined in a technically complex manner (outlined below) that is not in real-time. Because a specific concentration threshold is

available and a small number of analysis are needed for confirming CF in each patient, accuracy is of paramount importance. High frequency real-time sweat electrolyte data is therefore not a priority for clinical diagnosis of diseases. However, in the area of treatment (e.g. rehabilitative exercise for CF patients) and monitoring, such analytical tools may also be of great interest.

For measuring the concentration of specific electrolytes in sweat, there are 3 necessary steps regardless of approach. These are sweat stimulation, sweat collection and/or storage and finally analytical measurement.

Sweat stimulation – Stimulating sweat for subsequent chemical analysis is most commonly performed by methods based on the electrochemical Gibson and Cooke technique from the 1950s[12]. Pilocarpine iontophoresis is based on introducing a cholinergic drug into the skin via an electrical current, thereby stimulating artificial sweating. For non-clinical purposes, running or cycling exercises generate sweat. Controlled thermal (sauna) methods may also be used[13]. Each approach has advantages and disadvantages. Sweating is an intrinsic part of exercise and so is of minimum extra inconvenience and discomfort for an athlete. Conversely, a sufferer of CF can only exercise for a very short period due to his lungs and may experience severe discomfort. Sufficient sweat for analysis may not be produced. The pilocarpine technique is therefore most often used clinically. However, specialist equipment and training is required and in some instances discomfort ranging from skin reddening to burns have been reported[14].

Sweat collection and/or storage – This stage has by far the greatest impact on the accuracy of electrolyte analysis. Sweating helps regulate body temperature via the evaporation of sweat from the skin surface. It is this evaporation that can easily lead to falsely high electrolyte concentration readings and is the single biggest potential source of error in this field of research. Electrolyte concentration varies greatly with the sampling site on the body, so once

chosen, this must be cleaned from previous deposits and isolated from run-off sweat from other areas e.g. Sweat originating from the head, rolling down the back[8]. Once sweating has started, an absorbent material is sealed against a chosen area with an impermeable plastic backing to prevent vapour loses. The absorbent material may be a gauze or cellulosic material like in the sweat patch PharmChem^{TM} system[15]. Such patches either swab sweat briefly or can be left on to accumulate sweat over hours. The sweat collected is carefully weighed, extracted and diluted for analysis. Volume losses with paper and gauze methods can be as high as 2% per minute[9]. Alternatively a container containing a known amount of water is sealed against the skin and sweat is accumulated in solution over a known time. Sweat may also be collected from greater portions of the body by plastic sleeves covering an entire arm or armpit. Knowledge of whole body sweat electrolyte concentrations is a desirable measurement parameter instead of regional analysis, however sweat collection can be very inconvenient[16]. In one instance a box lined with plastic contains an exercise bicycle. All equipment and the subject are carefully washed before exercise. Following exercise, the box and equipment, clothes and the subject are again washed. In this way all electrolytes produced on the body are collected for analysis in a laborious fashion. The whole body approach is confined to very specialist applications. The Macroduct system goes some way towards eliminating evaporation losses and contamination by surrounding dried sweat[17]. In a previous study on CF conducted by our research group, iontophoresis on the wrist was followed by applying the macroduct system[18]. This consists of a coiled length of capillary tubing mounted on a wrist strap. A small orifice on the underside is held in place against the skin and sweat enters and mixes within the coil. Here it may stay until required for analysis with minimal evaporation losses. Due to the small collection orifice, contamination is minimised. One particular drawback of this and the other collection techniques mentioned above, as relevant to this paper, is that temporal data cannot be obtained conveniently.

Analytical measurement – Stored sweat samples are usually extracted and diluted quantitatively prior to analysis (indirect analysis). Sodium in sweat is mostly analysed by Flame Photometry techniques[9]. Another major technique used for indirect sodium analysis is based on Ion Selective Electrodes (ISEs)[19, 20]. Our own group measured sodium amongst other parameters in sweat using the Macroduct collector and a solid state ISE array and reference electrode[18]. Although the results were promising, sweat was measured after collecting a limited volume, meaning that replicate and temporal data can never be obtained in this way. Others have focussed on sodium's main counter ion in sweat, namely chloride using ISEs[21-23]. These methods as usual used iontophoresis for sweat stimulation with the analysis taking place at a later time. Invariably, evaporation was cited as the main error source regarding accuracy. Regarding precision, the availability of insufficient sweat volume caused the most variability and noise in the analytical signal, despite allowing a reasonable period for sweat accumulation. To date the US Cystic Fibrosis Foundation (CFF) has not approved ISEs for diagnosing CF, presumably due to the difficulties mentioned above. More recently, sodium quantification in sweat was carried out using Capillary Zone Electrophoresis (CZE)[15, 24]. Additional techniques for measuring sweat ions include titration based methods such as amperometric-coulometric determination of chloride using a chloridometer[25]. Conductivity and Osmolarity measurements have also been used[9]. Both are largely confined to screening in clinical applications and are not approved by the CFF. Wescor's sweat conductivity analyzer is one such technique of note[26]. Sweat was induced by 60 minutes of exercise and collected by closed-pouch collector. The analysis reflects total ionic content given in "NaCl equivalent" units (non-standard units). Due to the non-selective nature of these techniques, there is often positive systematic error. Although useful as a simple clinical screening method (for CF), it cannot yield quantitative specific analyte data in real-time.

To the best of our knowledge the only on-skin electrochemical probes for specific electrolytes commercially available are those for measuring pH such as the skincheck1™ (www.hannainst.co.uk). Our own research group has achieved some success with real-time monitoring of sweat pH in a wireless fashion[27]. In a similar study an attempt was made to monitor sodium in sweat wirelessly. Although compact solid state technology was in use, the sodium data (ISE based) revealed non-Nernstian slopes, severe drift and poor reproducibility. This is attributed to the fact that solid state ISE development is still ongoing (both working and reference electrodes) and has not reached the refinement of their classic inner solution based counterparts. Our research in this field continues[28-31].

We present our ISE based Sweat Sensor Belt (SSB) with which we aim to consolidate sweat collection and analysis conveniently in a single device and for the first time access real-time electrolyte data via continuous monitoring.

Experimental

Sweat Sensor Belt (SSB)

The Sodium Sensor Belt (SSB) consists of an impermeable plastic platform holding the ISEs and sweat wicking materials (pump), together with a potentiometer. The assembly is held against the subject's lower back during analysis by belt. The SSB is shown in Figure 1.

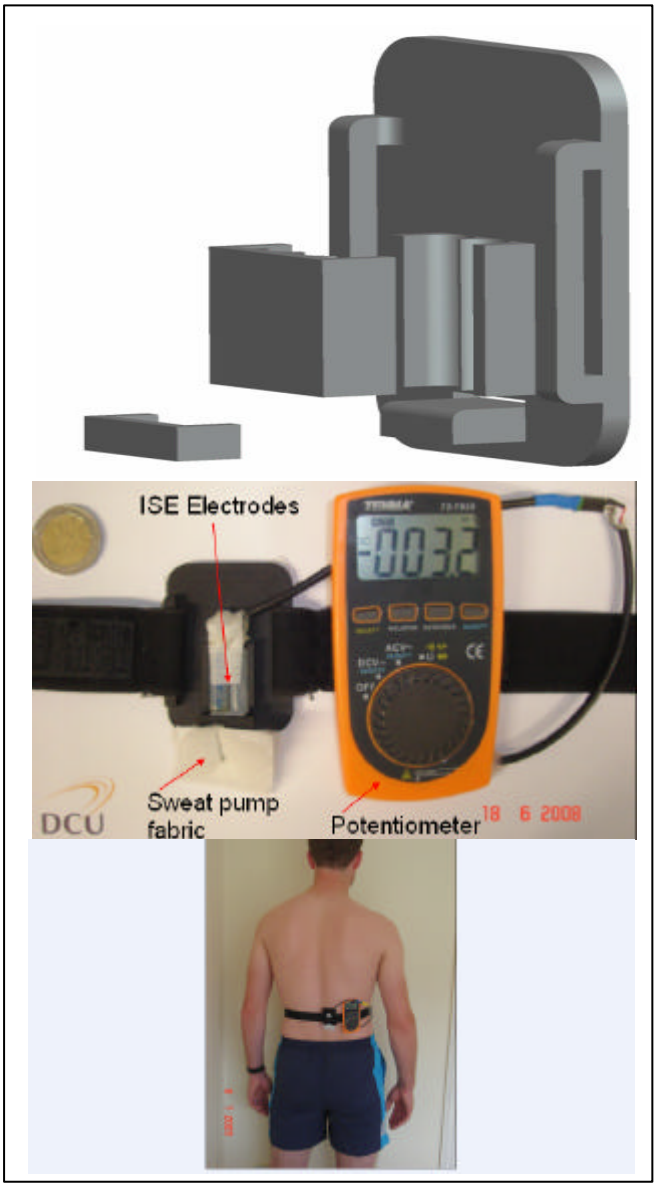

Figure 1. The Sodium Sensor Belt (SSB) showing (from top to bottom) a schematic of the impermeable holder platform with lower orifice, the entire sensor assembly showing potential read-out and the SSB mounted onto the lower back.

The platform consisted of impact grade acrylonitrile butadiene styrene (ABS) plastic platform fabricated via a prototyping 3D printer. This light, rigid yet rather porous plastic was rendered impermeable by immersion in dimethylketone for 15 minutes. The fluid handling system is based on the use of fabrics with inherent moisture wicking properties through

capillary action. A patch of polyimide/lycra® blend (www.sofileta.com), common in sportswear (from now referred to as just lycra), was glued to the reverse side of the platform for contact with human skin. This wicks fresh sweat produced through an orifice in the platform towards the sensor tips and finally into a terminal waste reservoir as shown in Figure 1. The reservoir material contains beads of Absorptex (www.smartex.ie) with a free swell capacity of 25g/g. A waste patch of 15×30mm was sufficient to absorb sweat/water for 3 hours. The platform served to press the patch against the skin to capture sweat efficiently as it emerges from skin pores and through the rim forming a seal against the skin, ensuring that only sweat from beneath the area of the platform was measured. This was necessary to prevent mixing of sweat on the back caused by sweat beads running down the subjects back. This arrangement also minimised evaporation losses prior to sensing.

The ISE electrodes were fabricated in-house using PVC tubing as barrels. The reference electrode was filled with 0.1M KCl on the day of analysis and the tip plugged with porous compressed foam or vycor 'thirsty glass' supplied by Biomedical Systems (code MF-2064). Electrodes were stored in appropriate filling solutions filling solution when not in use.

All chemicals were supplied by Sigma and were analytical grade. The electrodes were connected to a potentiometer (Tenma 72-7935) supplied by Farnell Ireland Ltd.

Potentiometric membranes were prepared using 250mg 2-Nitrophenyl octyl ether, 125mg PVC, 6.5mmol kg^{-1} 4-*tert*-Butylcalix[4]arenetetraacetic acid tetraethyl ester (Sigma 420484) and 2.7mmol Kg^{-1} potassium tetrakis(4-chlorophenyl) borate dissolved in dry THF and evaporated slowly. The well known calixarene ionophore was developed by members of our research group (ref). The electrochemical cell had the following arrangement:

Ag| AgCl| 0.1M KCl| saturated fabric patch| PVC membrane| 0.1M NaCl| AgCl| Ag.

Membranes were conditioned in 0.1M sodium chloride for 12 hours and deionised water for half an hour prior to ISE titrations. Two calibrations before and two after (total 4) were

carried out in bulk solutions prior to each exercise trial and the mean value used. This also ensured that the sensor was functioning properly. The Calibration range was based on standards 20, 40, 60, 80 and 100 mM NaCl and chosen to cover commonly encountered sodium concentration in sweat. Inter-conversion between sodium activity and concentration values was carried out according to Debye-Hückel equations.

Cycle exercise trials

The SSB was strapped to the lower back after calibration. Time and potential values were noted periodically during trials. The lycra sweat pump was dampened a little with de-ionised water to keep the ISE tips moist at the start of each trial and to give reasonably stable starting potentials.

A standard exercise bicycle was used. For the convenience and safety of the subjects who volunteered for the trials, the pace and duration of each trial was self-selected. Healthy subjects typically cycled for 1 hour. Sufferers of Cystic Fibrosis (CF) cycled for considerably less time.

AAS method

Pre-weighed 1cm^2 patches of lycra were soaked in either NaCl calibration standards (same solutions as used for the ISE calibration) or used to swab sweat samples from the lower back. The volume of liquid absorbed was estimated by re-weighing and assuming a density of 1.0g/ml. Extraction followed and involved shaking each patch twice in 20ml de-ionised water for 15 seconds. Following this, 10ml water was added to the combined extract. All solutions were analysed in triplicate according to the manufacturers' manual guidelines (Varian). A wavelength of 589.6nm was used.

Results and Discussion

The use of Ion Selective Electrodes (ISEs) is ideal for selectively measuring cation and anion concentrations in aqueous based samples including sweat. Our group has much experience with ISEs, from developing new ionophores to their application including environmental, industrial and physiological measurements[18, 32-36]. One of the most successful of these ionophores is a calix[4]arene based sodium ionophore (Sigma), which we use in the SSB.

In our original design of the sensor ISE, we realised that in conjunction with the PVC based working electrode, the reference electrode tip should provide efficient ionic conductivity between the inner filling solution and the passing sweat on the lycra patch with which the sensor makes contact. We initially used a compacted polyurethane foam plug to serve this function. The sensor response was excellent, showing Nernstian calibration slopes. However the bleed rate of inner filling solution when in contact with the lycra sweat pump was unacceptably high with all filling solution drained before a single exercise trial could be completed. Next we tried vycor glass tips. This is a type of porous glass sometimes referred to as 'thirsty glass'. Over a 3 month period of trials, no discernible liquid loss occurred from the reference electrode. The resulting calibration slopes were sub-Nernstian but constant at around 50mV per decade of sodium activity. Even after a 3 month period of sensor trials, Nernstian calibration slopes could be obtained when the vycor tips were exchanged for foam, demonstrating that the ISE still worked. Figure 2A shows an example of the calibration repeatability typically achieved, based on the four calibrations for a single trial.

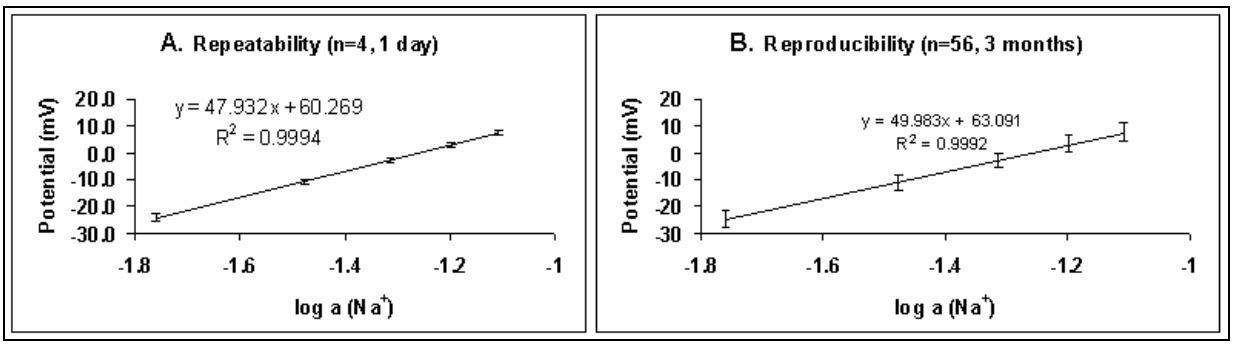

Figure 2. Precision in terms of repeat calibrations. A. Typical repeatability (n=4, 1 day). B. Reproducibility (n=56, 98 day period). a=activity.

To represent typical daily precision achievable, we take an intermediate sodium standard (40mM, or more accurately a Debye-Hückel based activity of 0.033) representing typical sweat sodium levels. For this standard, a mean potential of –10.8±0.8mV was obtained (n=4) as shown in Figure 2A. Given the slope of the line, this translates into a repeatability of 0.033±0.001 in terms of activity or a % relative standard deviation (% RSD) of 3.0%. The representation of activity is appropriate here to show that activity coefficients are considerably below unity when approaching a sodium chloride concentration of 0.1M. In terms of reproducibility over a 98 day period and a total of 56 calibrations, the mean absolute voltages and precision error bars are shown in Figure 2B. In this case, the sodium standard of activity 0.033 gave a mean potential of -11.1±2.7mV. This translates into a reproducibility of 0.033±0.004 or a % RSD of 12.1% (n=56). Clearly, the frequency and rigour of calibration must be chosen according to the required precision of any eventual sensor application (e.g. Obtaining absolute concentrations is analytically more demanding than monitoring concentration change). Good linearity was achieved, R^2 exceeding 0.99 in all cases. A darkening of the vycor reference tips was observed over time possibly due to bacterial growth. Contact with the sweat matrix possibly contributed to this. In future, an antibacterial agent could be added to the storage solutions.

Atomic Absorption Spectroscopy (AAS) was chosen as a reference and validation method to assess the performance of our SSB. Pre-weighed 1cm^2 patches of lycra were soaked in each of the NaCl calibration standards and re-weighed. Following an extraction and dilution procedure, the samples were analysed in triplicate by calibrated AAS to give the results shown in Table 1 below.

Standard (mM)	**Observed [Na] (mM)**	**% Recovery**
20.0	20.9	104.5
40.0	40.5	101.3
60.0	58.1	96.8
80.0	81.6	102.0
100.0	103.7	103.7

Table 1. AAS % recovery (accuracy) values as determined by the analysis of lycra patches soaked in the specified NaCl standard.

It can be seen that good accuracy is achieved with all results within a 95-105% accuracy specification. The same procedure was employed but this time sweat was swabbed directly onto lycra patches from exposed skin at intervals during a cycling trial. Samples were taken from the immediate vicinity of our belt mounted sensor on the lower back and immediately sealed into plastic containers to prevent evaporation and other sample perturbation. AAS analysis took place as soon as possible after a trial. Just prior to sample extraction for this, the same lycra patches were analysed on the bench by direct contact with the ISEs which were temporarily removed from the SSB. This served to directly compare our electrochemical sensor performance to the AAS reference measurements using real-life samples. The results of this exercise are shown in Table 2.

Trial time (minutes)	ISE [Na] (mM)	AAS [Na] (mM)
18	56.8	71.8
26	68.2	66.7
31	70.5	66.3
37	61.5	70.9
43	82.6	73.1
47	60.3	63.2
50	59.4	58.5
52	83.8	72.6
58	76.3	68.4
Mean	**68.8**	**67.9**

Table 2. Sodium concentrations measured from sweat swabs by both AAS and the ISE from the SSB during exercise trial.

It is apparent that the ISE and AAS results obtained are similar from Table 2. Furthermore a paired *t*-test suggests there is no significant difference between the two methods as the calculated t value is 0.301 and the probability of this result, assuming the null hypothesis, is 0.771 (n-1=8 degrees of freedom).

A total of 4 healthy and 4 subjects with CF took part in trials (all male). Useful data was only obtained from trials where the subject produced enough sweat, based on individual physiology and willingness to exercise with sufficient vigour for periods exceeding 30 minutes. The SSB was strapped to the subject's lower back. Potential values were noted from a digital read-out beside the sensor platform as shown in Figure 1. Typically, following the visual appearance of sweat beads on the subjects back, the potential rose until a concentration plateau was reached about 15 minutes after this. Figure 3 shows examples of sodium concentration change with time during cycling exercise trials.

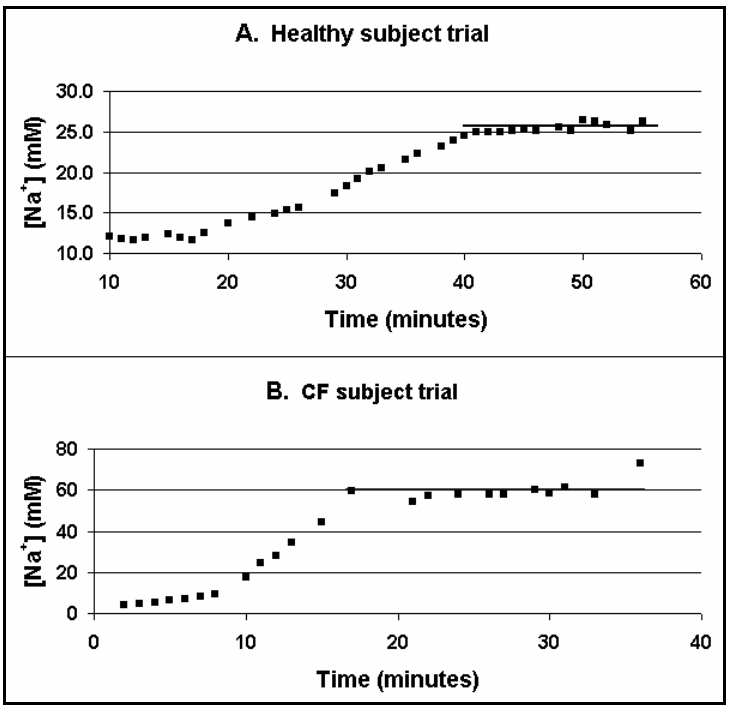

Figure 3. Cycle exercise trials showing rising sodium concentration with time. A concentration plateau is reached as indicated. Example of A. Healthy subject trial and B. CF subject trial.

For all full trials, the plateau sodium concentrations changed very little for the remaining duration of the trials. A maximum self-selected trial time of around 1 hour was typical for subjects of normal fitness. The constant nature of the sodium concentration at this stage suggested that the initial small quantity of de-ionised water (dampened lycra) was no longer causing a dilution effect. Secondly the plateau suggests that significant evaporation or sodium accumulation was not occurring and fresh sweat was being analysed as desired. This is bolstered by the fact that for all trials, concentrations fell within the range of other literature values for the lower back. Nimmo et al. state a mean of 26.2±19.4mM for lower back sodium concentration for cycling males[8]. Sawka et al. suggest 35mM as a universal mean of human sweat sodium[1]. Table 3 summarises the sodium plateau concentrations observed for all trials.

Subject	Trial end time (min)	Health	SSB Plateau $[Na^+]$ (mM)	AAS swab $[Na^+]$ (mM)
1	55	N	25.4±0.6	-
2	58	N	32.5±1.5	*68±5
3	60	N	18.0±1.2	-
4	60	N	24.8±1.2	72±5
5	36	CF	54.5±4.4	-
6	40	CF	61.9±12.5	284±71

N=healthy male CF=male with Cystic Fybrosis AAS swabs taken on bare skin beside SSB
*Based on AAS values from Table 2

Table 3. Cycle exercise trial results showing SSB plateau sodium concentrations. Proximal sweat swabs were analysed by AAS in some cases.

The high SSB sodium concentrations of around 60mM as observed for the CF subjects in Table 3 is in agreement with the literature, whereby concentrations around a diagnostic threshold of 60mM suggests CF prevalence[12]. The precision of SSB data is best for healthy subjects probably due to a higher number of plateau data points as longer trial times are possible. Only 2 of the 4 subjects with CF managed to complete a successful trial, and this trial is considerably shorter than for healthy subjects. The nature of CF prevents sufferers from performing physical exercise of the same duration and intensity as healthy individuals due to mucus build-up in the lungs which impairs intensive physical activity.

For all methods of sweat electrolyte analysis, the sweat collection and storage step is perhaps the greatest single source of error as sweat bead movement, regional intermixing and rapid evaporation cause large unwanted (for the analytical chemist) fluctuations in sample concentrations: A 2.5 hour marathon runner of 70kg, secretes about 2l of sweat per hour, 80% of the water evaporating (cooling function) and 20% drips away (wasted)[37]. An altered sweat sample means no useful correlation to physiological fluids like plasma can ever be made. The ultimate goal of sweat electrolyte analysis is after all to conveniently and accurately get a reflection of the bodies internal critical electrolyte concentrations. Our strategy of locally capturing and analysing these considerable sweat streams directly after excretion has considerable analytical potential. The results also seem to suggest that the

sweat pump appears to be effective at delivering fresh sweat sample past the sensor ISE tips and on to the absorptex waste compartment.

The AAS reference method, through the analysis of sweat swabbed directly from the bare skin demonstrates how vital a robust sampling strategy is. Although the AAS component of the analytical method was shown to be accurate (Table 1), the associated sampling approach was wholly inadequate. The quality of the AAS results was in sharp contrast to those obtained using the SSB. The AAS sodium concentrations in Table 3 are all much higher than the corresponding SSB values and unlike the SSB values, are outside reasonable ranges as suggested by the literature. These high values are most likely due to rapid evaporation of sweat leading to sodium accumulation on the skin. The poor precision of the AAS samples (Table 3) further reflects the ever-changing concentrations and suggest mixing of sweat from different body regions (dripping and rolling sweat) combined with the evaporation effects. The great variety and ongoing search of new sweat processing methods in the literature attest to the importance of a good sampling approach.

The reversibility of the SSB was investigated next. The SSB was mounted in the usual upright position on a waterproof surface. Sodium standards of varying concentration were delivered to the absorbent patch on the reverse side of the sensor by peristaltic pump. The flow rate was 17µl/min to approximately mimic the rate of excretion of sweat given the surface contact area of the sensor[1]. The standard supply was only removed from the SSB for line purging at each concentration change. The experiment was conducted over a 3 hour period, repeatedly cycling through the entire concentration range from 20-100mM NaCl. Figure 4 shows the resultant plot.

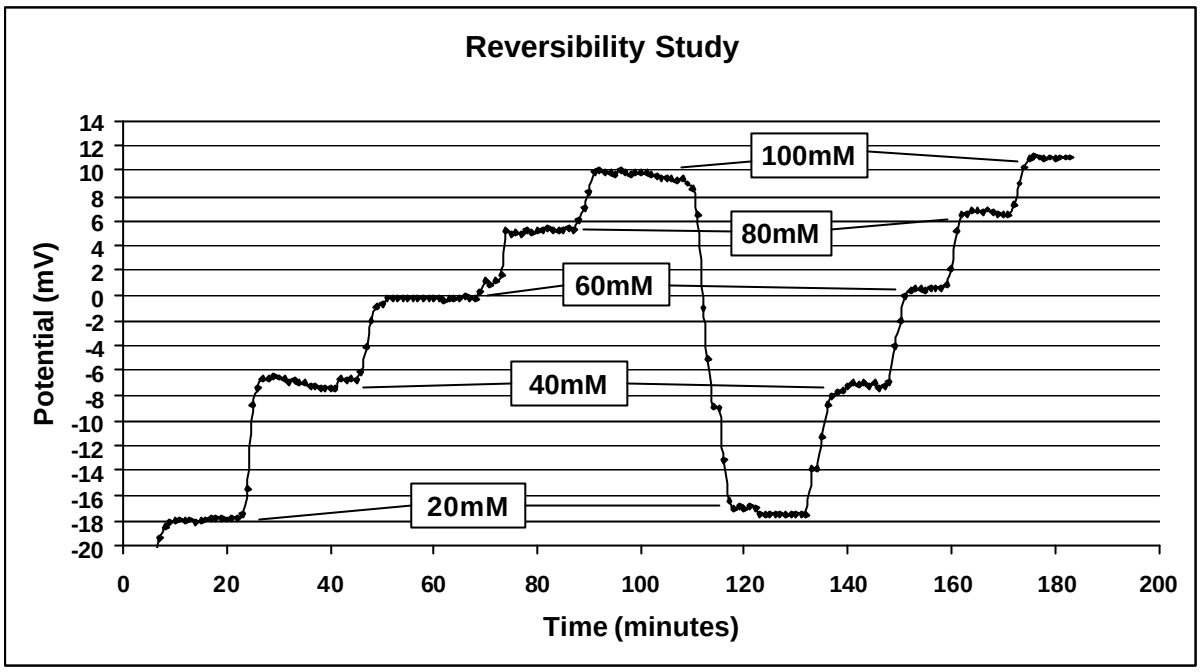

Figure 4. The reversibility of the SSB is proven over a 3 hour fluid collection period.

Even after a 3 hour period of continuous sweat pumping, good reversibility was observed with little evidence of hysteresis, sodium accumulation or evaporation within the analysis timeframe. The potential typically changes no more than ±1mV for repeat analysis of any concentration. The Absorptex waste terminal demonstrated considerable swelling suggesting an effective sweat wicking action. The range of concentration change and trial duration is lower in real-life trials proving the sensor has adequate reversibility.

We wished to find out whether the pressure with which the ISE tips were pushed against a sample surface made a difference to the analytical measurement. This matter is related to whether one can freely interchange between analysing a bulk solution and a solution soaked fabric patch. In light of assertions that pressure had to be controlled carefully,

Bray et al. reported that for volumes =1µl between skin and sensor, the pressure with which the electrode was pushed to the sample had no significant impact on the potential output. It was also revealed that authors who initially stated that the applied pressure was critical when

using ISEs, demonstrated that they had insufficient sweat volumes available for reliable readings[22]. Insufficient liquid would impair thorough sample contact with the sensor tips, hence introducing error. In using our ISE based sensor, we found that within a reasonable operational range, pressure made no difference to the analytical measurements. Consequently, our ISEs yielded the same absolute potential values for calibration standards whether placed in bulk solution or held against standard soaked lycra fabric.

In general, potential values observed were very stable, no doubt thanks in part to the luxuriously high sodium activities characteristic of sweat analysis (>0.01), activity levels at which all ISEs function very well. Indeed, state of the art ISE research is mainly concerned with obtaining reliable potentials many orders of magnitude below this.

What most analytical methods for sweat analysis have in common is that there is a considerable time delay between the emergence of sweat from the skin and its analysis. For any such approach, evaporation and other sample perturbation will always remain a major source of analytical error. The strategy of minimising the time delay between sweat emergence on the skin surface and analysis – analysing fresh sweat, inherently reduces error.

For clinical conditions, concentration thresholds and ranges are better defined then for healthy individuals. For example as relevant to our current study, sodium concentrations at or above 60mM Na^+ are indicative of CF disease[12]. It is only possible to assign an absolute value here because of the striking difference to 'normal' Na^+ levels in humans – it is approximately double typical healthy values. Interestingly, unlike in CF diagnosis, no absolute thresholds of sodium concentration (or other sweat ions) have been set indicating healthy electrolyte levels in the body or the onset of under (or over) hydration in humans and animals. The literature contains few 'recommended' or 'healthy' absolute electrolyte concentrations in sweat because as stated before, if using electrolytes in sweat to assess the state of other physiological fluids, the greatest variation occurs depending on the body area

where sweat is collected in addition to varying greatly from individual to individual for reasons that are not fully understood[8]. The former variable is dealt with to an extent by carefully sampling sweat from a specific area of the body (e.g. lower back). The issue of individuality clearly persists as shown by our study as well as others. Nimmo et al. measured sodium concentration on the lower back of 10 healthy males performing a similar cycling exercise routine as our subjects[8]. Results indicated sodium concentrations in the range 26.2±19.4mM, a variation of ±74%. The best strategy therefore may be to determine baseline or 'healthy' electrolyte levels for each individual. In terms of the SSB, this may correspond to the individual sodium plateau concentration. It is then the *deviations* from the individual plateau concentrations over time, which may serve for example as an indicator of hydration level. It is through high numbers of data points conveniently gathered in real-time, that we can begin to understand this aspect of physiology better.

Conclusions

Our exploratory analysis of sweat electrolytes via in-situ measurements using our SSB - in real-time - may open up new applications in research areas such as athletic performance and healthcare. Our exercise trials yielded absolute values of sodium concentrations in sweat, in agreement with literature ranges and verified using an AAS reference method. In future we would like to monitor the onset of dehydration, and for this exercise trials would have to be extended by perhaps X minutes at a Y level of exercise. Appropriate health safeguards and special consent from subjects would be required.

A large number of cations and anions can be selectively analysed according to the many ionophores available for use in ISEs. These could simply replace our current sodium selective ionophore or used as part of a sensor array. The miniaturisation and ongoing efforts to produce all solid state ISEs within our group will play a part in this work. Such

miniaturised solid state formats may be better suited for placement in closer proximity to the skin, reducing the delay time between excretion and analysis of sweat. In addition to inherently reducing sample evaporation and perturbation, it may render our sensor better for clinical diagnosis and monitoring such as in the case of CF, where only relatively short periods of exercise can be expected of patients and correspondingly lower sweat volumes are available for analysis.

In parallel, we may apply wireless sensing technology (e.g. Bluetooth) for improved data handling, similar to a wireless solid-state device for sweat pH analysis currently in testing.

Our goal then is to build a compact sensor for the analysis of sweat electrolyte concentrations. The data generated should be of high temporal resolution, recorded conveniently and autonomously, resulting in truly individualised applications.

References

(1) Sawka, M. N.; Burke, L. M.; Eichner, E. R.; Maughan, R. J.; Montain, S. J.; Stachenfeld, N. S. *Medicine and Science in Sports and Exercise* **2007**, *39*, 377-390.
(2) Maughan, R. J.; Shirreffs, S. M. *Science & Sports* **2004**, *19*, 234-238.
(3) Yoshida, T.; Takanishi, T.; Nakai, S.; Yorimoto, A.; Morimoto, T. *European Journal of Applied Physiology* **2002**, *87*, 529-534.
(4) Sanders, B.; Noakes, T. D.; Dennis, S. C. *European Journal of Applied Physiology* **1999**, *80*, 318-323.
(5) Maughan, R. J.; Shirreffs, S. M. *Journal of Sports Sciences* **1997**, *15*, 297-303.
(6) von Duvillard, S. P.; Braun, W. A.; Markofski, M.; Beneke, R.; Leithauser, R. *Nutrition* **2004**, *20*, 651-656.
(7) Brouns, F.; Saris, W.; Schneider, H. *International Journal of Sport Nutrition* **1992**, *2*, 229-238.
(8) Patterson, M. J.; Galloway, S. D. R.; Nimmo, M. A. *Experimental Physiology* **2000**, *85*, 869-875.
(9) Constantinescu, M.; Hilman, B. C. *Laboratory Medicine* **1996**, *27*, 472-477.
(10) Meyer, F.; Baror, O.; Macdougall, D.; Heigenhauser, G. J. F. *Medicine and Science in Sports and Exercise* **1992**, *24*, 776-781.
(11) Morimoto, T.; Slabocho.Z; Naman, R. K.; Sargent, F. *Journal of Applied Physiology* **1967**, *22*, 526-&.
(12) Gibson, L. E.; Cooke, R. E. *Pediatrics* **1959**, *23*, 545-549.
(13) Amatruda, T. T.; Welt, L. G. *Journal of Applied Physiology* **1953**, *5*, 759-772.
(14) Kirk, J. M. *Archives of Disease in Childhood* **2000**, *82*, 425-427.

(15) Appenzeller, B. M. R.; Schummer, C.; Rodrigues, S. B.; Wennig, R. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2007**, *852*, 333-337.
(16) Shirreffs, S. M.; Maughan, R. J. *Journal of Applied Physiology* **1997**, *82*, 336-341.
(17) Miller, M. E.; Cosgriff, J. M.; Schwartz, R. H. *Clinical Chemistry* **1985**, *31*, 1715-1716.
(18) Lynch, A.; Diamond, D.; Leader, M. *Analyst* **2000**, *125*, 2264-2267.
(19) Barbour, H. M. *Annals of Clinical Biochemistry* **1991**, *28*, 150-154.
(20) Northall, H.; York, G. A. *British Journal of Biomedical Science* **1995**, *52*, 68-70.
(21) Szabo, L.; Kenny, M. A.; Lee, W. *Clinical Chemistry* **1973**, *19*, 727-730.
(22) Bray, P. T.; Clark, G. C. F.; Moody, G. J.; Thomas, J. D. R. *Clinica Chimica Acta* **1977**, *80*, 333-338.
(23) Bray, P. T.; Clark, G. C. F.; Moody, G. J.; Thomas, J. D. R. *Clinica Chimica Acta* **1977**, *77*, 69-76.
(24) Hirokawa, T.; Okamoto, H.; Gosyo, Y.; Tsuda, T.; Timerbaev, A. R. *Analytica Chimica Acta* **2007**, *581*, 83-88.
(25) Legrys, V. A.; Burnett, R. W. *Archives of Pathology & Laboratory Medicine* **1994**, *118*, 865-867.
(26) Boisvert, P.; Candas, V. *European Journal of Applied Physiology and Occupational Physiology* **1994**, *69*, 176-178.
(27) Morris, D.; Coyle, S.; Wu, Y. Z.; Lau, K. T.; Wallace, G.; Diamond, D. *Sensors and Actuators B-Chemical* **2009**, *139*, 231-236.
(28) Desmond, D.; Lane, B.; Alderman, J.; Glennon, J. D.; Diamond, D.; Arrigan, D. W. M. *Sensors and Actuators B-Chemical* **1997**, *44*, 389-396.
(29) McGraw, C. M.; Radu, T.; Radu, A.; Diamond, D. *Electroanalysis* **2008**, *20*, 340-346.
(30) Chumbimuni-Torres, K. Y.; Rubinova, N.; Radu, A.; Kubota, L. T.; Bakker, E. *Analytical Chemistry* **2006**, *78*, 1318-1322.
(31) Sutter, J.; Radu, A.; Peper, S.; Bakker, E.; Pretsch, E. *Analytica Chimica Acta* **2004**, *523*, 53-59.
(32) Cadogan, A. M.; Diamond, D.; Smyth, M. R.; Deasy, M.; McKervey, M. A.; Harris, S. J. *Analyst* **1989**, *114*, 1551-1554.
(33) Diamond, D.; Svehla, G.; Seward, E. M.; McKervey, M. A. *Analytica Chimica Acta* **1988**, *204*, 223-231.
(34) Diamond, D.; McKervey, M. A. *Chemical Society Reviews* **1996**, *25*, 15-&.
(35) Diamond, D.; Nolan, K. *Analytical Chemistry* **2001**, *73*, 22A-29A.
(36) Oconnor, K. M.; Arrigan, D. W. M.; Svehla, G. *Electroanalysis* **1995**, *7*, 205-215.
(37) Maughan, R. J. *International Journal of Sports Medicine* **1992**, *13*, S132-S135.