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Rafael Hoekstra, Pascal Blondeau, Francisco J. Andrade\*

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Department of Analytical Chemistry and Organic Chemistry. Universitat Rovira i Virgili (URV), Campus Sescelades, c/.  
Marcel·lí Domingo, 1, Tarragona, 43007, Spain.

rafael.hoekstra@urv.cat

pascal.blondeau@urv.cat

\* Corresponding author: [franciscojavier.andrade@urv.cat](mailto:franciscojavier.andrade@urv.cat)

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# IonSens: a wearable potentiometric sensor patch for monitoring total ion content in sweat

Rafael Hoekstra, Pascal Blondeau, Francisco J. Andrade\*

Department of Analytical Chemistry and Organic Chemistry. Universitat Rovira i Virgili (URV), Campus Sescelades, c/.  
Marcel·lí Domingo, 1, Tarragona, 43007, Spain.

\* e-mail: Francisco Andrade franciscojavier.andrade@urv.cat

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## Abstract

A sensor for monitoring total ion activity is described, and its performance as a wearable device for monitoring the total ion levels of sweat is evaluated. The sensor works by tracking changes in the Donnan potential generated across a Nafion® membrane. This cation-exchange polymer was cast on a paper coated with carbon-ink, making the platform elegantly simple. Analytical parameters during calibration in aqueous solution include a sensitivity of  $56.3 \pm 1.0$  mV /dec.a(Na<sup>+</sup>) and a standard deviation between standard electrode potentials of 5.3 mV (N = 5) for first time use. By integrating a paper-based pseudo-reference electrode, a miniature disposable electrochemical cell (the “IonSens” device) was created and demonstrated as a wearable sensor. Potentiometric measurements estimating the total ion activities were validated against conductivity measurements. Recoveries of eleven raw sweat samples were determined to be  $95.2 \pm 6.6$  % (n = 3). The perspiration conductivity profile of an athlete during exercise was monitored in real-time and visualized on a mobile phone application connected via Bluetooth®. The excellent reproducibility of the electrode without any conditioning is noteworthy and lends itself to applications including -but not limited to- the monitoring of total ion activity in sweat.

## Highlights

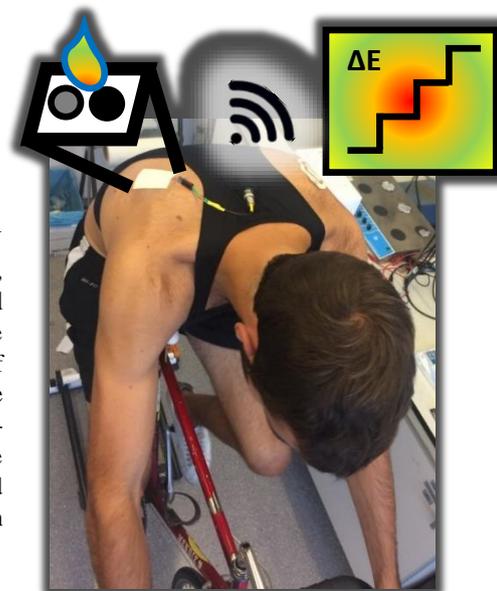
**Keywords:** conductivity, ion-exchange membrane, solid contact potentiometry, wearable sensor

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## 1. Introduction

The development of body sensing networks [1] using wearable sensors to monitor physiological parameters in real time and in real settings is becoming possible due to the exponential progress in electronics and communication technologies. These platforms may have a significant impact in areas such as medical care [2], sports performance [2], homeland security, etc. Today, a wide range of devices that can monitor and wirelessly transmit physical parameters (heart rate, body temperature, movements, etc.) are available. Tools to generate chemical and biochemical information have not progressed at the same pace, creating an increasing gap that is fueling the interest for the development of wearable chemical and

biochemical sensors. For this reason, since the pioneering works of Diamond *et al.* and Wang *et al.* this topic has been receiving a continuously increasing amount of attention in analytical chemistry [1-5]. The challenge is multifaceted, since wearable devices must work under very stringent dynamic conditions- including mechanical stresses, vibrations, temperature changes, etc.- and must adapt to the end-users needs without interfering in their routines. Given these constraints, the analytical problem must be reframed beyond the traditional performance-focused parameters, in order to include other equally relevant factors such as simplicity of operation, low power consumption, robustness, size, ergonomics and cost. The need to simultaneously meet all these requirements may offset the advantages of many well-established lab-based



techniques. Therefore, the search for alternative detection approaches is an essential task in order to develop truly wearable (bio)chemical systems.

A good example of the complexity of this challenge is the analysis of sweat, a biological fluid that provides opportunities for the non-invasive monitoring of physiological information [5–7]. Electrolyte levels in sweat can be correlated with physical activity, physiological processes and some health-related issues, such as cystic fibrosis. Traditional lab-based methods require sampling sweat using absorbing pads, a step that is not free from problems (contamination, evaporation and changes on sample composition, etc.) and that stresses the advantages of using wearable devices. Total electrolyte levels are usually estimated by monitoring the sweat conductivity due to the overwhelming simplicity of this technique. However, many of these advantages vanish when dealing with wearable systems. Conductimetric measurements strongly depend on the cell geometry (electrode distance, spatial arrangement, shape and size), a factor that is easy to control in a lab, but that is harder to maintain in a wearable, flexible environment. To overcome this problem, a miniaturized, rigid wearable conductivity cell, as reported by Liu *et al.* for the real-time monitoring of athletes' sweat during exercise [6], can be employed. While this solution is effective, it requires coupling to a sweat pumping and transport system. This increases the complexity and reduces the robustness of the overall approach. Clearly, wearability favors the use of detection schemes that are more impervious to these geometrical constraints.

Colorimetric systems have been widely explored [8, 9], but electrochemical detection provides significant advantages. In particular, potentiometric sensors have shown significant benefits for the development of wearable devices, due to their simplicity of instrumentation and operation, robustness, low power consumption and ease of miniaturization. Potentiometry displays a great deal of versatility in the design of cells, thus allowing the construction of patches [10], yarns [11], band aids [12] and even tattoos [13] for the detection of different kind of ions. Multi-ion detection [5, 14–16] and more recently- the successful development of flexible potentiometric sensors [17–19] emphasize the importance of this technique in the field of wearable devices.

It is worth mentioning that despite being a century-old technique, periodical waves of innovation have renewed the interest on potentiometric sensors. From the introduction of polymer-based electrodes in 1967 [20, 21], to the *silent revolution* of the solid-state technology in the 1990s [22]- invigorated later by the use of nanomaterials [23]- the past decade has seen creative advances in the use of novel substrates such as paper [24, 25], rubber [26], cotton [11], commercial carbon fibres [10], and plastics such as polyethylene terephthalate (PET) [5] to build potentiometric sensors. This wide range of substrates has

increased the versatility of potentiometry across various applications.

The range of polymeric materials used in sensing membranes has also been expanding -the most widely-used being plasticized PVC [22]. While this material has proven to be highly versatile, limitations remain such as poor control over pink noise or  $1/f$  noise, high impedance, issues with water layer formation, and the high desolvation energy required for recognition of hydrophilic anions. Furthermore, these membranes are prone to fouling by biomolecules and cells, which often causes issues in analysis in biological media [27]. Because of these limitations, significant research has been devoted to find alternative polymeric substrates, such as polyurethane [18], conductive polymers such as PANI [12] and polypyrrole [28], and ion-exchange polymers such as Nafion® (from now on simply Nafion) [29–31]. Heng and Hall have also explored 'self-plasticizing' acrylates which obviate the addition of toxic plasticizers and avoid their leaching [32], and Poplawski *et al.* have reported the use of silicone rubber without plasticizer for Na<sup>+</sup>-ISEs [33]. More recently, Bakker *et al.* have described the use of sparingly-selective ion-exchange Donnan exclusion membranes. Their work included studies of Nafion- the sulfonated tetrafluoroethylene based fluoropolymer-copolymer- using commercially available membranes and inner-solution electrodes. Interestingly, they emphasized that selectivity should be less dependent on the ion lipophilicity (as described by the Hoffmeister series), and instead depend primarily on coulombic interactions [31]. Michalska *et al.* reported solid-contact permselective (Nafion) membranes upon conducting polymers (poly(pyrrole)) as early as 2001 [34]. The cationic responses they observed were limited to electrolyte concentrations below 1 mM, which they acknowledged as limiting its applied utility.

Nafion membranes have been extensively used in electrochemical sensors, particularly in voltammetric and amperometric detection. It has been shown that this negatively charged fluoropolymer acts as an effective permselective barrier against anions, reduces the biofouling and improves the stability of the system. More recently, the successful use of Nafion membranes in potentiometric biosensors has been also demonstrated [30]. In most of these cases, Nafion was used to improve the performance of the detection, but was not directly involved in the detection of substances. Nevertheless, in light of recent reports, this material offers a good opportunity to develop a potentiometric system that responds generically to the overall activity of the ions dissolved in solution.

This work aims to introduce, optimize and validate a wearable potentiometric sensor for monitoring the total ion activity in sweat. First, a disposable solid-contact potentiometric sensor that responds to the total electrolyte levels is presented. This sensor is built by casting a Nafion

membrane on a paper substrate coated with a carbon ink. Due to the nature of Nafion as an ion-exchange membrane, this system is shown to respond in a sparingly-selective way to different types of ions, as has already been reported for similar electrodes with inner-solutions [31]. The performance of the working electrode to sodium (the predominant cation in sweat) is then expanded to other monocations, before extending the media to an artificial sweat solution of mixed electrolytes. Analytical parameters for different type of ions show a Nernstian response with very little difference between cations. To validate this result, the potentiometric response is compared to that obtained with a lab conductimeter. Thereafter, the system is integrated with a pseudo-reference electrode in the form of a wearable patch and applied to the direct monitoring of total electrolyte levels in sweat during exercise. The results show that the system can be used as an alternative approach to conductivity, with advantages in overall simplicity and flexibility of form factor.

## 2. Material and methods

### 2.1. Reagents

All solutions were prepared with doubly distilled water (18.2 M $\Omega$  cm) from a Milli-Q water system (Millipore Corporation, Bedford, MA). Artificial sweat was composed following a minor modification of European Standard number EN1811:2012 by dissolving 0.5 % w/v NaCl (85 mM), 0.1 % w/v KCl (13 mM), 0.1 % w/v urea (16 mM) and 0.1 % w/v sodium lactate (11.1 mM) into doubly distilled water, and adjusting the pH to  $5.4 \pm 0.3$  with HCl (100 mM). Membranes were cast from Nafion® 117 of circa 5% in a mixture of lower aliphatic alcohols and water. These analytical-grade salts, as well as the Nafion and methanol were obtained from Sigma-Aldrich. Polyvinyl butyral (PVB) B-98 was purchased from Quimidroga S.A. (Barcelona, Spain).

### 2.2. Instrumentation

Electromotive force (EMF) was monitored with a high input impedance ( $10^{15}$   $\Omega$ ) EMF16 multichannel data acquisition device from Lawson Laboratories, Inc. (Malvern, USA). When performed in the lab, EMF measurements were recorded in stirred solutions at room temperature ( $26 \pm 4$  °C) while making successive additions of analyte unless otherwise stated. Double-junction 3 M Ag/AgCl/KCl (type 6.0726.100) reference electrodes from Metrohm AG containing 1 M LiOAc as ionic salt bridges were used for potentiometric measurements unless otherwise stated.

A conductivity meter (GLP 32, Crison Instruments S.A., Spain) was used to calibrate and validate conductivity measurements.

The *Go Wireless* Electrode Amplifier by Vernier Software and Technology (OR, USA) was used to make wireless measurements with the wearable device for on-body measurements. Data was sent via a Bluetooth connection to a mobile phone running the Vernier Android app.

### 2.3. Electrode construction

Number 5 Whatman™ filter papers (GE Healthcare Life Sciences) were painted with screen-printable electrically conductive ink (122-49, Creative Materials, Inc., MA, USA) to form the carbon conductive substrate of working electrodes and with Ag/AgCl ink (113 – 09, Creative Materials, Inc., MA, USA) for reference electrodes. Platinum and gold papers were made by sputtering a Number 5 filter paper with a 100 nm layer of the metal using an ATC Orion 8-HV (AJA International Inc., MA, USA) at 150 W, 3 mTorr, 20 sccm Argon as RF magnetron source in the case of platinum, or a sputtercoater (K575XD, Emitech, France) at 30 mA for 12.5 minutes in the case of gold.

In a method similar to that previously reported [35], the electrodes were encased within a 1.5 x 2 cm mask of polyester (ARCare 8259, Adhesives Research, Inc., PA, USA) with circular apertures of 2.1 mm and 2.9 mm diameter exposed for deposition of working and reference electrodes respectively (Figure 1). This orifice exposed the electrochemically active surface of the electrode. The working electrode was completed by drop-casting 10  $\mu$ L of Nafion in 5 equal portions, allowing for evaporation of solvent between additions. Adapting the work previously published by our group [25, 36], the reference electrode was completed by adding some 1-3 mg of finely ground NaCl crystals to the Ag/AgCl surface before drop-casting 30  $\mu$ L of the reference cocktail (10 mg NaCl, 120 mg PVB, 1 mL MeOH), in three equal aliquots, allowing for evaporation of the bulk of the solvent between additions. These membranes were dried overnight at 4 °C.

For experiments in which a conventional double-junction reference electrode was used, no conditioning of the membranes was performed. However, when the PVB pseudo-reference electrode was integrated, both electrodes were conditioned by immersion in a stirred solution of 1 M NaCl for at least 3 hours followed by rinsing with artificial sweat solution diluted 1:10 for 5 minutes.

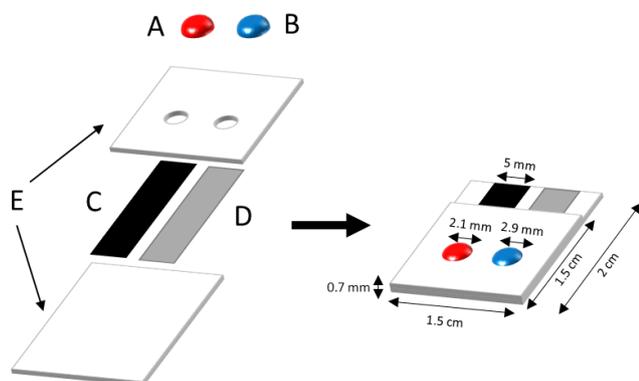


Figure 1. Illustration of the elements comprising the IonSens device. A Nafion membrane (A) and reference membrane (B) were drop-cast over carbon paper (C) and Ag/AgCl paper (D) respectively. These papers were protected by a mask (E).

## 2.4. On-body measurements

For on-body dynamic measurements, a polycarbonate membrane (reference: PC 100 47 TL, Albet) filter was applied in order to avoid direct contact between the membranes and the skin while allowing permeation of sweat. Connection to the wireless instrument was made with copper wires attached to the conductive papers with conductive epoxy (CW2400, Circuitworks). All exposed connections were insulated and waterproofed by wrapping in teflon tape, cotton padding and electrical insulation tape. The sensor was affixed to the skin on the shoulder beneath a Hansaplast Aquaprotect XXL sterile plaster (8 x 10 cm). Data was collected and visually displayed upon a mobile phone running the Android version of the Vernier Graphical Analysis app, version 3.1.1 (build 8526) (Vernier Software & Technology).

## 3. Results and discussion

### 3.1. Analytical performance of solid-contact ion-sensitive electrode based on Nafion

The response of Nafion to different type of ions has already been described using inner-solution electrodes [31]. This approach clearly cannot be implemented in wearable systems. Therefore, the first step of this research is focused on finding a suitable strategy to build and characterize solid-contact, flexible and ideally disposable electrodes. To this end, paper was used as a substrate and different types of coating materials- namely, platinum, gold and carbon- were used for testing. The metals were sputtered on the paper, while the carbon was applied in the form of an ink, and the Nafion membranes were cast on the electrochemically active window as described above (Figure 1). As a first approach, considering the predominant component of sweat, the responses of these

electrodes to NaCl were evaluated. The corresponding time-trace and calibration plot are displayed in Figure 2 and the analytical parameters summarised in Table 1 (as well as in S1 and S2). For clarity, the first 15 minutes are not shown in the time trace as no appreciable change in EMF took place, (they are included in the supporting information).

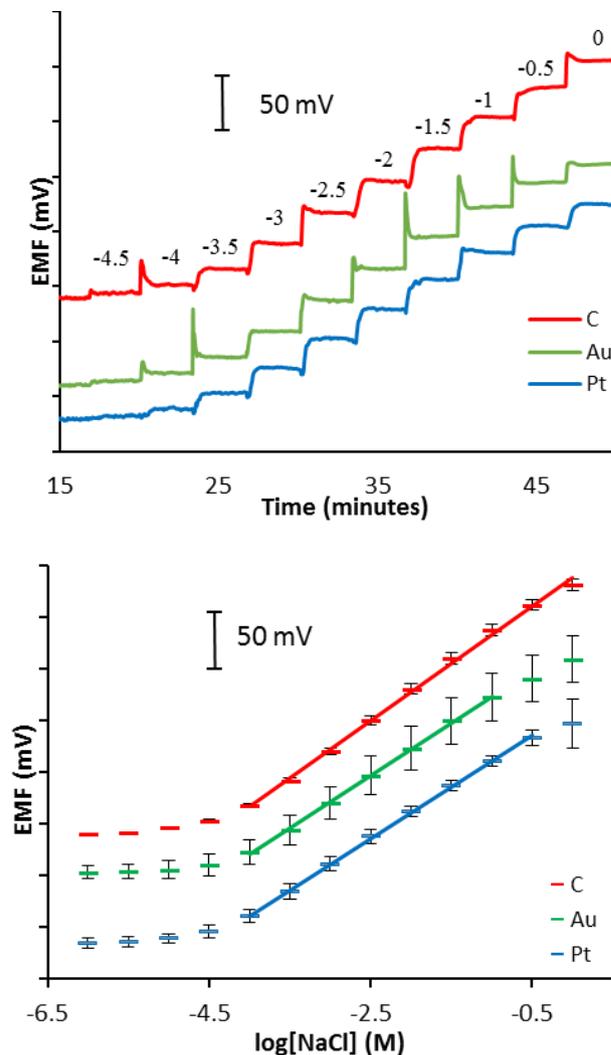


Figure 2. Time trace (above) and calibration plot (below) comparing response of working electrodes based on carbon, gold and platinum papers.

Once the electrodes are initially immersed, the signal rapidly rises to reach a steady-state. At a low ionic strength such as 0.1 mM, the signal will take approximately 10 minutes to stabilize; for stronger tonicities, the signal will stabilize within a couple of minutes. As the membrane is hydrophilic, the electrode can be removed from solution and reused shortly thereafter without the need to wait for rehydration.

After this initial time, the electrodes remain remarkably stable, with a resting potential that depends on the electrode material, and that responds to the mixed potential of the system, modulated by the Nafion membrane.

The comparison of the sensitivities shows that for each one of the substrates an approximately Nernstian response to addition of sodium was observed. This is consistent with the results already reported, that attribute this trend to the change in the Donnan potential of the membrane [31]. Although electrodes upon all substrates tested showed somewhat similar performance, those upon carbon were selected to continue this study. Firstly, this was because this material displayed the greatest upper limit of linear range, which is desirable for applications in undiluted biological fluids such as sweat, whose composition easily reaches a sub-molar range. Additionally, of the three, carbon is known to be insensitive to redox active molecules, which are known to be present in biological fluids. Furthermore, it has been already shown that metal coatings on flexible substrates are more sensitive to cracking upon mechanical stress, leading to fissures that severely affect the electrical characteristics of the material. Last– but not least– carbon is a very low-cost substrate and it can be applied by direct printing, simplifying the potential mass manufacturing of the sensors. For all these reasons, carbon ink was selected as the conductive substrate of choice.

Table 1. Comparison of key analytical parameters between the three substrates ( $N = 3$ ).

Substrate	C	Au	Pt
Sensitivity (mV/dec)	$55.3 \pm 1.0$	$51.1 \pm 4.6$	$49.8 \pm 0.2$
Standard potential (mV)	$588.2 \pm 6.0$	$524.5 \pm 28.3$	$510.2 \pm 5.6$
Linear range (mM)	0.1 – 1000	0.1 - 100	0.1 – 316
Limit of detection (uM)	$48.3 \pm 5.0$	$55.0 \pm 4.1$	$40.3 \pm 5.1$
Response time (s)	$17 \pm 6$	$22 \pm 14$	$18 \pm 6$

An important factor affecting the usability of a wearable device is the simplicity of its operation, which in the case of chemical sensors finds in the calibration step, a major challenge. Potentiometry offers very attractive features, since the highly reproducible Nernstian sensitivity (as shown above) reduces the problem to the stabilization of the standard potential. It has been shown that single-point calibrations can be successfully applied [37]. More recent works on the stabilization of the standard potential by chemical [38] or instrumental [39] approaches have been closing the gap towards the development of truly calibration-free potentiometric sensors. The instrumental approach proposed by Bobacka *et al.* has been recently explored by our group for the development of wearable systems [10].

The Nafion-coated carbon ink electrodes developed in this work show a decent reproducibility of the standard potential, of  $\pm 6$  mV ( $n = 5$ ) as shown in Table 1. This may

be due in part to the mechanism of response of the electrode and the particular structure of Nafion. This polymer contains a lipophilic teflon ( $-\text{CF}_2\text{CF}_3$ ) group, which has been reported to adhere to metallic (in this case, carbon) surfaces. On the other hand, the hydrophilic sulfonate groups ( $-\text{SO}_3^-$ ) have very strong interactions with water molecules. The structure and properties of Nafion are not yet fully understood and they continue to receive extensive investigation. It is accepted, however, that hydrophilic domains formed by the sulfonate groups create nano-channels that strongly interact with water. It is also understood that the aqueous phase partially percolates through the porous nanostructures into the membrane. The activity of water in the membrane, as well as the concentration of oxygenated species that may lead to the electrode resting potential through a mixed potential mechanism are also modulated by the Nafion membrane. All these factors may contribute to the stabilization of the response that is observed when this polymer is used. From a practical point of view, these results encourage the development of calibration-free, wearable sensors.

The mechanism of generation of the Donnan potential in this type of membranes is well known and has been extensively described in the literature [40, 41]. Briefly, taking into account the ion-exchange between monovalent cations, Figure 3 illustrates this process and the potential drops at each interface.

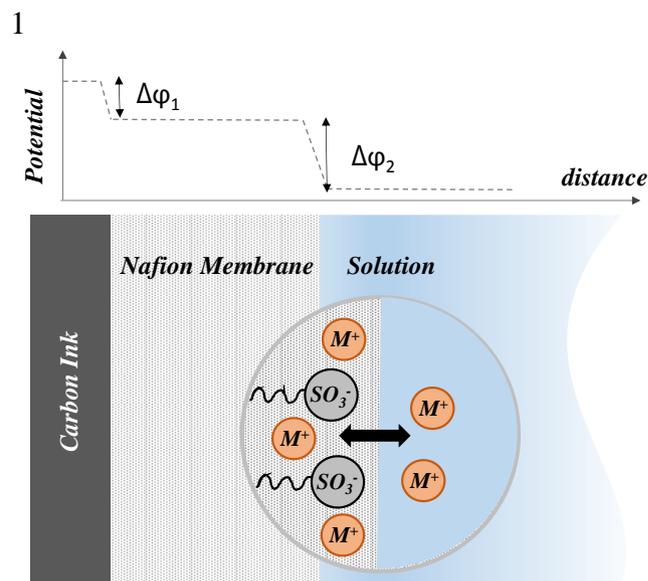


Figure 3. Ion-exchange between the solution and Nafion interface between cations,  $M^+$ , with sulfonate groups immobilized within the Nafion.

Drawing on the work of Michalska [34], the two main contributions to the electrical potential are at Carbon ink-Nafion ( $\varphi_1$ ) and at the Nafion-solution interface ( $\varphi_2$ ). The potential generated at the electrode ( $E_{el}$ ) is then:

$$E_{el} = \varphi_1 + \varphi_2$$

Making the reasonable assumption that  $\varphi_1$  is independent of the electrolyte concentration, this simplifies to:

$$E = \text{constant} + \varphi_2$$

It has been shown that this can be rearranged in the form of the Nernst equation:

$$E = E^{0'} + \frac{RT}{F} \ln a_{\text{Cations, Solution}}$$

This, treatment, however, is an approximation, since Nafion presents a very complex structure, with hydrophilic nanopores across the membrane segregated from the hydrophobic domains. Despite of this highly inhomogenous structure, the Nernstian dependence is phenomenologically observed.

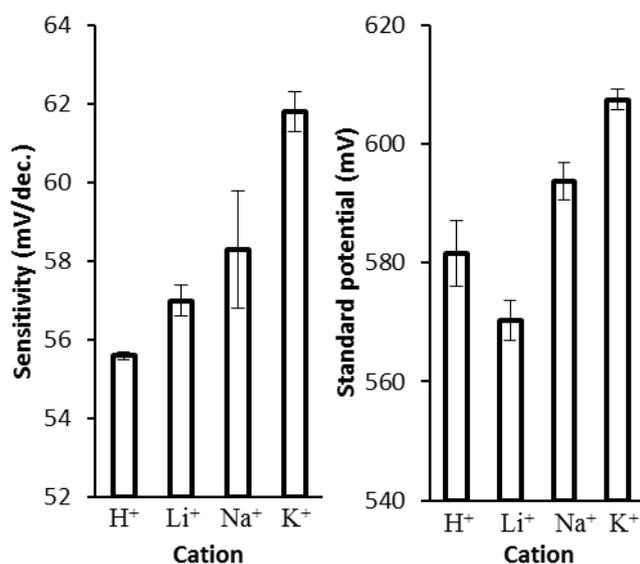


Figure 4. Comparison of the working electrode's response to a series of monocations. Left: sensitivities to each cation. Right: standard potentials to each cation.

### 3.2. Affinity amongst cations

Not only was the reproducibility between electrodes very good, but also the successive calibrations with the same cation (such as sodium) show similar figures of merit (S2). Therefore, experiments were conducted in order to evaluate the response of the sensor to different monovalent ions. When electrodes were tested in parallel against different monocations, it was observed that the Nafion membrane -while not highly selective- did indeed exhibit a subtle affinity amongst cations in accordance with the Hoffmeister series. This is reflected in both the sensitivities and the standard potentials (Figure 4). With the exception of the outlying standard potential during calibration with HCl, the observed trend shows an increment of values towards greater sensitivity and greater absolute value of response to the cations of the lower periodic rows. Although this tendency aligns with the

Hoffmeister series, it is not necessarily due to differences in lipophilicity, as indicated by Bakker *et al.* [31]. Michalska *et al.* have also reported lower sensitivities for the lower primary cations, observing the lowest sensitivity with Li<sup>+</sup> [42].

It is also worth noting that reusing an electrode for the calibration of distinct monocations leads to significant changes in the standard electrode potential as one cation species is exchanged for another. However, this memory effect seems to be negligible for practical applications as an approximation of response to monocations can be made by combining the results of all calibrations with the four different salts to give an overall sensitivity of  $58.3 \pm 2.5$  mV/dec.<sub>[MCl]</sub> and a standard electrode potential of  $581.3 \pm 34.3$  mV (N = 11).

Using these parallel calibrations with the four electrolytes mentioned, logarithms of potentiometric selectivity coefficients were calculated by treating the data as though it had resulted from the separate solution method, as electrode memory made reuse of the same electrodes unviable. Given that prior to any conditioning, Nafion contains protons as the exchangeable-ions to the immobile sulfonate groups, protons were (arbitrarily) selected as the primary analyte for these calculations (Table 2). All logarithms of potentiometric selectivity coefficients were between -0.6 and 0.5. While these affinities are minor, they do exceed those reported by the group of Bakker by an order of magnitude (in their study sodium was the primary ion) [31]. However, as mentioned in Section 1, the bespoke membranes used in this study were not in contact with an inner solution which may have had a role in the observed differences in affinities.

With the objective of constructing a wearable sensor which neither requires conditioning nor calibration, this working electrode is very attractive (as it requires neither). However, as will be seen, the integration of a reference electrode to complete the sensor currently still necessitates both of these steps.

Table 2. Potentiometric selectivity coefficients approximated from a separate solution method treatment of parallel calibrations of each electrolyte.

Ion (A)	Ion (B)	$\log K^{\text{pot}}_{A,B}$
H <sup>+</sup>	H <sup>+</sup>	0
	Li <sup>+</sup>	$-0.54 \pm 0.07$
	Na <sup>+</sup>	$0.38 \pm 0.10$
	K <sup>+</sup>	$0.44 \pm 0.13$

### 3.3. Total ion activity as a proxy for conductivity in artificial sweat solutions and integration of a pseudo-reference electrode

While the experimental evidence has revealed a weak affinity between monocations, the general response to all ions encourages the exploration of this electrode as a proxy for conductivity ( $\kappa$ ) measurements. Experiments where the

conductivity and EMF were both read across a range of dilutions of artificial sweat to establish the relationship between the two techniques were conducted. While this could in principle be done using a sodium ion-selective electrode, the point of difference here is that we expect the relationship to also hold for mixed electrolyte solutions.

Furthermore, in order to move towards a wearable device, a paper-based pseudo-reference electrode was integrated into the platform using a modification of the method reported by our research group [25, 36]. Although this showed a sensitivity  $-6.9 \pm 1.7$  mV/dec.<sub>(Artificial Sweat)</sub> ( $N = 3$ ) across the one decade of change in ionic strength when tested against a conventional double-junction Ag/AgCl/KCl reference electrode, this response contributed to the total sensitivity of the paper-based cell. Accordingly, the sensitivity of the IonSens cell increased to approximately 60 mV per decade of conductivity. The ability of the IonSens system to approximate conductivity in mixed electrolyte solutions was tested using ten dilutions of an artificial sweat solution. At each increment, the conductivity was also recorded with a commercial instrument and the results are shown in Figure 5. This confirmed that both instruments- the commercial conductivity meter and the bespoke IonSens device- had a predictable relationship to dilutions of a mixed electrolyte artificial sweat solution.

It is worth mentioning at this point that the system does not show any response to the neutral molecules tested, namely glucose, hydrogen peroxide and urea. As sweat is predominated by ions, the global reading of these is suggested to provide a snapshot of composition (Table 3).

Table 3. Typical sweat constitution components on the scapula [43]

Ion	Concentration of ions
Na <sup>+</sup>	41.10 ± 24.80 mM
Cl <sup>-</sup>	38.10 ± 24.10 mM
K <sup>+</sup>	3.49 ± 1.23 mM
Lactate	7.65 ± 1.49 mM
HCO <sub>3</sub> <sup>-</sup>	1.80 ± 2.51 mM
pH	5.82 ± 0.81

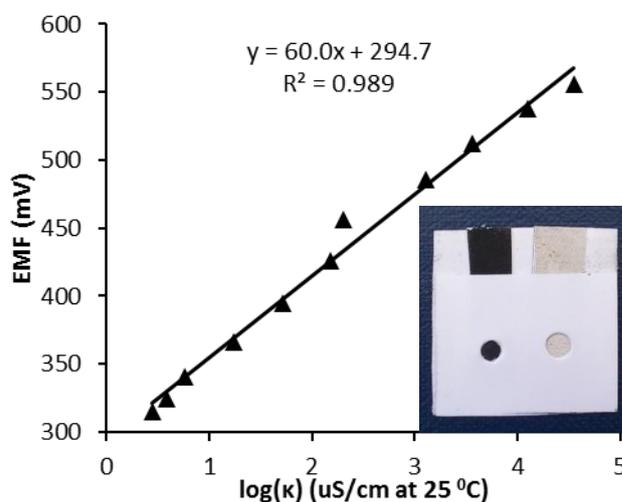


Figure 5. Plot validating the relationship of EMF to the logarithm of conductivity in a series of dilutions of artificial sweat. The inset is a photograph of the IonSens cell.

### 3.4. Determinations in real sweat samples

Real sweat samples were collected inside a plastic vial, by sliding it across the skin of the upper body of 11 athletes after playing basketball. These were analyzed by the benchtop conductivity meter and in triplicate with IonSens cells (on the lab bench). Prior to each determination, the IonSens cells were calibrated with 2 points of artificial sweat and rinsed with milli-Q water. An exemplary time trace of these experiments is shown in Figure 6A. The protocol was: a 2-point calibration with artificial sweat was made, the sensor was dried, the sample was introduced, the sensor was dried, and a final 2-point calibration was made. It is notable that the potential was not stable, but rather decreased for several analyses- in the figure, this can be seen for sensor 1. In this, the potential through the sample could be recorded at a maxima (marked 'M') or at the subsequent steady-state value (marked 'S'). Related to this, the initial or final calibration could be considered in order to evaluate the sample. A pattern was observed from this: using the initial calibration led to appropriate recoveries relative to the maxima; whereas the steady-state potential- which followed some minutes- later gave an appropriate recovery relative to the final calibration. The reasons for this phenomena- observed with some sensors but not with others- is not yet understood.

By using the initial calibration and taking the maxima of the potential generated by the sample, all eleven raw sweat samples were analyzed and validated with a conductivity meter as recovering  $95.2 \pm 6.6$  % of the conductivity. These analyses were made in triplicate and are summarized in Figure 6B. (The complete data is included in S3). It is worth noting that the error introduced by the logarithmic response of potentiometric method also applies to these measurements in which conductivity was estimated from a potentiometric measurement. Although

the pH of sweat varies widely- typically ranging from 4.5 to 6 for eccrine sweat while thermal apocrine sweat has higher pH values [44]- this did not impact the recoveries (see Table 2 of SI for sample pH values). As described in Section 3.2, the working electrode responds to free protons as it does other monovalent cations.

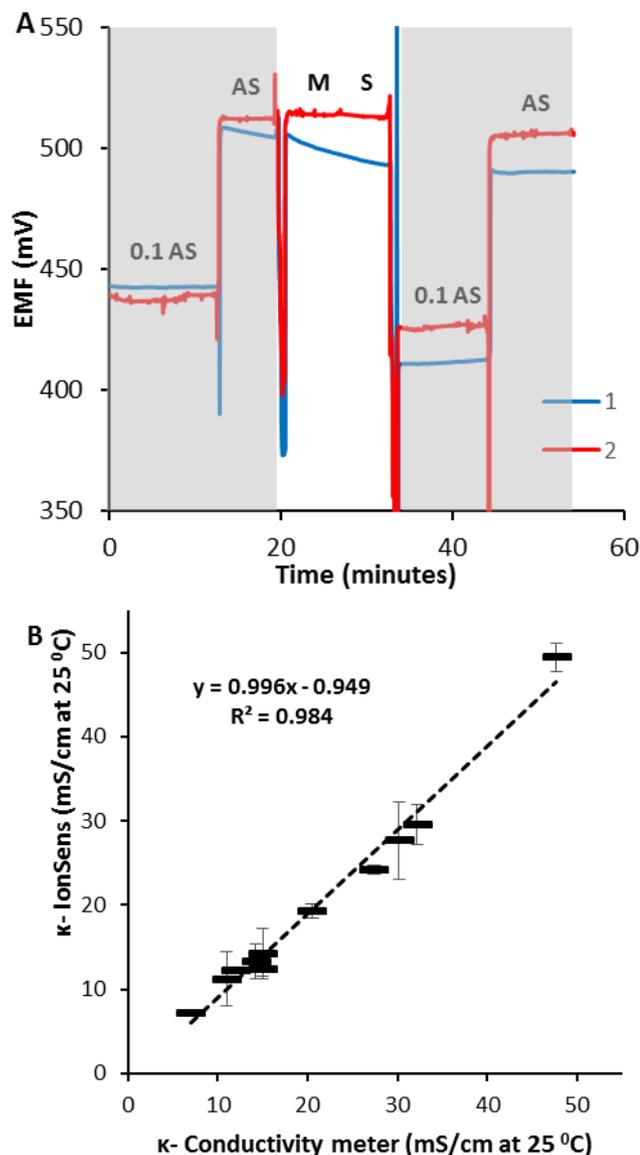


Figure 6. A: time traces of two IonSens cells (labelled 1 and 2). Grey background indicates a calibration with artificial sweat, white background indicates a sweat sample. 'AS' indicates artificial sweat, '0.1 AS' indicates artificial sweat diluted 10x, 'M' indicates the signal maxima, 'S' indicates the signal at steady-state. The noise seen at times 15 minutes, 21 minutes, 35 minutes and 45 minutes are due to the sensor being momentarily dry between changes of solution. B: validation of conductivity determinations by IonSens in real sweat samples against those made by a commercial conductivity meter.

### 3.5. A wearable device for on-body dynamic monitoring of sweat conductivity

In moving from static steady-state detection to real-time on-body tracking of chemical information with a wearable device, several new challenges arise. Very practical issues include the electrical isolation and waterproofing of sensitive elements such as paper electrodes, as well as the need for flexibility of sensors to respond to the stretching and bending of the body during movement. If not managed appropriately, these elements can each introduce noise and error into measurements. Additionally, there is a challenge in fluidics- to truly represent real-time analysis, sweat must be allowed to flow freely across the sensing surface.

In this on-body trial, an IonSens device was affixed beneath a plaster to the shoulder of a cyclist riding a stationary bicycle and the sensor's dynamic response during 35 minutes of exercise was monitored. Data was transmitted via Bluetooth from a small device in the athlete's clothing to a mobile phone app (see S4 for photo of the full device). Perspiration was evident after 10 minutes of exercise, after which time sweat samples were collected at 5 minute intervals from bulk sweat of the shoulder. These were subsequently tested 'off-body' (on the lab bench) using the same sensor in order to validate the on-body measurements.

The time trace recorded during exercise is reproduced in Figure 7 and the corresponding conductivities measured statically in the on-bench analyses are superimposed at the time corresponding to when the sample was collected. The (calculated) conductivity rose from the initiation of exercise until  $t = 8$  minutes, at which time the signal stabilized and sufficient sweat was present for bulk collection from the shoulder. While during these first minutes of exercise the electrode membranes were already hydrated, the athlete's skin was dry so no bulk liquid connected the circuit. It is therefore notable that the signal is not dominated by the noise associated with an open circuit. This attractive result may be attributable to the membrane impedances being 1-3 orders of magnitude lower than those typical of PVC (see S5), as well as being hydrophilic. A positive bias in the dynamic recording (of  $141 \pm 17\%$ ) as compared to the static measurements can be seen, although there is a preliminary correlation in the profiles of the two series. Variations in the sample collected manually from the shoulder as compared with the sample exposed to the sensor may have contributed to the bias. Additionally, mixing with previous sweat and evaporation may have been factors. Ultimately, these challenges are common to the validation of wearable sensors.

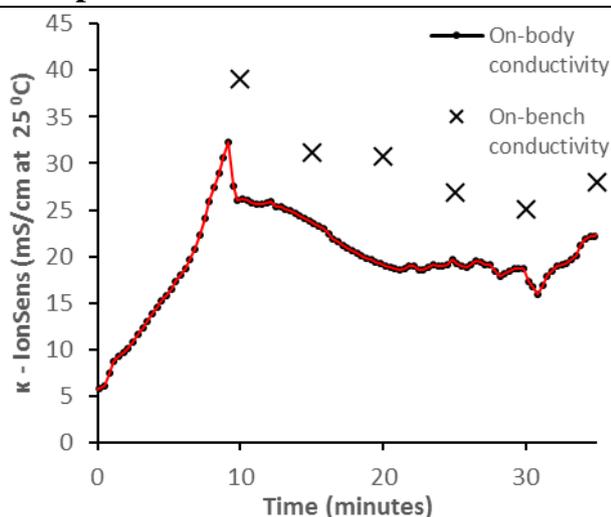


Figure 7. Plot of conductivity values generated by the on-body IonSens device during 35 minutes of exercise. Subsequent on-bench measurements were made at five minute intervals and the values are superimposed at the corresponding times.

#### 4. Conclusions

Nafion has been demonstrated to monitor total ion concentration within a solid-state electrode. This electrode- a drop-cast membrane upon carbon ink- is attractive for its simplicity, low-cost and reproducibility. Determinations in both synthetic solutions and real sweat samples have been validated by a commercial instrument to show correlation with conductivity. Furthermore, by fashioning the electrode into a wearable device with the inclusion of a pseudo-reference electrode, a low-impedance, low-consumption, paper-based conductivity sensor was realized. Through a Bluetooth connection to a mobile phone app, its utility for real-time gathering of chemical data was demonstrated by tracking the conductivity profile of an athletes' sweat during exercise. In contrast to the more accurate but bulky and expensive high-end instruments, this wearable offers a new value proposition to the user.

Sweat is a parameter rich in physiological information. It has been reported that the concentration of sodium and chloride varies significantly depending on sweat rate, while potassium and calcium concentrations remain unaffected [45]. Sweat rate and composition also varies regionally over the surface of the body while pH values can span some four orders of magnitude [43]. One approach is to multiplex sensors to monitor multiple analytes such as in the work of Gao *et al.* [5]. Another proposition is to take a single global reading that incorporates as much information as possible as in the case of conductivity readings. The IonSens device offers such a global reading. In this way, the IonSens platform 'plugs in' to the vision of chemical sensors being embedded into a

person's environment to gather meaningful information about the chemical gestalt of the individual.

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#### 4. Conflict of interest

The authors have no conflict of interest.

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