

Fully Organic Graphene Oxide-based Sensor with Integrated Pump for Sodium Detection

Jingfeng Huang^{1,2,3}, James Harvey^{1,2,3}, Hu Chen^{1,2,3}, Steve Faulkner³, James King³
Myra A. Nimmo^{2,3} and Alfred Tok I. Y.^{1,2}

¹*School of Materials Science and Engineering, Nanyang Technological University,
50 Nanyang Avenue, Singapore, 637553, Singapore*

²*Institute for Sports Research, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798, Singapore*

³*School of Sport, Exercise and Health Sciences, Loughborough University,
Ashby Road, Loughborough, Leicestershire, LE11 3TU, U.K.*

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Abstract: Sweat is produced by the body naturally during physical activity and this fluid can be analysed in real-time to reflect the body's hydration and electrolyte status. This paper reports a new type of organic disposable sensor pump that integrates sweat collection and sodium (Na⁺) ion sensing into cotton threads. This integration allows the sensor platform to be small, portable and wearable; thus allowing potential advantage to interface with the human body during field exercises enabling the provision of real-time data for immediate intervention. The sensor uses a sodium-selective Ion Selective Electrode (ISE) modified graphene oxide transducer intertwined with a thread pump. In this paper, we present the characterisation, synthesis and sensing data of this sensor.

1 INTRODUCTION

During exercise, sweating can lead to the loss of electrolytes such as sodium and potassium (Baker et al., 2009). With increasing sweat rate, only Na⁺ and Cl⁻ (from extracellular compartments) concentrations in sweat tend to increase while Ca²⁺ (from intracellular space) decreases; K⁺ and Mg²⁺ (from intracellular space) remain unchanged (Costill, 1977). Electrolyte loss through urine during exercise is small because of decreased urine formation and increased renal Na⁺ reabsorption. Deficits of 5% to 7% of the body's Na⁺ and Cl⁻ ions can be lost through sweat in comparison to less than 1.2% loss for K⁺ and Mg²⁺. (Costill, 1977). Measurement of sodium and potassium ions in sweat can be used to predict changes in serum (Baker et al., 2009). Thus sodium levels in sweat can be also used to predict exercise-associated hyponatremia. Therefore, sodium measurement in sweat is important and essential. In this paper, we present the characterisation, synthesis and sensing data of a novel disposable, organic, low-cost, graphene-based and pump-integrated sodium sensor suitable for real-time sensing of sweat sodium concentration in field

conditions.

2 RESULTS AND DISCUSSION

2.1 Idea of Fully Organic Sodium Sensor

From a list of currently available sodium portable sensors (Table 1), there is a niche to develop a disposable and low-cost sodium sensor suitable for real-time sensing in field conditions.

There is a demand for such a sensor platform in the fast-growing biosensor market which is projected to reach US\$12 billion by 2015 propelled by the growing population and health issues (GIA, 2012).

Increasingly graphene is the transducer material of choice because of its ability to detect single molecule binding events (Schedin et al., 2007). This is due to its exceptional low-noise electronic material property. Graphene, being a 2-dimensional material with high surface area to volume ratio, is also very sensitive to small perturbations on its surface and these perturbations change its electrical

Table 1: Comparison of practical and portable sodium sensors.

Na ⁺ Sensors	Minimum Sample Volume	Useful Range	Precision	Size	Disadvantage
Wescor brand –Sweat Check Conductivity Meter (to be used with Macroduct system)	8µl	0-150mM	2% from 75-110mM	10x20x16 cm; 1kg	-Non-real time -Not suitable for field testing
Wescor brand – Neonatal Sweat Analysis system	Needed but not indicated	3-200mM	<1% from 25-150mM	19x13x5 cm; 0.5kg	-Not suitable for field test
Centre for sensor web technologies – Wearable sodium sensor (Schazmann et al., 2010)	Needed but not indicated	0mM-100mM	3% RSD in lab testing	Not stated	-Requires special plastic molds - Requirement to store electrode in special liquids when not in use -Not disposable, bacteria growth observed
BIOTEX—Biosensing Textiles for Personalised Healthcare Management (Coyle et al., 2010)	Needed but not indicated	Not stated	Not stated	Not stated	-Requirement to store electrode in special liquids when not in use. -Not real-time as contact between fabric and electrode is poor

resistivity drastically (Huang et al., 2013b).

Briefly, graphene is a single atom thick film made up of carbon atoms connected in a sp² hybridized network. It has novel properties such as size tunable band gap and high carrier mobility. Graphene has already demonstrated that it is able to detect single gas molecules (Schedin et al., 2007). Graphene can be obtained via several methods, including chemical vapour deposition (Huang et al., 2013c), mechanical exfoliation (Novoselov et al., 2004) and chemical methods (Larisika et al., 2012). However, the chemical method has several advantages: low-cost, scalability and aqueous processability. Using graphene from the chemical route can lower the cost of sensors down to less than £1 per chip. Graphene produced via the chemical route contains several oxygen groups even after reduction and is thus sometimes termed reduced graphene oxide or reduced aqueous graphene (Huang et al., 2013d). In later discussions, we term the product as reduced graphene oxide (RGO). RGO-based bio-sensors have also been reported to be able to detect single bacteria (Mohanty and Berry, 2008), cell-activities (He et al., 2010) and label-free DNA (Ohno et al., 2009). The graphene transducer

can be used in an optical, amperometrical or acoustical sensor setup.

This paper uses the amperometric field effect transistor detection setup (Figure 1) as it can eliminate the need for expensive, bulky, highly-specialised equipment in diagnostic screening tests.

For selectivity, any receptors (e.g. sodium ionophore) can be attached to the amperometric graphene transducer to enable detection of a large variety of biological molecules with biomedical significance in real time.

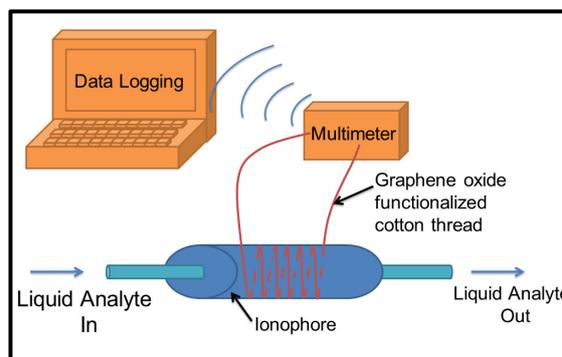


Figure 1: Schematic of amperometric field effect transistor (FET) biosensor setup.

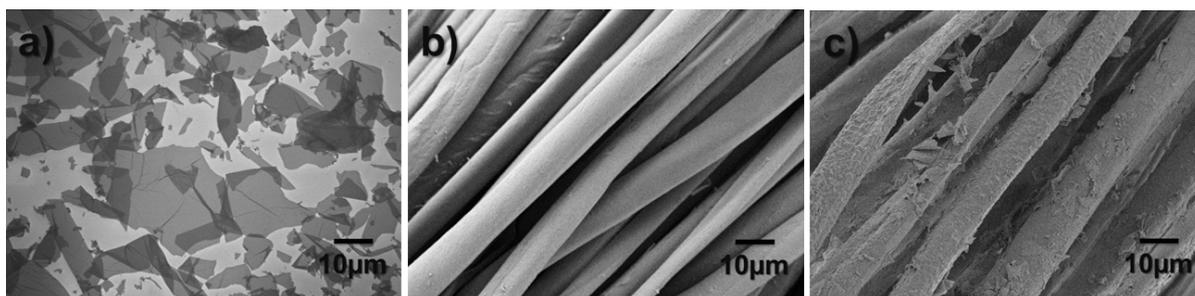


Figure 2: Field emission scanning electron microscopy images of (a) graphene oxide flakes on silicon dioxide wafer (b) cotton thread after plasma treatment (c) graphene oxide on cotton thread taken under similar magnification.

2.2 Integrating Sweat Collection

Methods for the collection of sweat include whole body washdown (WBW) believed to be the most accurate and reliable with analysis undertaken later. However this method is limited to cycling and requires a controlled laboratory setting. Alternatively, regional skin collection is relatively simple, practical for field studies and is able to accurately and reliably predict WBW sodium concentrations (Patterson et al., 2000). Regional skin collection methods with occlusive coverings have been reported to result in falsely high electrolyte readings because of electrolyte leeching from the stratum corneum of the skin (Weschler, 2008). However, if the skin can be kept dry by a sweat wicking material, it could be possible to obtain reliable estimates of local sweat electrolyte concentrations. A wicking sensor can thus prevent reabsorption of Na^+ back into the body. It will then be possible to measure the true Na^+ excretion. After these considerations, we designed a novel RGO-based, amperometric sensor platform with an integrated pump which can be used for real-time sensitive detection of sodium ions.

2.3 Fabrication and Characterisations

Special large Graphene Oxide (GO) flakes, a precursor to RGO, were prepared by modified Hummer's method from natural graphite flakes (3-5mm). Briefly, 2g of graphite flakes were mixed with H_2SO_4 (12ml) and stirred for 5 hours at 80°C . The mixture was ultra-sonicated for 1 hour and diluted with DI water (500ml). The suspension was then filtered using $0.2\mu\text{m}$ filter to obtain dry pre-oxidized graphite powders. To fully oxidize the graphite, H_2SO_4 (120ml) and KMnO_4 (15g) was added to the powder and stirred for 2 hours before diluting with 950ml of DI water slowly in ice-bath and finally stopping the reaction using H_2O_2 (20ml).

The upper portion of the solution was collected, filtered and washed to remove remaining metal ions and acid. This forms the stock GO solution containing large GO flakes used in subsequent experiment. To image the large GO flakes, GO solution was drop-cast on silicon dioxide flat substrate and scanning electron microscopy was used (Figure 2a). Using this method, the average size of the GO flakes are $700\mu\text{m}^2$ and this is 7 orders of magnitude larger (Larisika et al., 2012) than existing literatures. Larger GO flakes exhibit remarkable lower sheet resistance, lower intra-flake resistance and higher hole carrier mobility which could result in a more sensitive sensor.

To prepare the cotton substrate for the RGO, cotton threads were treated with oxygen (25%) plasma. This treatment removes natural wax and increases oxygen moieties on the surface of the thread, making subsequent chemical functionalization possible. After a 50 minute plasma treatment, the structure of the cotton thread is still intact (Figure 2b). Then (3-Aminopropyl) triethoxysilane (2% v/v ethanol) was used to functionalise the cotton surface to make it positively charged. The functionalised thread was then submerged in the GO stock solution for 30 minutes. As the surface of GO is negatively charged, during the incubation period, the GO will be electrostatically attached onto the thread.

It had been reported that GO can also be coated directly onto the cotton thread using a drip-and-dry method (Shateri-Khalilabad and Yazdanshenas, 2013). However, attaching the graphene using the electrostatic attraction method allows self-assembly and termination, thus allowing controlled 1-2 layers of GO to be adhered to the thread (Figure 2c). The 2-dimensional material characteristics of GO is preserved with 1-2 layers of GO as the hole or electrons carriers can only move along the planar direction. Having more than two layers of GO will allow the carriers to travel in parallel (3-

dimensional) and decreases the sensor's sensitivity to any perturbations from the ionophore.

After the attachment of GO to the thread, a chemical reduction is needed to remove oxygen moieties from the carbon backbone of GO and restore its electrical conductivity. The thread is placed in a sealed petri dish with 500 μ l of hydrazine monohydrate and left overnight at 70°C. The hydrazine vapor produced will reduce the GO into RGO. This RGO-lined cotton thread forms the RGO transducer. The exposed RGO transducer is sensitive to all molecules, such as H₂O (humidity) and NO₂ (gas) molecules. Therefore a coat is needed to protect the RGO transducer from the environment.

To achieve selectivity of sodium on the RGO surface, a sodium ionophore cocktail was coated onto the RGO transducer. Briefly, bis(1-butylpentyl) adipate, sodium ionophore (71733) and poly(vinyl chloride) high molecular weight, (Sigma-Aldrich, St Louis, USA), were dissolved in tetrahydrofuran solvent and then the ionophore cocktail was left overnight to stabilise before coating on the RGO transducer. The ionophore used is an electrically neutral, lipophilic ion-complexing agent of small relative molar mass. Two coatings of sodium ionophore were applied to the RGO transducer to ensure complete coverage.

The ionophore-covered RGO transducer was then fixed across a polyethylene terephthalate (PET) platform and attached to a multimeter (Agilent U1273A and E3ABAG) via copper wire and clips. Another plasma-treated thread was coiled around the

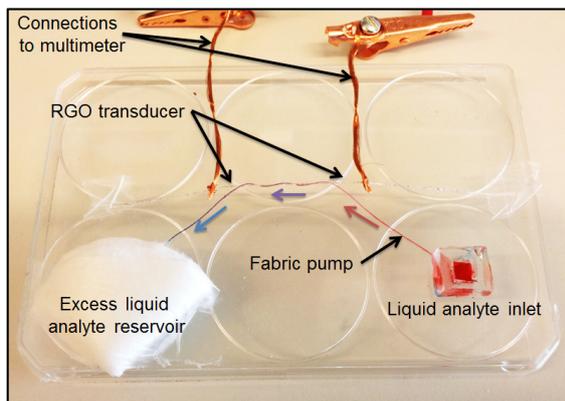


Figure 3: The graphene oxide based sensor with integrated fabric pump. (For interpretation of the colored dyes on the figure, kindly refer to the online version of this article).

RGO transducer. This allows the liquid analyte to flow through it to reach the transducer (figure 3).

One of the thread ends is attached to a silicone rubber liquid analyte inlet and the other to a cotton

wool excess liquid analyte reservoir. The large collection reservoir allows constant flow of liquid analyte through the thread. This thread acts as a pump using capillary action and no external power source is needed (Reches et al., 2010; Li et al., 2009). After the sensing setup is completed, colored dyes are used to detect the movement of liquid analyte.

2.4 Pump Testing and Sodium Sensing

From Figure 3, it can be observed that the blue and then red colored dyes travel cleanly along the fabric pump. The wicking rate increases exponentially with increasing plasma treatment time on the cotton thread ca. 3mm/min, 10mm/min and 50mm/min after 10, 30, 50 minutes of oxygen plasma treatment respectively. In the experiments, 50 minutes of plasma treatment on all cotton thread was used. Then the colored dyes were replaced with sodium analyte for sensing data collection.

To test the sodium detection window of the sensor, different concentrations of sodium chloride solution were added to fill the analyte inlet and allowed to flow through the thread pump. The liquid analyte travels via capillary action along the thread pump towards the ionophore-coated transducer. The ionophores selectively extract sodium ions into the hydrophobic membrane phase and transport these ions across the barrier by carrier translocation. The ions carried across the ionophore will then cause perturbations on the RGO surface and change the electrical resistivity. This change in electrical property can be detected by a multimeter and the data is then sent to a data-logger. A sensing window is thus obtained and presented in Figure 4.

As the fabricated RGO transducer is a p-type semiconductor (Huang et al., 2013a), when positive sodium ions travel through the ionophore onto the surface, it induces a positive gating effect (Huang et al., 2013b) and increases the RGO's electrical resistance. The electrical resistance changes according to the change in the concentration of sodium chloride.

As the concentration of the sodium chloride increased from 0mM to 90mM, the electrical resistance through the RGO transducer increased from 615.2 to 638.5k Ω correspondingly. A 90mM sodium ion concentration contributed a 3.9% increase in electrical resistance across the RGO transducer.

Patterson et al (Patterson et al., 2000) reported whole-body sweat Na⁺ concentrations of 24.1 \pm 15.0 mM (mean \pm SD). Na⁺ levels at specific regions

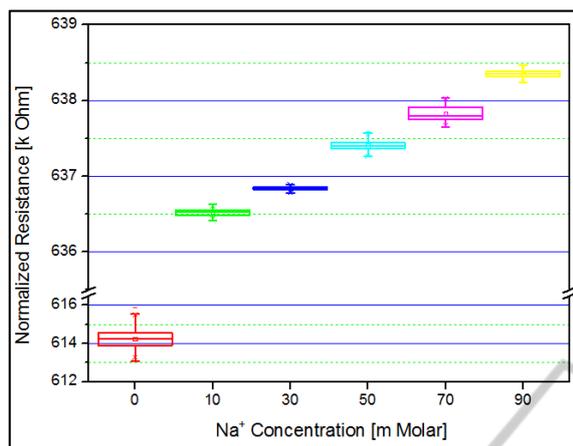


Figure 4: Normalized electrical resistance output vs. sodium ion concentration input.

(such as the forearm, chest and scapula) were consistently higher (mean Na⁺ concentration at each site: 42.2±25.8, 47.6±25.7 and 42.2±24.8 mM respectively). Therefore, our sensor operates within the typical physiological range. As can be seen from the large standard deviations for sweat Na⁺ levels, sweat composition can vary widely between individuals. Our sensor is therefore of interest in the field of exercise science: real-time monitoring of sweat Na⁺ losses allows for the development of athlete-specific rehydration strategies.

In theory, the lower detection window of the reported transducer is only limited to the sodium ionophore applied and electronic amplification equipment used, as graphene had already been shown to detect 1 single molecular binding events (Schedin et al., 2007). As a comparison, a single liter of 10mM of sodium chloride solution contains 6.023×10^{26} number of sodium ions. With such high sensitivity, this RGO-based sensor can also be modified with antibodies or other ionophores to detect other specific ions or biomolecules of interest to the community. The presented sodium sensor could potentially be used to diagnose dehydration in the ageing population and used in the study of the biology of sweat in older people. Future work on the disposable and low-cost real-time fully-organic sensor platform would include optimizing the fabrication technique for mass-production and sensing for other ions or proteins.

3 CONCLUSIONS

During exercise, one of the body's major ionic deficits of concern is Na⁺. Measurements of sodium

ions in sweat had been reported previously to predict changes in serum. In this paper, we have shown the fabrication, characterisation and physiologically-relevant sodium-ions detection limits of a novel RGO-based sensor with an integrated pump that is fully organic, low-cost and disposable. As the sensor is small, light and wearable, it has enormous potential to be integrated onto the human body during field training to obtain real-time data for immediate intervention.

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REFERENCES

- Baker, L. B., Stofan, J. R., Hamilton, A. A. & Horswill, C. A., 2009. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *Journal of Applied Physiology*, 107, 887-895.
- Costill, D. L., 1977. Sweating: Its Composition and Effects in Body Fluids. *Annals of the New York Academy of Sciences*, 301, 160-174.
- Coyle, S., King-Tong, L., Moyna, N., O'gorman, D., Diamond, D., Di Francesco, F., Costanzo, D., Salvo, P., Trivella, M. G., De Rossi, D. E., Taccini, N., Paradiso, R., Porchet, J. A., Ridolfi, A., Luprano, J., Chuzel, C., Lanier, T., Revol-Cavalier, F., Schoumacker, S., Mourier, V., Chartier, I., Convert, R., De-Moncuit, H. & Bini, C., 2010. Biosensing Textiles for Personalised Healthcare Management. *Information Technology in Biomedicine, IEEE Transactions on*, 14, 364-370.
- Gia, 2012. Biosensors in Medical Diagnostics. *Global strategic business report*. CA.
- He, Q., Sudibya, H. G., Yin, Z., Wu, S., Li, H., Boey, F., Huang, W., Chen, P. & Zhang, H., 2010. Centimeter-Long and Large-Scale Micropatterns of Reduced Graphene Oxide Films: Fabrication and Sensing Applications. *ACS Nano*, 4, 3201-3208.
- Huang, J., Harvey, J., Chen, H., Fam, W. H. D., Nimmo, M. A. & Tok, I. Y. A., 2013a. Growth of Graphene Oxide Using Ethanol CVD. *Procedia Engineering*.
- Huang, J., Harvey, J., Fam, W. H. D., Nimmo, M. A. &

- Tok, I. Y. A., 2013b. Novel Biosensor for Interleukin-6 Detection. *Procedia Engineering*.
- Huang, J., Larisika, M., Fam, W. H. D., He, Q., Nimmo, M. A., Nowak, C. & Tok, A. I. Y., 2013c. The extended growth of graphene oxide flakes using ethanol CVD. *Nanoscale*, 5, 2945-2951.
- Huang, J., Larisika, M., Nowak, C. & Tok, I. Y. A., 2013d. *New Methods in Aqueous Graphene (Graphene Oxide) Synthesis for Biosensor Devices*.
- Larisika, M., Huang, J., Tok, A., Knoll, W. & Nowak, C., 2012. An improved synthesis route to graphene for molecular sensor applications. *Materials Chemistry and Physics*, 136, 304-308.
- Li, X., Tian, J. & Shen, W., 2009. Thread as a Versatile Material for Low-Cost Microfluidic Diagnostics. *ACS Applied Materials & Interfaces*, 2, 1-6.
- Mohanty, N. & Berry, V., 2008. Graphene-Based Single-Bacterium Resolution Biodevice and DNA Transistor: Interfacing Graphene Derivatives with Nanoscale and Microscale Biocomponents. *Nano Letters*, 8, 4469-4476.
- Novoselov, K. S., Geim, A. K., Morozov, S. V., Jiang, D., Zhang, Y., Dubonos, S. V., Grigorieva, I. V. & Firsov, A. A., 2004. Electric Field Effect in Atomically Thin Carbon Films. *Science*, 306, 666-669.
- Ohno, Y., Maehashi, K., Yamashiro, Y. & Matsumoto, K., 2009. Electrolyte-Gated Graphene Field-Effect Transistors for Detecting pH and Protein Adsorption. *Nano Letters*, 9, 3318-3322.
- Patterson, M. J., Galloway, S. D. R. & Nimmo, M. A., 2000. Variations in Regional Sweat Composition in Normal Human Males. *Experimental Physiology*, 85, 869-875.
- Reches, M., Mirica, K. A., Dasgupta, R., Dickey, M. D., Butte, M. J. & Whitesides, G. M., 2010. Thread as a Matrix for Biomedical Assays. *ACS Applied Materials & Interfaces*, 2, 1722-1728.
- Schazmann, B., Morris, D., Slater, C., Beirne, S., Fay, C., Reuveny, R., Moyna, N. & Diamond, D., 2010. A wearable electrochemical sensor for the real-time measurement of sweat sodium concentration. *Analytical Methods*, 2, 342-348.
- Schedin, F., Geim, A. K., Morozov, S. V., Hill, E. W., Blake, P., Katsnelson, M. I. & Novoselov, K. S., 2007. Detection of individual gas molecules adsorbed on graphene. *Nat Mater*, 6, 652-655.
- Shateri-Khalilabad, M. & Yazdanshenas, M. E., 2013. Fabricating electroconductive cotton textiles using graphene. *Carbohydrate Polymers*, 96, 190-195.
- Weschler, L. B., 2008. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. *Journal of Applied Physiology*, 105, 1376-1377.