Lab on a Chip

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Cite this: *Lab Chip*, 2018, **18**, 2279

Ion sensing with thread-based potentiometric electrodes†

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Potentiometric sensing of ions with ion-selective electrodes (ISEs) is a powerful technique for selective and sensitive measurement of ions in complex matrices. The application of ISEs is generally limited to laboratory settings, because most commercially available ISEs and reference electrodes are large, delicate, and expensive, and are not suitable for point-of-use or point-of-care measurements. This work utilizes cotton thread as a substrate for fabrication of robust and miniaturized ISEs that are suitable for point-of-care or point-of-use applications. Thread-based ISEs selective for Cl^-, K^+, Na^+, and Ca^{2+} were developed. The cation-selective ISEs were fabricated by coating the thread with a surfactant-free conductive ink (made of carbon black) and then coating the tip of the conductive thread with the ion-selective membrane. The Cl^- ISE was fabricated by coating the thread with an Ag/AgCl ink. These sensors exhibited slopes (of electrical potential *vs.* log concentration of target ion), close to the theoretically-expected values, over four orders of magnitude in concentrations of ions. Because thread is mechanically strong, the thread-based electrodes can be used in multiple-use applications as well as single-use applications. Multiple thread-based sensors can be easily bundled together to fabricate a customized sensor for multiplexed ion-sensing. These electrodes require volumes of sample as low as 200 μL. The application of thread-based ISEs is demonstrated in the analysis of ions in soil, food, and dietary supplements (Cl^- in soil/water slurry, K^+ and Na^+ in coconut water, and Ca^{2+} in a calcium supplement), and in detection of physiological electrolytes (K^+ and Na^+ in blood serum and urine, with sufficient accuracy for clinical diagnostics).

Received 4th April 2018,
Accepted 28th June 2018

DOI: 10.1039/c8lc00352a

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Introduction

Sensing of ions is an integral part of management of water, the environment, a range of industrial activities (*e.g.*, agriculture, food processing, and process control),[1–3] and healthcare (*e.g.*, determining the concentrations of physiological electrolytes present in blood serum).[3] For measurements of ions, samples are usually sent to central laboratories where technicians and robots perform the analyses, and report the results to the customer or the healthcare provider. This process is problematic in several circumstances including (i) medical emergencies where rapid access to results is important, (ii) uses where convenient access to reliable laboratories is not possible, and (iii) in analyses in rural and resource-limited areas where not only access to reliable laboratories is limited, but also the cost of the analysis is not affordable by the patient or the customer. There is thus a substantial need for affordable and rapid sensing of ions at the point of care or point of use for a range of types of applications.

A few commercial ion-sensors suitable for point-of-care analysis of blood electrolytes are available.[4–6] Microfabrication has allowed development of "chips" (cartridges), and use of hand-held clinical analyzers such as the i-STAT for multiplexed potentiometric detection of physiological electrolytes.[6] Development of hand-held clinical analyzers was a

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† Electronic supplementary information (ESI) available: Details of instrumentation, preparations of solution, calibrations of sensor, measurements of resistance, measurements of electrolytes in serum and urine, measurements of chloride in soil, and SEM imaging. See DOI: 10.1039/c8lc00352a

major advancement for point-of-care analysis of physiological electrolytes, but the high cost of the instruments and the consumables hinders their use in resource-limited areas, and their application for ion sensing is limited to analysis of physiological electrolytes only; thus ion-sensing at the point-of-use for agricultural, environmental, and quality control applications remains an unmet need.

This work utilizes potentiometry for fabrication of portable ion sensors. In this technique, the electrical potential of an indicator electrode (an ion-selective electrode, or ISE) provides a quantitative measurement of the thermodynamic activity of an ion in the sample. This potential is measured with respect to a reference electrode that maintains a constant and sample-independent electrical potential throughout the experiment. Six characteristics of potentiometric sensors make them suitable for point-of-care or point-of-use analyses. (i) These sensors usually have short response times (<1 s),[3] and thus can provide rapid analysis of concentrations of ions in the samples. (ii) Potentiometric sensors can selectively detect the target ion in a complex matrix, and thus do not require coupling with separation techniques. The sensors typically have selectivities to the target ion that are 10^2 to 10^6 times greater than to interfering ions,[3,7,8] and the field of molecular recognition offers many opportunities for developing new selectivities. Selectivity is crucial because most analyses are performed in complex samples (*i.e.*, food, serum, urine, impure water) containing several types of ions. (iii) ISEs selective for a wide range of ions have already been developed.[3,7,8] (iv) The sensing mechanism is non-destructive to the ISE, and the ISE can be reused for multiple analyses. (v) Multiplexed ion-sensing with potentiometric sensors can be readily accomplished. Since potentiometric measurements are performed under zero-current condition, the electrical potential of multiple ISEs can be measured simultaneously relative to one reference electrode in the solution. This capability eliminates the need for complicated electronic circuits for the control of electrical voltage and current in multiple channels of a potentiostat. Multiplexed ion-sensing decreases the time of the analysis, when determination of the concentration of a panel of ions is required (*i.e.*, in the analyses of physiological electrolytes, Na^+, K^+, Ca^{2+}, Cl^- in serum). (vi) Since the measured signal is an electrical voltage, the analysis can be performed at the point-of-use using low-cost portable electrochemical detectors[9,10] (with no need for complicated and expensive instrumentation).

Despite these advantages, most commercially available potentiometric ion-selective and reference electrodes are large, delicate (made with glass enclosures), expensive ($100–500), require large sample volumes (10–100 mL), and must be operated by skilled users; thus, they are unsuitable for applications that are time-, resource-, and cost-limited.[3,11,12] We and others have adopted paper as a low-cost substrate for point-of-care potentiometric ISEs.[11,13–27] Paper-based potentiometric sensors are useful in point-of-care analysis, but they are most suitable for single-use applications because of poor mechanical strength of wet paper and moderate mechanical stability of dry paper. The ability to reuse devices (which enables calibration of each individual sensor) is important operationally and economically in many applications. The mechanical strength of paper can be reinforced by using plastic masks and covers that allow the paper-based ISE to be reused,[15,20] however, this approach increases the size of the electrode and decreases the convenience of their use, specifically in multiplexed analyses.

This work utilizes thread for fabrication of potentiometric sensors that are affordable, simple-to-use, reusable, require small volumes (<500 μL) of sample, and offer customizable multiplexing capabilities, and thus are suitable for many types of analyses. Thread is inexpensive, light, flexible, and mechanically strong, and comes in diameters of smaller than 100s of microns (and thus does not require micropatterning to define channel sizes). Thread is available in a variety of materials (*e.g.*, nylon, cotton, polyester, wool, polyolefin, and many others); the material can thus be selected to have properties appropriate to the specific application. Thread can be manipulated by industrialized processes (*e.g.*, weaving and knitting) into complex structures. Some of these capabilities have been demonstrated for thread in previous work by us, and others.[28–35]

Andrade *et al.* have pioneered the use of thread as a substrate for fabrication of potentiometric sensors.[36,37] They employed cotton thread impregnated with carbon nanotubes, and later carbon fiber thread, for fabrication of wearable potentiometric sensors selective for pH, NH_4^+, and K^+.[36,37] The utility and capabilities of the thread-based potentiometric ion-sensors as diagnostic and analytical tools (rather than as wearable sensors) for clinical analyses or analyses in resource-limited circumstances has not been realized. This work (i) demonstrates that different types of thread (cotton, nylon, polypropylene) and a wide range of ion-to-electron transducer materials (conductive polymers, different types of carbon) and conductive inks can be used to fabricate thread-based ISEs, and thus shows the versatility of this technology, (ii) develops a surfactant-free carbon-based conductive ink to allow fabrication of durable thread-based ISEs with improved reproducibility and stability in their electrical potential (to enable rapid and accurate ion sensing with these electrodes), (iii) develops a customizable multiplexed ion-sensor with the thread-based ISEs for measurements in small volumes of sample (∼200 μL), and (iv) demonstrates the application of thread-based ISEs in ion-sensing in food and dietary supplements (K^+ and Na^+ in coconut water, and Ca^{2+} in a calcium nutritional supplement), in soil (Cl^-), and for multiplexed analysis of physiological electrolytes (K^+ and Na^+) in human serum and urine (with sufficient accuracy to allow clinical diagnosis).

Experimental

Materials

Potassium ionophore I, sodium ionophores X, calcium ionophore II, potassium tetrakis(4-chlorophenyl)borate (KTPClB), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 2-nitrophenyl octyl ether (*o*-NPOE), high molecular weight poly(vinyl chloride) (PVC), and tetrahydrofuran (THF, inhibitorfree, for HPLC, purity ≥99.9%) were purchased from Sigma-Aldrich. Blood serum (from human male AB plasma, USA origin, sterile-filtered) and urine (human, pooled) were purchased from Sigma-Aldrich and the experiments did not involve interaction with live subjects. ESI† lists the suppliers of other materials and chemicals used.

Measurements

Unless noted otherwise, we measured the response of the sensors (measurements on five identically-prepared electrodes) at room temperature with respect to a free-flow double-junction Ag/AgCl reference electrode (with a movable ground glass sleeve junction, 1.0 M lithium acetate bridge electrolyte) purchased from Mettler Toledo. The ESI† lists the details of instrumentation, preparation of solutions, calibration of sensors, measurement of resistance, measurement of K^+ and Na^+ in serum and urine, and SEM imaging of ink-coated thread.

Membrane compositions

We prepared the ion-selective cocktail (the precursor of the ion-selective membrane) by dissolving a total of 1.0 g of membrane components in 2.5 mL of THF. We stirred the ion-selective cocktail vigorously for at least three hours prior to use. The potassium-sensing membrane consisted of 660 mg of *o*-NPOE, 330 mg of PVC 330 mg, 10 mg of potassium ionophore I, and 2.23 mg of KTPClB (ionophore to KTPClB molar ratio of 2 : 1). The sodium-sensing membrane consisted of 660 mg of *o*-NPOE, 330 mg of PVC, 10 mg of sodium ionophore X, and 4.46 mg of NaTFPB (ionophore to NaTFPB molar ratio of 2 : 1). The calcium-sensing membrane consisted of 660 mg of *o*-NPOE, 330 mg of PVC, 10 mg of calcium ionophore II, and 3.59 mg of KTPClB (ionophore to KTPClB molar ratio of 3 : 1). We added 4 μL of 1.0 M $CaCl_2$ (aqueous) to the Ca^{2+} ion-selective cocktail to avoid the need for conditioning of the Ca^{2+} ISEs.[20]

Fabrication of thread-based ISEs

We cut the cotton thread (1 mm thickness) into 8 cm pieces. We prepared the carbon black ink by grinding (five minutes, with a mortar and pestle) 0.2 g of carbon black powder, 0.533 g of *o*-NPOE, 0.267 g of PVC, 1 mL of THF, and 4 mL of cyclohexanone. We applied the ink to the cotton thread immediately after mixing and did not store the suspension. We used a polystyrene-tipped swab to apply the carbon black ink to the thread and allowed the ink to dry overnight at ambient temperature to generate electrically-conductive thread. We attached one end of the conductive thread to a paper clip, dipped 3 cm of its other end into the ion-selective cocktail, and hung the thread vertically to allow the ion-selective membrane (ISM) to set overnight at room temperature. We sealed the ISM-coated conductive thread using either of two approaches (Fig. 1): (i) we inserted the ISM-coated conductive thread into a 7 cm heat-shrinkable tubing, leaving 0.5 cm of thread exposed at each end, and used a heat gun to heat the heat-shrinkable tube for 5–10 s to form a tight fit around the thread, and (ii) Using a brush, we painted 7 cm of thread with nail polish, leaving only 0.5 cm of the ink-coated and ISM-ink-coated thread uncovered at each end.

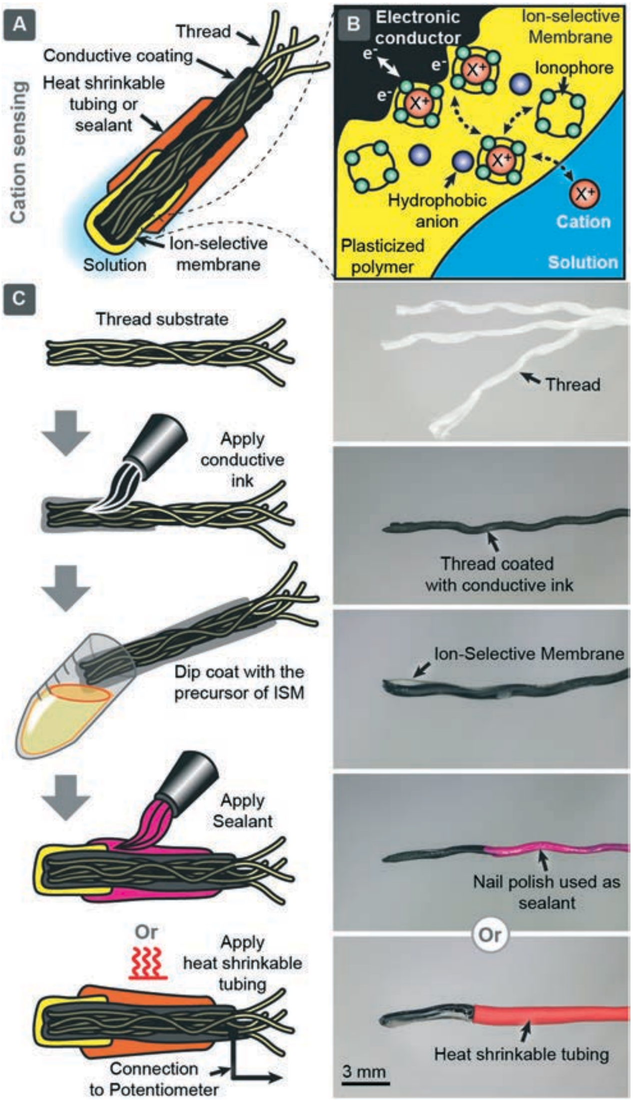

Fig. 1 Design (A), working principle (B), and fabrication process (C) of thread-based cation-selective electrodes.

To fabricate the Cl^- ISE, we used a polystyrene-tipped swab to apply the Ag/AgCl ink to the thread and allowed the ink to dry overnight at ambient temperature. We then painted 0.5 cm of the Ag/AgCl-coated thread with this solution (as shown in Fig. 5) and allowed the nail polish to dry at ambient temperature for two hours. We then inserted the Ag–AgCl-coated thread into a 7 cm heat-shrinkable tubing, leaving 0.5 cm of the thread exposed at each end, and used a heat gun to heat the heat-shrinkable tube for 5–10 s to form a tight fit around the thread.

Results and discussion

Design of the cation-selective ISEs

Fig. 1A and B show the sensing components and design of thread-based cation-sensing ISEs. We performed ion sensing using an ion-selective membrane (an ISM), which is a hydrophobic membrane composed of a polymer (to provide mechanical support), plasticizer, a receptor that selectively binds to the target ion (referred to as the ionophore), and a hydrophobic ion used as a counter-ion to the target ion (referred to as the ionic site). We deposited the ISM on thread, coated with a conductive substrate (referred to as the ion-to-electron transducer[27]) and measured the electrical potential of this conductive substrate relative to a reference electrode. The relationship between the potential of the ISE (referred to as electromotive force or emf), and the activity of the analyte is described by the Nernst equation (emf = $E^\circ + (RT/zF)\ln a$), where E° represents the standard potential, R the universal gas constant, T the temperature, F the Faraday constant, z the charge, and a the activity of the ion.[3] An order of magnitude change in the activity of the ion with charge z, results in 59.2 mV/z change in the potential of the ISE (59.2 mV/z is referred to as the Nernstian slope).[3]

Thread is composed of aligned fibers, the spaces between these fibers provide a surface area to contain the conductive substrate and the ISM (the components needed for the fabrication of the ISE), while the thread provides mechanical support for the ISE. Fig. 2 shows the SEM image of bare cotton, ink-coated-thread, and ink-ISM-coated thread. The mechanical strength, light weight, and narrow diameter of the thread enables fabrication of sensors that are light, portable, compact, and easy to use. The conductive substrate affects the performance of the ISE.[3,27] A variety of materials have been employed to develop ISEs with stable electrical potentials, including derivatives of conductive polymers (*e.g.*, polythiophene[38–40] and polypyrrole[41]), nanomaterials,[42–44] and different types of conductive carbons (*e.g.*, carbon nanotubes, carbon black, graphite, and colloid-imprinted mesoporous carbon).[45–48]

Commercial conductive inks (based on carbon graphite and PEDOT:PSS), the previously reported aqueous carbon nanotube ink,[36] and carbon fiber[37] all provided conductive thread and subsequently thread-based ISEs with the theoretically-expected Nernstian behavior (the ESI† lists the results). The performance and durability of these ISE was, however, not suitable for a reusable sensor for rapid sensing of ion in the field or the clinic due to (i) large drifts in the emf of the ISEs (>0.2 mV min^{-1}), (ii) poor mechanical properties of the ink-coated thread (the ink cracked with slight mechanical stress, *i.e.*, bending, of the thread—an event which led to breaking the conductivity along the thread—or the ink did not adhere well to the thread and the carbon particles fell off during mechanical stress or

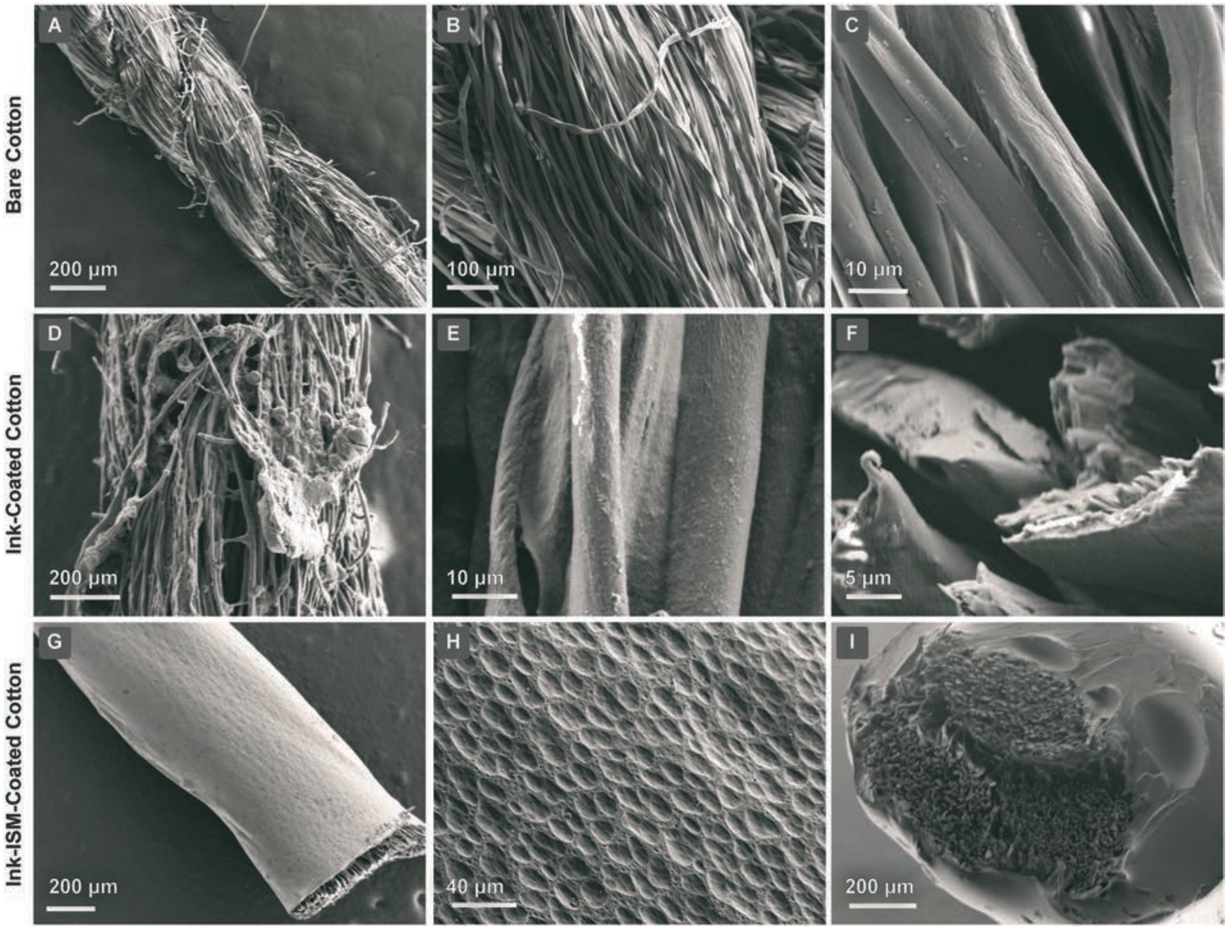

Fig. 2 SEM images of bare cotton thread (A–C), cotton thread coated with the carbon black ink (D–F shows the cross-section), and cotton thread coated with carbon black ink and the ISM (G–I show the cross-section).

handling of the thread), and (iii) poor sensor-to-sensor reproducibility in the emf of the ISEs (>5 mV).

To fabricate thread-based ISEs, we developed an ink with carbon black using an organic polymer (PVC + *o*-NPOE as plasticizer) as the ink binder. We made this selection for seven reasons: (i) carbon black is porous and has a large ratio of surface area to volume, and high specific capacitance; this high specific capacitance establishes a stable interfacial potential between the ion-to-electron transducer and the ISM, and minimizes the drift in the emf of ISEs with carbon-based materials.[27,45,46] (ii) Carbon black is inexpensive and widely available. (iii) Using a plasticized polymer matrix maintains flexibility of the carbon black-polymer composite, and the thread coated with this ink maintains its conductivity after mechanical stress. (iv) The carbon can be mechanically dispersed in the polymer and plasticizer and surfactants were not used in the fabrication. Surfactants could degrade the performance of ISEs.[49,50] (v) The polymer and plasticizer contain the carbon black particles, adhere well to the thread, and avoid shedding of carbon particles from the ink-coated thread. (vi) The ISM adheres well to the ink-coated thread because the ink has a similar composition to the ISM (both have PVC + *o*-NPOE as the polymer support). (vii) The ink can be developed with simple equipment (a mortar and pestle) and with different types of carbon if carbon black was not accessible, it thus can be employed for fabrication of thread-based ISEs in resource-limited settings.

Fabrication of sensors

Fig. 1C shows the fabrication of thread-based ISEs. We applied the carbon black ink to the cotton thread, and once the ink was dry (after evaporation of the solvent), we measured the resistance of the thread coated with the carbon black ink, and obtained 3.3 ± 0.9 kΩ cm^{-1}. To ensure that the ink-coated thread maintains its conductivity upon mechanical stress, we bent the ink-coated thread 180° five times and confirmed no significant change in the electrical resistance of the ink-coated thread. We did not observe any shedding of carbon particles from the ink-coated thread during mechanical stressing or handling of the thread. We dipped one end of the conductive thread in the ion-selective cocktail (ISM dissolved in THF, details in the Experimental section) and allowed the THF to evaporate overnight at room temperature.

We used two approaches (which are both in principle simple and do not require complicated equipment) for sealing the thread that was covered with the conductive ink and not the ISM (Fig. 1C): (i) we applied a solution of nail polish to the exposed ion-to-electron transducer, leaving only 0.5 cm uncovered at one end to make the electrical connection to the potentiometer, and 0.5 cm of the ISM uncovered at the other end to contact the sample solution. The surface area of the ISM that is exposed to the sample does not affect the potentiometric measurement (electrical potential does not depend on the surface area of the indicator electrode). We chose the contact area of 5 mm simply for convenience in fabrication. We used nail polish as sealant, because it is hydrophobic, widely accessible, dries rapidly, and has good adhesion to the thread. Nail polish can be replaced by solutions of other hydrophobic polymers. Different colors of nail polish can be used to color-code sensors that are selective to different ions. (ii) We used heat-shrinkable tubing to cover the ISM-ink-coated thread, leaving only 0.5 cm of the ISM-ink-coated and ink-coated thread at each end. Heat-shrinkable tubing was also used by Andrade *et al.* for fabrication of a wearable potentiometric sensor, and has been used successfully for decades to insulate and protect electrical connections and to make water-impermeable seals.[36,51–54] We used different colors of heat-shrinkable tubing to color code the sensors selective to different ions. The heat-shrinkable tube adds to mechanical strength of the thread-based sensor and thus makes handling of the sensor easy (and suitable for diagnostic applications). We used heat-shrinkable tubing for fabrication of the sensors that are characterized in this work. Approach (i) would be useful for fabrication of thread-based electrodes where exposure of the ISM to heat can result in degradation of the sensing components and malfunction of the sensor.

Results

Fig. 3A shows the response of K^+-selective ISE made with the carbon black ink and cotton thread. The sensors exhibited Nernstian slopes (53.6 ± 0.7 mV per decade) and good electrode-to-electrode reproducibility ($E°$: 495.9 ± 2.3 mV). We varied the ratio of carbon to polymer content to optimize the electrode-to-electrode reproducibility in the $E°$ of the sensors. The ink that contained 0.2 g of carbon black, 0.533 g of *o*-NPOE, and 0.267 g of PVC (carbon black comprised 20% of the total weight of the dried ink) provided ISEs with the best reproducibility in the values of $E°$. This composition allowed the ink to adsorb onto the thread homogenously and to form the conductive thread with a reproducible surface area. Increasing the carbon content to 50% increased the reproducibility of the $E°$ to 6 mV and caused the ink to flake off when the ink-coated thread was mechanically stressed. Decreasing the carbon content to 5% or 10% increased the resistance of the thread coated with the ink with no consistent improvement in the $E°$ reproducibility. Carbon black has previously been used as an ion-to-electron transducer for fabrication of ISEs,[47,55] but has not been incorporated into an ink for fabrication of paper- or thread-based ISEs.

In addition to carbon black, other types of porous carbon can also be incorporated in this ink. For other types, however, the carbon to polymer ratio needs to be tuned to obtain the optimal performance of the ISE. For example, the ESI† shows that a conductive ink comprised of 5 wt% carbon nanotubes, 32 wt% PVC, and 63 wt% *o*-NPOE provided thread-based K^+ ISEs with a Nernstian response (slope: 53.8 ± 0.3 mV per decade, $E°$: 278.4 ± 1.9 mV) and good potential stability (emf drift of −0.06 mV min^{-1}, measured over 30 minutes in an aqueous 100 mM KCl solution).

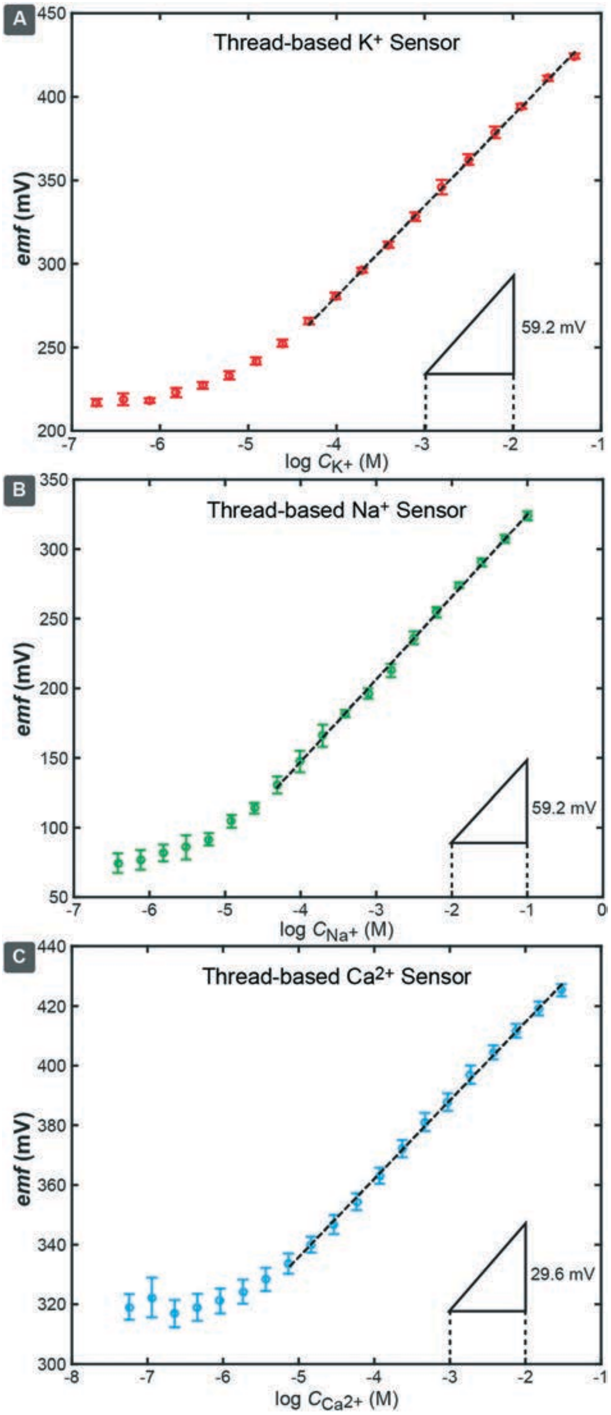

Fig. 3 Response of thread-based K^+ (A), Na^+ (B), and Ca^{2+} (C) selective electrodes made with cotton and carbon black ink. We show the average and standard deviation of emf of five identically prepared electrodes. The triangles represent the theoretically-expected Nernstian slope.

Fig. S3† demonstrates that thread made of other materials such as nylon and polypropylene can also be used for fabrication of potentiometric thread-based sensors with Nernstian responses (the ESI† lists the results). We used cotton thread for fabrication of the sensors in this work because cotton absorbed the inks more homogenously than the nylon and polypropylene thread and resulted in sensors with better reproducibility in the slope and *E*° than did the nylon and polypropylene thread.

Fig. S4† shows the drift in the emf of K^+ ISEs made with the carbon black ink and cotton thread. We evaluated the potential stability of the sensors using a protocol that would represent how they would be used in the field. We selected a set of sensors that were not exposed to or soaked in deionized water or a KCl solution (that is, we had not conditioned the sensors). We placed the sensors in 100 mM KCl, and recorded their emf for 30 minutes. The sensors exhibited excellent potential stability (0.15 mV min^{-1} over five minutes, and 0.01 mV min^{-1} over 30 minutes). Most of the drift in the emf occurred within the first minute of the sensor's exposure to the KCl solution, and the potential drift was less than 0.05 mV min^{-1} after minute one. This profile indicates that rapid and accurate point-of-care ion-sensing with thread-based ISEs can be accomplished.

Fig. 3B and C show the response of Na^+- and Ca^{2+}-selective ISEs to Na^+ and Ca^{2+}. The response of the Na^+ (slope: 59.1 ± 1.1 mV per decade, *E*°: 384.0 ± 3.4 mV) and the Ca^{2+} (slope: 26.3 ± 0.5 mV per decade, *E*°: 467.2 ± 2.6 mV) were close to the theoretically-expected Nernstian slopes of 59.2 and 29.6 mV per decade. To ensure that the sensors maintain their function after fabrication and while in storage, we measured the response and emf stability of a K^+ -selective ISE four months after its fabrication, and confirmed that there is no statistically significant change in the analytical performance of the sensor over that interval (the ESI† lists the data).

Reusability of the cation-selective ISEs

Potentiometry is a non-destructive technique; the ISM and the reference membrane are, in principle, not damaged during most measurements, and ISEs are reusable.[3] We demonstrated that thread-based ISEs are also reusable by measuring the calibration curves (Fig. 3) with one set of electrodes and changing the concentration of Na^+, K^+, and Ca^{2+} in the test solution. The ISM is hydrophobic and fills in the spaces between the fibers of thread, and also covers the length of the thread that is exposed to the sample, so aqueous solutions are not trapped in the fibers of the thread. There might be some residual sample left on the surface of the ISM which can be washed away simply by rinsing the area that was exposed to sample, and the sensor can be reused immediately and placed in a different test solution. To further demonstrate the reusability of the thread-based sensors, we successively placed a Na^+ sensor in different solutions (1 and 10 mM Na^+) and showed that the sensor responds correctly and reversibly (Fig. 4).

Design of the thread-based chloride ISE

Chloride sensing can be accomplished by using the redox system of $Ag_{(s)}/AgCl_{(s)}/Cl^-_{(aq)}$, where the electrical potential of Ag/AgCl is determined by the activity of chloride ions in the sample. Fig. 5 shows the design and fabrication of the thread-based Cl^- ISE. We measured the resistance of the

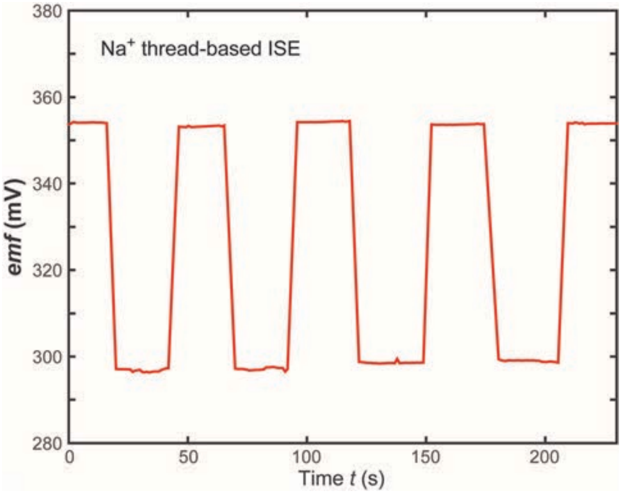

Fig. 4 Reusability of the Na^+ thread-based ISE. A thread-based Na^+ selective ISE, successively immersed in 1 mM and 10 mM NaCl. For each solution change, we rinsed the electrode by deionized water and dried it gently with tissue paper.

thread saturated with Ag/AgCl ink to ensure conductivity over the length of the thread (electrical resistance $<10\ \Omega\ \text{cm}^{-1}$, Table S1† lists the values). Fig. S9† shows the SEM image of Ag/AgCl-coated thread, confirming that thread can be used as a matrix to support the Ag/AgCl ink. We applied a nail polish solution to thread (as shown in Fig. 5C) as a wicking barrier to ensure that when this sensor is placed in an aqueous solution, the solution does not wick up to the other end of the thread and interfere with the measurement of electrical potential. We then enclosed the ink-coated thread (that contain the wicking barrier) in a heat-shrinkable tubing, leaving 0.5 cm of thread exposed at each end (for contact with the sample, and for electrical connection). We used heat-shrinkable tubing as an electrical insulator and a physical barrier (to avoid short-circuits) when the thread-based Cl^- ISE is bundled with other thread-based electrodes. The area of the surface of the thread coated with Ag/AgCl ink that is exposed to sample does not affect the potentiometric measurement, and we chose the contact area of 5 mm simply for convenience in the fabrication process.

We measured the response of thread-based Cl^- ISEs relative to a commercial reference electrode; a stable potential (less than 0.1 mV min^{-1} drift) was obtained approximately 100 s after exposing the sensor to Cl^- containing solutions. Thread-based Cl^- ISEs have a function that is similar to an AgCl-coated Ag wire and exhibited Nernstian slope in a range of 100 mM to 0.1 mM Cl^- (Fig. 6). We obtained 0.5 mV or better electrode-to-electrode reproducibility in the emf of the thread-based Cl^- ISEs. Fig. S9† shows that other types of thread (nylon and polypropylene) provide Cl^- ISEs with a similar performance to the cotton-based Cl^- ISE.

Analysis of chloride levels in soil

To demonstrate application of the thread-based Cl^- ISE for in-field ion sensing, we used this sensor to measure the level of chloride in soil. Detection of chloride levels in soil is important for maintaining the health and the proper growth of plants in agricultural fields.[56,57] Depending on the type of the crop, different chloride levels are required to foster growth as well as maintain the ideal crop yields.[57] Fluctuations in the level of chloride in soil can arise through multiple causes such as runoff from deicing roads or highways, rainwater, irrigation, and fertilization.[58,59] Sensors for in-field analysis of chloride facilitate management of Cl^- levels in the environment and agricultural fields.

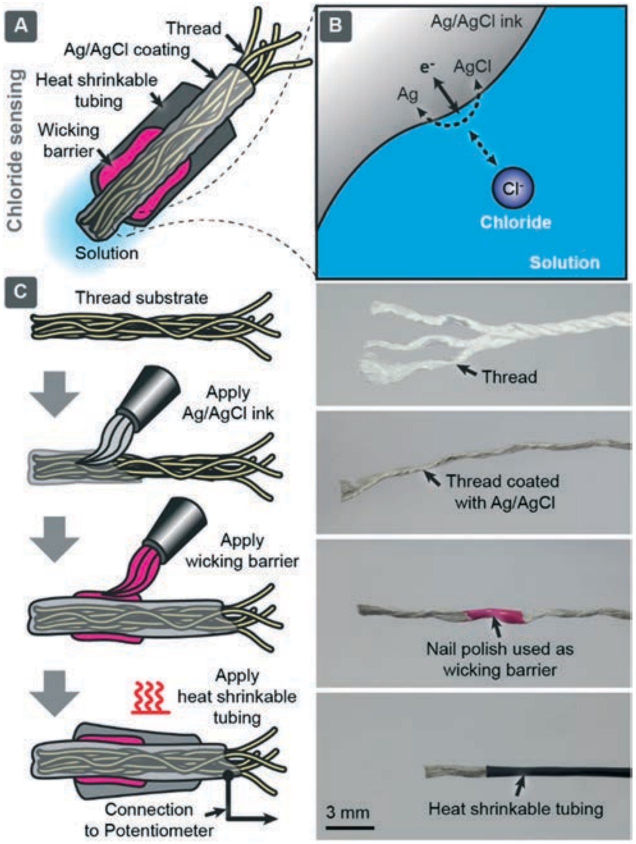

Fig. 5 Design (A), working principle (B), and fabrication process (C) for the thread-based Cl^- ISE.

To confirm that the thread-based Cl^- sensor can accurately determine chloride levels in soil, we washed the soil with deionized water, and immersed in a solution with known concentrations of chloride. We dipped the thread-based sensor in the slurry of the soil and aqueous solution and recorded the emf after two minutes. We converted the emf values to the concentration of Cl^- using the calibration equation established in Fig. 6B. Table S4† lists the results and demonstrates that the thread-based Cl^- sensor can accurately (≈5% error) determine Cl^- levels in soil and in aqueous solutions.

Analysis of K^+, Na^+, and Ca^{2+} in food and dietary supplements

To demonstrate an application of thread-based ISEs, we detected K^+ and Na^+ in coconut water (advertised as a good

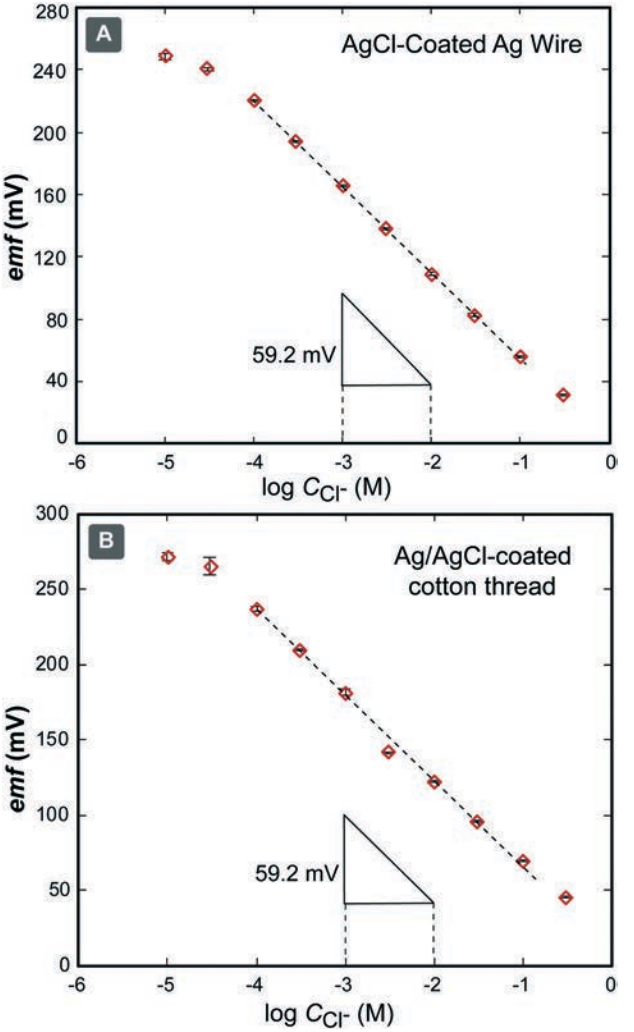

Fig. 6 The Nernstian response of AgCl-coated Ag wire (A) and Ag/AgCl-coated cotton thread (B) to Cl^-. We show the average and standard deviation of emf of five identically-prepared electrodes. Error bars (five replicates) are shown, but are mostly concealed by data symbols.

source of electrolytes and suitable for hydration) with the following protocol. We randomly picked a K^+ and a Na^+ sensor from a batch of sensors, calibrated them (as explained in the ESI†) to determine the equation for conversion of the emf to the concentrations of K^+ and Na^+, and then measured the emf of these sensors and three additional K^+ and Na^+ sensors (which were not calibrated and were from the same batch) in coconut water. The sensors that were not calibrated prior to the analysis resulted in a K^+ concentration of 34 ± 3 mM and a Na^+ concentration of 19 ± 1 mM. These values were close to the concentrations measured by the sensors that were calibrated (37 mM K^+ and 18 mM Na^+). The label of the juice specified that the product contained 470 mg potassium and 70 mg sodium in 240 mL (one serving) which is equivalent to 50 mM K^+ and 12 mM Na^+, and is in agreement with the concentrations determined by the thread-based ISEs.

To measure Ca^{2+} in a calcium dietary supplement (the product label specified that each pill contained 500 mg of calcium), we used the Ca^{2+} thread-based ISE with the same calibration protocol as above (calibrate a representative sensor to determine the calibration equation, use a different sensor for the measurement in sample), and obtained 442 ± 76 mg for the amount of calcium in each pill (details in the ESI†). These demonstrations show that calibration of each individual sensor is not required in field use for applications that can tolerate approximately 10% error in the analysis. Several approaches have been suggested for further improving the electrode-to-electrode reproducibility in the potential of ISEs, including application of an electrical potential or current pulse to ISEs after their fabrication,[60] short-circuiting the ISE with a reference electrode immersed into the same solution for several hours,[61] and doping the ISM with the lipophilic Co(II)/Co(III) redox buffer.[62–64] All these approaches (which we have not explored in this work) can in principle be adopted for further improving the electrode-to-electrode reproducibility in the potential of the thread-based ISEs.

Multiplexed analysis of K^+ and Na^+ in blood serum and urine

To demonstrate multiplexed ion-sensing and accurate analysis with thread-based ISEs (in a complex matrix), we used these electrodes to measure the levels of Na^+ and K^+, in human blood serum and urine samples. Electrolytes play a vital role in regulating human physiology, and abnormal levels of these ions can lead to serious and life-threatening conditions (*e.g.*, hypokalemia can cause muscle weakness, ileus, and cardiac arrhythmias; hyperkalemia can cause cardiac arrest).[65–67] Concentrations of electrolytes in serum are important indicators of the status of patient's health and are checked in routine blood tests where the appropriate "access to the laboratories" is available.

Because the normal concentration range of physiological electrolytes is very narrow (Table S3† lists this range in serum), the tolerable error (4 mM for Na^+ and 0.5 mM for K^+) in their analysis is very small.[68] To accomplish this accuracy without calibration of each sensor, the sensor-to-sensor $E°$ reproducibility should be less than 0.7 mV.[27] Unfortunately, the sensor-to-sensor reproducibility in the $E°$ of the thread-based ISEs developed in this work (~3 mV) is not sufficient for a calibration-free operation, and the sensors must be calibrated before the analysis of physiological electrolytes. The sensors have sufficient selectivity to allow simultaneous calibration and multiplexed ion-sensing (to shorten the time of the analysis).[8] To provide quantitative values, the selectivity of the Na^+ ISE to Na^+ is 10^2 times greater than that for K^+ or Ca^{2+}, and the selectivity of the K^+ ISE is 10^4 times greater to K^+ than that for Na^+ or Ca^{2+}.[8] Due to the high selectivity of potentiometric sensors for the target ion, and the compact design of the thread-based ISE bundle, multiplexed ion-sensing in small volumes of sample can thus be accomplished with thread-based ISEs (Fig. 7A and B).

We made a bundle of the K^+ and Na^+ sensors, and simultaneously calibrated the sensors in three standard solutions containing different levels of Na^+ and K^+ to cover the

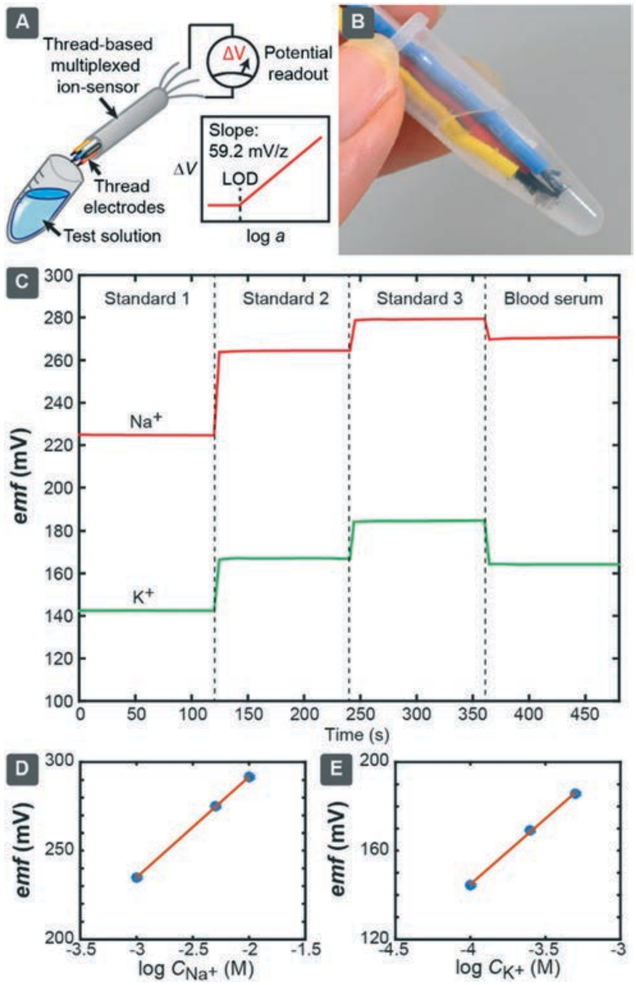

Fig. 7 Multiplexed ion-sensing with thread-based ISEs. A and B show the ISE bundle. C shows the emf traces of K^+ and Na^+ thread-based ISEs in standard solutions and blood serum, D and E show the corresponding calibration curves. Standard 1 contained 0.1 mM KCl and 1 mM NaCl, standard 2 contained 0.25 mM NaCl and 5 mM KCl, and standard 3 contained 0.5 mM NaCl and 10 mM KCl.

physiological range of these ions in blood serum and urine. We had fabricated the sensors a week in advance and had not exposed them to any aqueous solutions prior to this measurement. Fig. 7C shows the emf traces of detection in blood serum and Fig. 7D and E show the corresponding calibration curves of the ISEs, which we used to convert the emf values to the concentrations of Na^+, and K^+ in blood serum and urine.

Since ISEs determine the thermodynamic activity and not the concentration of ions, we performed the measurements in diluted (20-fold) urine and serum to ensure that activity and concentration could be approximated as the same. Potentiometric sensors can be used for measurement in whole blood or serum, but an appropriate correction factor needs to be introduced to account for differences between activity and concentration, protein binding, and ion-pair formation.[69] To provide comparison with ISEs that were fabricated according to a well-established method in the literature,[8,13,70] we fabricated conventional ISEs with inner-filling solutions (the ESI† lists the details)[8,13,70] and used these ISEs for evaluating the performance of thread-based ISEs. We determined the concentration of Na^+ and K^+, with the same protocol as we did with the thread-based ISEs. We calculated the differences between concentrations of electrolytes determined with thread-based ISEs and with conventional ISEs.

Table 1 lists the results and the errors (calculated as $(C_{\text{thread-based ISE}} - C_{\text{conventional ISE}})/C_{\text{conventional ISE}}$). The values measured with the thread-based ISEs (relative to the commercial reference electrode) had excellent agreement with concentrations determined with conventional ISEs. The errors for K^+ and Na^+ are within the tolerable error in the measurement of ion concentrations required by the U.S. FDA.[68] This demonstration confirms that the potential stability of the thread-based ISEs is sufficient to allow rapid and accurate measurement of the concentration of physiological electrolytes.

Conclusions

With the goal of developing sensors for accurate analysis of ion concentrations at the point-of-care and point-of-use, we have incorporated thread as a substrate, and carbon black as ion-to-electron transducer material for development of reusable potentiometric ion-sensing devices with multiplexing capabilities, and high potential stability (<0.1 mV min^{-1} drift in the emf) and good sensor-to-sensor $E°$ reproducibility (<5 mV). The thread-based ISEs provide an alternative to existing commercial ISEs which are expensive, large, delicate, and require large volumes of sample. Thread-based ISEs are a versatile technology and a variety of ion-to-electron transducer materials (conductive polymers, different types of carbon such

Table 1 Errors in the measurement of K^+ and Na^+, in blood serum and urine with thread-based ISEs (three measurements)

	Concentrations measured relative to the commercial reference electrode (mM)		
	Thread-based ISEs	Conventional ISEs	Relative error calculated as: $(C_{\text{thread-based ISE}} - C_{\text{conventional ISE}})/C_{\text{conventional ISE}}$
Blood serum			
K^+	4.6 ± 0.23	4.5 ± 0.04	1.5%
Na^+	141.8 ± 1.98	140.4 ± 3.77	1.0%
Urine			
K^+	41.3 ± 0.67	41.4 ± 0.71	−0.2%
Na^+	99.0 ± 2.90	101.0 ± 1.19	−1.9%

as graphite, carbon black, carbon nanotubes) and different types of inks can be employed for their fabrication.

The thread-based ISEs have several characteristics that makes them suitable for affordable analysis at the point of care. (i) The electrodes are robust, compact, lightweight, and portable. (ii) The fabrication of the electrodes does not require use of stencils, masks, and screen printing or microfabrication techniques. It thus offers the potential to provide a simple manufacturing process which can be carried out in resource-limited settings. (iii) The high mechanical strength of thread makes it a suitable substrate for fabrication of robust sensors; thus, the thread-based electrodes can be utilized for single or multiple analyses (one sensor can be reused). (iv) These ISEs have easily and broadly adaptable multiplexing capabilities. ISEs selective for different ions can be bundled together to allow multiplexed ion-sensing in small volumes of sample. The ISE bundle can easily be disassembled, and the individual ISEs can be used again to fabricate a different ISE bundle (for sensing of a different set of ions). (v) The electrodes can be used for continuous measurements (*e.g.*, real-time monitoring of electrolyte levels during surgery or in process monitoring), and can be embedded in flow cells. (vi) Even though we have only shown application of these sensors for detection of four ions (Cl^-, K^+, Na^+, Ca^{2+}) in soil, serum, urine, a dietary supplement, and fruit juice, they are in principle adaptable for analyses of other clinically or environmentally relevant ions (*e.g.*, Li^+, Mg^{2+}, NH_4^+, NO_3^-, S^{2-}, Cd^{2+}, Pb^{2+}) by using the appropriate composition of ISM developed for these ions.[3,7,8] Numerous ionophores for selective binding to other ions have been demonstrated based on concepts from molecular recognition and host–guest chemistry.[3,7,8] Many of the well-established ionophores are commercially available; this availability makes it convenient to develop ISEs for detection of a range of ions, and designs of new structure-selective ionophores will certainly be possible when needed. (vii) The sensors can be interfaced with a web-connected portable electrical reader for the voltage readout. Examples of such a reader has been developed in prior work by us and others.[10,71,72] An electronic reader will be necessary for the successful transition of these sensors from the laboratory to the field and point-of-care measurements in an environment in which mHealth is ubiquitous.

While this work was focused on development of the thread-based ISEs, successful field use of the sensors requires fabrication of a thread-based reference electrode to enable analysis with a full thread-based cell. Due to their compact design and simple fabrication, thread-based ISEs have applications beyond point-of-care ion sensing and can be employed as a versatile tool for analysis in research and development, and for *in situ* monitoring of biological and organic processes where the reactant or the product is an ionic species.

Contributions of authors

M. P. S. Mousavi contributed to conceptualization, writing, and analysis. A. Ainla contributed to writing. E. K. W. Tan contributed to device fabrication and data collection. L. Yuan performed the SEM imaging. The undergraduate students, H. H. Sigurslid, N. Arkan, and M. C. Yip contributed to device fabrication and data collection. C. K. Abrahamsson contributed to conceptualization. M. K. Abd El-Rahman and Y. Yoshida contributed to writing and conceptualization. S. Homer-Vanniasinkam contributed to medical evaluation. G. M. Whitesides supervised.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

We acknowledge Mahiar Max Hamedi for fruitful conversations on sensing with thread-based devices. We acknowledge Dr. Maria-Nefeli Tsaloglou (Diagnostics for All, Inc.) and Shekar Shetty (Nano Terra Inc.) for providing information on common practices in the analysis of physiological electrolytes in blood serum and urine. This work was supported by DTRA grant number HDTRA1-14-C-0037, as well as by the Wyss Institute for Biologically Inspired Engineering. The SEM imaging was performed at the Center for Nanoscale Systems (CNS), Harvard University. A. A. thanks the Swedish Research Council (VR) for a postdoctoral fellowship. L. Yuan acknowledges financial support from NSF award # CHE-1506993. N. Arkan was supported by the Harvard REU program under NSF award # DMR-1420570. E. K. W. Tan acknowledges the Trinity Hall, University of Cambridge, for financial support. Y. Yoshida was supported by the overseas education exchange program for faculty members of the Kyoto Institute of Technology.

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