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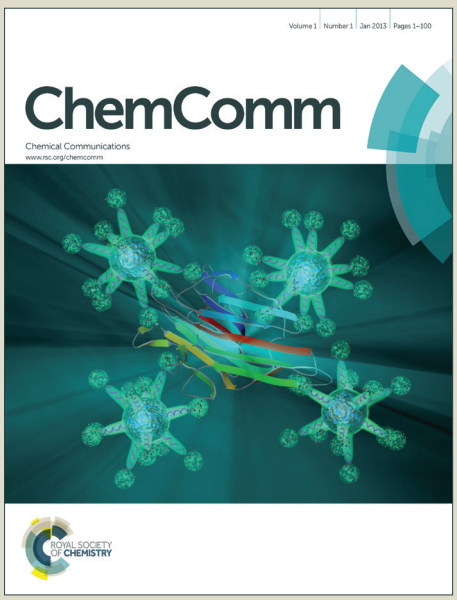

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Paper-based plasticizer-free sodium ion-selective sensor with camera phone as a detector

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Xuewei Wang, Yu Qin and Mark E. Meyerhoff*

An ionophore-based ion-selective optode platform on paper is described for the first time with a sodium optode as the example. Cellulose paper is shown to be an excellent substrate for adsorption of the required chromoionophore, ionophore, and ion exchanger species. These adsorbed components form a hydrophobic phase that enables heterogeneous optical ion sensing in the absence of any plasticizer or organic polymer phase.

Ion-selective electrodes/optodes (ISEs/ISOs) are chemical sensors based on a water-immiscible phase doped with an ion-specific ionophore. Various ISEs/ISOs have been developed for direct detection of over 60 inorganic, organic, and biological ions as well as indirect detection of numerous gases, neutral solutes, chemical/biochemical reactions, and bio-recognition events.[1-8] Such sensing capabilities make ISEs/ISOs useful in important analytical fields such as clinical chemistry, environmental analysis, and food safety control. Especially in electrolyte (Na, K, Ca, Cl, etc.) determination in biological fluids such as blood and urine, ISEs/ISOs have completely replaced classical atomic spectroscopy techniques and are used routinely within instruments in all hospitals around the world.

Point of care testing (PoCT) is a type of medical testing outside of a lab and has played an important role in public health over the past few decades.[9] A new wave in PoCT is the development of sensors that are transportable, portable, and use small volumes of sample, all with ultralow cost and with reduced requirements for instrumentation. Such sensors will largely benefit the healthcare in resource-limited settings such as in patient homes, at accident scenes, and in underdeveloped countries/areas. For this purpose, paper-based sensors and sensing microfluidics have attracted considerable interest in recent years.[10-14] Paper is very cost-effective and easy to manufacture, transport, handle, and store. More importantly, the capillary action of porous paper enables the development of pump-free microfluidics with easily fabricated channels on paper. Indeed, various paper-based sensing platforms have been reported for ions, metabolites, enzymes, proteins, nucleic acids, and bacteria.[13]

The concept of ISEs has also already been adapted to paper. Classical ion-selective polymeric membranes have been applied to a conductive material-coated paper to obtain solid contact working electrodes, and a separate reference electrode is used for potentiometric measurement.[15-18] Recently, a more compact ISE device has been developed using wax-patterned paper with integrated working electrode and reference electrode.[19,20] Compared to ISEs, ISOs allow colorimetric sensing based on vision or portable cameras and have a unique potential to develop a simple and compact ion sensing platform in an essentially power and instrument-free mode. Although direct blood sample analysis by ISO-based colorimetric method is not as feasible as ISE-based potentiometry, blood separation strategies for paper microfluidics hold promise to overcome this limitation.[21] To our knowledge, ISOs based on the typical proton-selective chromoionophore have not been reported on paper. However, an optical sensor configuration on paper that may be applicable for ISOs has been developed, in which filter papers are stacked with a plasticized PVC membrane functionalized by a copper ion carrier and a copper ion-sensitive dye.[22] In all of these electrode and optode systems, paper mainly functions as a physical substrate for the solid contact, polymer membrane or solution, and classical ISE/ISO membranes based on plasticized or self-plasticized polymers are used. Unfortunately, such polymeric films are quite hydrophobic, and this prohibits the wicking flow of sample across the membrane area, which has been observed when an aqueous sample is attempted to be driven from a hydrophilic channel into an area where the polymeric sensing membrane resides.[22] This problem may hinder the development of truly pump-free paper microfluidics, which is important for integrated multi-ion analysis based on one small volume of sample and one piece of paper with different sensing spots.

Department of Chemistry, University of Michigan, 930 N. University, Ann Arbor, MI 48109-1055, United States.
E-mail: mmeyerho@umich.edu

Here, we report a new paper-based ISO platform that does not employ any additional hydrophobic polymers and plasticizers. The sensing components (chromoionophore, ionophore, and ion-exchanger) are directly adsorbed on cellulose paper via intermolecular forces. The hydrophobic micro-environment that is formed enables ion sensing in analogy to classical ISOs, while aqueous solution can still spread easily on the optical sensing area. In this communication, a sodium ISO is examined initially as an example to demonstrate the feasibility of this plasticizer-free paper-based ISO.

To fabricate the paper-based Na^+ selective optode, 0.58 mg of chromoionophore 1 (CH1, ETH 5294 (Sigma-Aldrich)), 1.1 mg KTFPB (potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (Sigma-Aldrich)), and 2.0 mg sodium ionophore VI (bis[(12-crown-4)methyl] dodecylmethylmalonate (Sigma-Aldrich)) were dissolved in 0.45 mL THF. Then, 2.5 µL of this solution was applied to a piece of Whatman filter paper (Standard, Grade 5, 200 µm in thickness). A sensing circle with a diameter of about 9/32'' was generated within several minutes after the THF evaporated. A Fisherbrand™ 96 well plate (clear polystyrene, flat bottom well) was used for incubation of the sensing paper with sample solutions and taking pictures. To fit the plate well, a handheld circle punch was used to cut a 1/4'' circle from the 9/32'' optode area. The small optode circle was put into the plate well and aqueous solutions (300 µL for each sample) were gently added to the wells to test the optode responses toward increasing concentrations of cations. Notably, polypropylene centrifuge tubes were used as the container for all aqueous stock solutions because disposable glass vials can release a significant amount of Na^+. A Samsung Galaxy cell phone was used to take pictures of the optical sensing paper from the bottom of 96 well plate. To avoid the color interference from variable ambient light, pictures were taken in a dark room, and only the LED flash of the built-in cell phone camera was used as the light source.[23]

Fig. 1 shows pictures of one Na^+-selective optode in 10^{-2} M

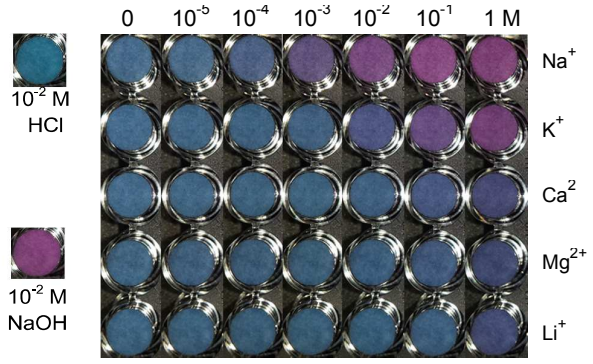

Fig. 1 Pictures of one paper-based Na^+-selective optode in 10^{-2} M HCl and NaOH solution, respectively, and five optodes (from top to bottom) in pH 7.4 Tris-HCl buffer containing increasing concentrations of NaCl, KCl, $CaCl_2$, $MgCl_2$, and LiCl, respectively.

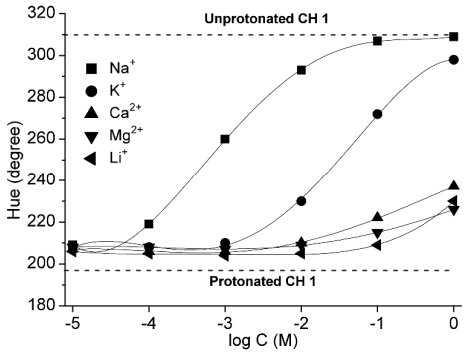

Fig. 2 Hue-based response curves of the paper-based Na^+-selective optode toward different cations. Dashed lines are Hue value of the optode in 10^{-2} M HCl (protonated CH 1) and NaOH (unprotonated CH 1).

HCl and 10^{-2} M NaOH, and five optodes in Tris-HCl buffer (pH 7.4, 0.1 M) and buffers containing increasing concentrations of chloride salts of Na^+ and interfering cations (K^+, Ca^{2+}, Mg^{2+}, Li^+). CH 1 is protonated in acidic aqueous solution and the paper optode is blue, while CH 1 is in its unprotonated form under basic aqueous pH conditions and the optode becomes purplish. In Tris-HCl buffer at pH 7.4, the optode is mainly blue, which indicates that most of the CH 1 indicator species is protonated. Upon contact with buffers containing increasing concentrations of NaCl, the optode color changes from blue to purple. Less significant color changes were observed for the potential interfering cations. The optode takes less than 6 min to reach 95% of the equilibrium response, but all pictures in Fig. 1 were taken after 10 min static incubation of the optode papers pieces with samples. The response time may be improved by optimizing the porosity and thickness of the paper and the density of modified sensing components.

For quantification purposes, optode pictures in Fig. 1 were analysed by ImageJ (http://rsbweb.nih.gov/ij/) to obtain the RGB (Red-Green-Blue) color coordinates. RGB coordinates were converted to HSV (Hue-Saturation-Value) coordinates and Hue was used for quantification since it has been found to provide excellent precision for quantitative colorimetric indication of chromoionophore deprotonation.[24] Fig. 2 shows the Hue values of the optode with fully protonated and unprotonated CH 1 (dashed lines) and Hue-based calibration curves for the different test cations. The dynamic range for Na^+ detection is 10^{-4} to 10^{-1} M. By using the Separation Solution Method, the optode selectivity coefficient can be calculated from the distance between half protonation points of different calibration curves. According to Fig. 2, the selectivity coefficients of this paper-based Na^+-selective optode are -1.7 over K^+ and < -3 over the other cations examined. Such selectivity is comparable to plasticized PVC-based sodium ISE using the same ionophore[25] and meets the selectivity requirement for Na^+ detection in both undiluted and diluted blood/serum samples (a maximum interference of 1%).[26] Since the response behavior (dynamic range and selectivity) of this optode is comparable to traditional

plasticized polymer-based optodes, the present plasticizer-free optode likely shares the same response mechanism as classical ISOs (ion-exchange of primary cation in aqueous phase with H^+ in organic phase to maintain electroneutrality)[5]. Recently, the Bakker group reported their surprising finding on a surface sensing layer-based optode on polystyrene microspheres.[27] Surface modification of these hydrophobic particles by chromoionophore, valinomycin, and cation exchanger was found to enable optical K^+ sensing without using any plasticizer. Based on these two new types of plasticizer-free optodes, we assume that a hydrophobic micro-environment can be formed by the highly hydrophobic sensing components themselves that mimics the plasticized polymer phase of traditional ISOs and enables the phase transfer-based heterogeneous sensing to occur.

One crucial question for the present paper-based optode is whether and how the sensing components can be attracted onto the surface of the paper. Unlike the hydrophobic polystyrene particles employed by Bakker and co-workers, the structure and property of cellulose paper is rather complex. Cellulose exists in both crystalline and amorphous form. In the crystalline form, cellulose molecules are tightly hydrogen-bonded and penetration of other chemicals into such region is prohibited, while the amorphous form is disordered and penetrable by water and other chemicals.[28,29] Therefore, the sensing components can access cellulose chains in their amorphous form and the outer surface of the cellulose crystallite. In the field of cellulose dyeing, it has been demonstrated that cellulose is able to attract dyes based on Van der Waals forces and an unusual synergistic π bond.[30-32] Especially, the existence of aromatic π electrons or more alkyl groups in the dye favors dye binding to the cellulose. The sensing components used here have either aromatic rings (KTFPB) or a long alkyl chain (CH 1 and sodium chromoionophore VI). Therefore, strong adsorption of the sensing components on the accessible cellulose area in paper is not surprising. In addition, although cellulose paper is usually considered highly hydrophilic because water readily adsorbs and spreads, its remarkable hydrophobic nature originating from some non-hydrogen bonding, and non-polar protons on cellulose haves also been well recognized.[33,34] This hydrophobic feature is expected to further enhance the residence of the ion sensing components on the filter paper owing to hydrophobic interactions when paper is soaked in water. Indeed, after soaking a paper circle with the 9/32'' sensing area in 1 mL Tris buffer for 24 h under gentle shaking, the level of KTFPB and CH 1 that leached into the aqueous phase are less than 2%, and the amount of sodium ionophore VI present in the aqueous phase is about 5%. These levels were determined by HPLC-MS (run on Agilent Q-TOF) with positive ion electrospray ionization (for CH 1 and sodium ionophore VI) and negative ion electrospray ionization (for $TFPB^-$). This experiment confirms the strong affinity of sensing components used here toward the filter paper, although this affinity may vary under different experimental conditions.

As mentioned above, the adsorption of these highly lipophilic components is expected to form hydrophobic micro-environment. To confirm that the hydrophilicity of the paper is perturbed by such an adsorbed layer, the contact angle of a hydrophilic solvent (glycerol) on the paper was determined. As shown in Fig. 3, the contact angle on a modified area of the paper (left) is indeed significantly largely than that on raw filter paper (full spreading, right), which indicates an enhanced hydrophobicity. Notably, the contact angle of water was not used because water spreading on modified paper is complete within several seconds although slower than that on unmodified paper. This indicates that significant capillary action is still enabled on the optode paper, which would make this sensing system fully compatible with microfluidic devices using patterned paper.

Fig. 3 A droplet of glycerol on a filter paper area (blue) modified with optode sensing ingredients (left) and on an unmodified paper area (right). Pictures were taken about 5 s after addition of the droplet and glycerol will further spread after longer time.

Similar to classical ISOs, the paper-based optode is reversible. When an optode was repeatedly exposed to 10^{-3} M and 10^{-2} M NaCl for 10 min, the Hue values (mean value ± standard deviation) for five measurements were 258 ±0.7° and 291±0.4° for 10^{-3} M and 10^{-2} M NaCl, respectively. As expected, it is necessary to rinse the plate well and paper optode with buffer after exposure to a high concentration of NaCl to avoid contaminating the low concentration sample. However, like most paper-based sensors, the paper-based ISO described herein is expected to be employed as a single-use strip.

In conclusion, the unique affinity of cellulose paper toward hydrophobic sensing components employed to prepare ISOs has been explored and an adsorption layer-based plasticizer-free ISO platform for detection of Na^+ has been demonstrated. Based on this platform, the development of a paper microfluidics device that enables simultaneous detection of multiple ions by using one drop of sample is ongoing in our lab. If a nanoporous cellulose paper is used,[35] the strong affinity of cellulose toward hydrophobic ionophores and ion-exchangers may be utilized to develop plasticizer-free ISEs in analogy to the Au-coated nanoporous membrane-based ISE[36] but with better compatibility with commercial ionophores. Work in this direction is also being pursued.

This work was financially supported by the National Institutes of Health (Grant EB-000784).

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