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Authors: Xuewei Wang; Qi Zhang; Changwoo Nam; Michael Hickner; Mollie Mahoney; Mark Meyerhoff

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Ionophore-based anion-selective optode printed on cellulose paper

Xuwei Wang,^{*,[a]} Qi Zhang,^[a] Changwoo Nam,^[b] Michael Hickner,^[b] Mollie Mahoney^[a] and Mark. E. Meyerhoff ^{*,[a]}

Abstract: We report a general anion sensing platform based on a portable and cost-effective ion-selective optode and a smartphone detector equipped with a color analysis app. In contrast to traditional anion-selective optodes using a hydrophobic polymer and/or plasticizer to dissolve hydrophobic sensing elements, the new optode relies on hydrophilic cellulose paper. The anion ionophore and a lipophilic pH indicator are inkjet-printed and adsorbed on paper and form a "dry" hydrophobic sensing layer. Porous cellulose sheets also allow the sensing site to be modified with dried buffer that prevents any sample pH dependence of the observed color change. A highly selective fluoride optode using an Al(III)-porphyrin ionophore is examined as an initial example of this new anion sensing platform for measurements of fluoride levels in drinking water samples. In addition to Lewis acid-base recognition, hydrogen bonding recognition is also shown to be compatible with this sensing platform.

Analysis of anions continues to attract considerable attention because anions play important roles in biological and environmental systems. Supramolecular recognition of anions has been achieved by electrostatic attraction, hydrogen bonding, Lewis acid-base coordination, anion- π interaction, and halogen bonding.^[1] However, most anion recognition systems only operate effectively in organic solvents/polymers/plasticizers or organic-aqueous mixtures, which often are incompatible with the targeted environmental or biological application.^[2] Ionophore-based ion-selective optodes (ISO), the optical counterpart of ion-selective electrodes (ISE), represent an elegant sensor configuration that takes advantage of supramolecular ion recognition in an organic phase, but detects the target anions in aqueous samples via a co-extraction equilibrium process between the phases.^[3] Such ISOs have been developed for inorganic anions such as Cl^- , F^- , CN^- , SCN^- , ClO_4^- , PO_4^- , CO_3^{2-} , SO_4^{2-} , NO_3^- , NO_2^- , and organic anions such as acetate, citrate, and salicylate.^[3] These optodes typically use a water-immiscible organic medium doped with anion receptors (ionophores). Upon contact with an aqueous sample, the anion analyte is extracted into the organic phase via ion recognition (e.g., hydrogen bonding or Lewis acid-base interaction) with the ionophore. A proton-sensitive dye (chromoionophore) is typically also doped into the organic phase as an optical read-out unit. Co-extraction of the target anion and a proton from the aqueous sample into the organic film protonates the chromoionophore

and can yield a visible change in color or fluorescence signal. Depending on the charge of the ionophore, an anion exchanger may be used to facilitate the anion extraction. Such optodes typically rely on use of an organic plasticizer, plasticizer-polymer mixture, or self-plasticized polymer as a medium to dissolve the sensing ingredients, and may be employed in different configurations, including films, micro/nano-particles, or liquid to satisfy requirements of different applications.^[3, 4]

Over the past decade, paper-based analytical devices (PADs) have been developed for a wide range of analytes including ions, metabolites, proteins, nucleic acids, and bacteria, providing new devices for environmental analysis and medical diagnosis, especially in resource-limited settings.^[5] Cellulose paper as a sensor substrate: i) has excellent sample wicking ability and allows pump-free microfluidics; ii) has strong ink absorbency and is highly compatible with inkjet printing technology; iii) is easy to be chemically modified (non-covalently or covalently); and iv) is simple to stack, carry, and transport. Ionophore-based ISEs have also been designed on cellulose paper^[6] and a new paper-based plasticizer-free Na^+ ISO has been proposed recently by our group.^[7] However, anion sensing chemistries typically employed in ISOs have not yet been adapted to a cellulose paper-based sensing scheme.

Herein, we report a novel type of anion-selective optode that uses adsorption of hydrophobic sensing ingredients directly on cellulose paper rather than their dissolution in a bulk organic phase matrix. Such optodes can be fabricated not only on paper but also other cellulose materials such as cloth (e.g., cotton fabric) and particles (e.g., cellulose nanocrystals), thereby enabling applications in creating wearable sensors and use for bioimaging. However, the present work only focuses on paper-based optodes and their application as an ultralow cost strip type sensor for detecting anions.

A large number of anion ionophores recognize targets based on metal-anion coordination, a type of Lewis acid-base interaction. Metalloporphyrin, metallophthalocyanines, metal-salophen complexes, and other organometallic compounds have been used for sensing anions such as Cl^- , NO_2^- , PO_4^{3-} , F^- , I^- , and salicylate.^[3,4,8,9] Herein, a fluoride-selective optode based on Al(III) octaethylporphyrin (Al[OEP]) as the ionophore^[9] is employed to demonstrate the new paper-based ISO concept.

^a Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA. E-mail: wangxue@umich.edu (X. W.); mmeyerho@umich.edu (M. M.)

^b Department of Materials Science and Engineering, Pennsylvania State University, University Park, PA 16802, USA.

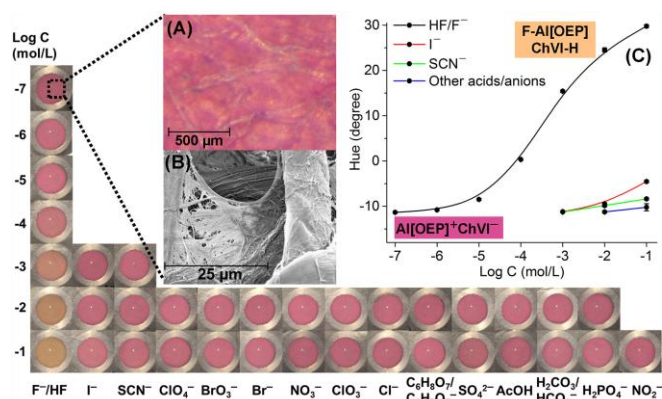


Figure 1. Pictures of optodes in 30 μL of 0.1 M β -alanine-phosphate buffer at pH 3.6 containing different anions/acids after an incubation time of 5 min. Inset A: microscopic image; Inset B: SEM image; Inset C: hue-based calibration curves (negative degrees were obtained by subtracting 360 from actual degrees).

In contrast to using a traditional spin coating or manual coating technique for optode fabrication, inkjet printing was used as a precise, rapid, and reproducible reagent deposition method.^[10] Equal moles of Al[OEP] and chromoionophore VI (4',5'-dibromofluorescein octadecyl ester; ChVI) dissolved in cyclohexanone were printed onto Whatman filter paper by a Dimatix inkjet printer. The optode is asymmetric and the sensing ingredients mainly reside on the side that faces the cartridge during printing (Figure S1). Images of the printed side taken by optical and scanning electron microscopes are shown in Figure 1 insets A and B, respectively. Initial attempts to confirm the chemical modification involved use of attenuated total reflectance Fourier transform infrared spectroscopy and energy-dispersive X-ray spectroscopy that can characterize films with penetration depths of 0.5–2 μm and 1–3 μm , respectively. However, no characteristic signals of the Al[OEP] or ChVI species could be identified on top of the cellulose background. In contrast, as shown in Figure 2, peaks for N (only existing in Al[OEP]) and Br (only existing in ChVI) were clearly observed using X-ray photoelectron spectroscopy (XPS) as a surface-sensitive technique. This suggests that the printing modification

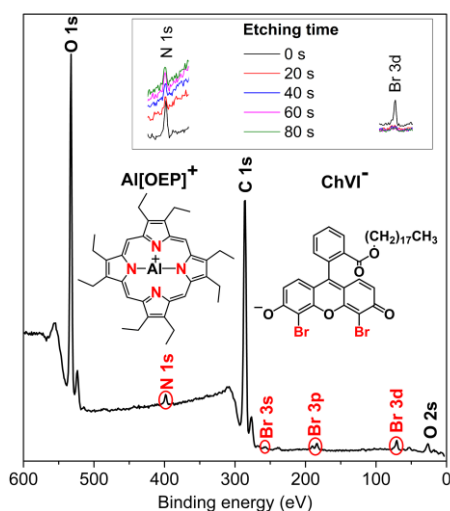


Figure 2. XPS survey spectrum of F^- optode (see Figure S2 for spectrum of raw filter paper). Inset: N 1s and Br 3d peaks after ion beam etching.

likely takes place primarily on the surface of the cellulose material. Using high resolution C1s spectra (Figure S3), the C-N bonds from Al[OEP] and an increase in C-C/C-H ratio resulting from ChVI with an 18-carbon chain were also observed after modification. In filter paper, cellulose chains organize into microfibrils that consist of crystalline and non-crystalline domains, and microfibrils further pack into fibers.^[11] Ink for optode fabrication could access and modify the surface of fibers with ease. However, crystalline domains are impenetrable toward water and most solvents, including cyclohexanone, because of substantial inter- and intra-molecular hydrogen bonding. Therefore, the crystalline bulk of cellulose hydrogen is difficult to dope with the sensing ingredients. In contrast, penetration of ink into the fiber bulk could take place through loosely-ordered non-crystalline domains. The thin layer surface modification and limited bulk modification through non-crystalline defects is further supported by the following observations: i) XPS depth profiling analysis (see inset of Figure 2) shows that a large portion of sensing ingredients were eliminated by only 20 s of ion beam etching (corresponding to an etching thickness of a few nm or less^[12]) but the amount of sensing ingredients levels off for the subsequent etching cycles (resulting from in-depth modification); ii) in microscopic pictures (Figure S1), large fibers show an accumulated reddish color on the surface, but only limited reddish domains from the fiber bulk.

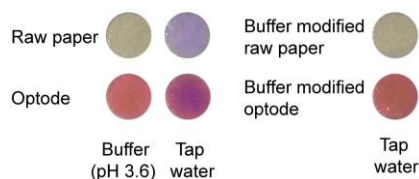
Furthermore, Al[OEP] and ChVI were found to remain on the cellulose paper in the presence of aqueous solutions. Indeed, after soaking the optode in β -alanine-phosphate buffer at pH 3.6 for 24 h with shaking, the amount of sensing compounds that leached from the paper were below 1% based on HPLC-MS analysis (run on Agilent Q-TOF). The preference of these compounds to adhere to cellulose paper rather than be solubilized in aqueous solution is likely due to both their low hydrophilicity and their strong adsorption on cellulose. Indeed, the distribution coefficients ($\log D$) of Al[OEP] cation and ChVI in its dissociated form are as high as 12.7 and 10.4, respectively (predicted by ChemAxon). The strong adsorption of these compounds on cellulose paper could be attributed to the hydrophobic interaction of these sensing compounds with hydrophobic domains within cellulose^[13] as well as intermolecular interactions such as hydrogen bonding and van der Waals forces^[14]

Figure 1 shows pictures taken by a smartphone for the fluoride sensing optodes in pH 3.6 buffer containing different concentrations of various anions/acids. To quantify the color change, the hue of each picture was extracted by the app “Color Mate - Convert and Analyze Colors” (Figure S4). Figure 1C shows the hue-based calibration curves for F^-/HF and other anions/acids. As can be seen, an increasing concentration of F^-/HF induces a significant increase in hue with a dynamic range of 10^{-6} M to 10^{-1} M. In contrast, up to 10^{-3} M of I^- and SCN^- , and up to 10^{-2} M of other anions do not induce a detectable color change, demonstrating the excellent selectivity of this paper-based fluoride selective optode.

It is known that Al[OEP] and ChVI dissolved in PVC-*ortho*-nitrophenyloctyl ether respond optically to F^- based on anion/proton co-extraction into the plasticized PVC.^[9] The selectivity and sensitivity of the adsorption-based ISO on paper are similar to its plasticizer-based equivalent, suggesting that the

paper-based optode likely follows the same mechanism. Indeed, the zeta potential of the fluoride optode described here is relatively constant when the F^-/HF concentration increases from 0 to 10^{-3} M (Figure S5). Therefore, the absorption layer maintains its electroneutrality upon contact with F^-/HF , confirming simultaneous binding of F^- and protons into the

Figure 3. Pictures of 10 μ L of pH 3.6 buffer solution or tap water on raw filter paper (top) and F^- optode (bottom). Optodes on the right have been modified with dry pH 3.6 buffer. 0.1 mg/mL bromophenol blue was added in aqueous solutions as a pH indicator, which is blue at pH >4.6 and yellow at pH <3.0.



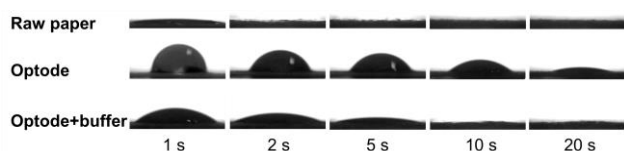
adsorbed layer. Although only the absorbance of ChVI at 537 nm was used in spectrophotometric fluoride sensor,^[9] both anion

ligation on Al[OEP] and protonation of ChVI contribute to the color change of the paper-based optode. Notably, a chromoionophore was used here because it has been found to be necessary to improve the response time and reversibility of anion optodes.^[9]

As expected, the co-extraction mechanism results in cross-sensitivity toward protons in aqueous samples. Such a pH dependence is a known limitation of all optodes based on proton-sensitive dyes. Also, metalloporphyrins as anion ionophores suffer from interference by higher levels of OH^- ^[15] (see Figure S6 for pH dependence of the fluoride optode). Therefore, metalloporphyrin-based optodes only function reliably under constant acidic pH conditions, although the pH of most real samples is near-neutral and variable (e.g., drinking water has a pH range of 6 to 8.5). Therefore, in this work, a solid buffer was fully integrated over the entire ISO to provide a pH-independent fluoride ion analysis. The optode was immersed into the buffer used in Figure 1 and dried at 37 °C for 20 min. No aggregated powders or crystals of buffer were observed by SEM (Figure S7), suggesting that the buffer was deposited on sensing paper in a homogeneous fashion. The effectiveness of such dry buffer to adjust pH of drinking water is confirmed via the results shown in Figure 3. Notably, modification of traditional optodes with buffer has not been reported previously probably because adsorption of a hydrophilic buffer on a non-porous hydrophobic membrane is difficult.

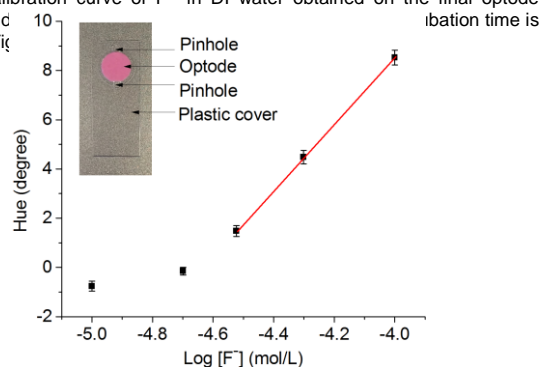
To prevent the optode from long term exposure to air (e.g., moisture and oxygen) and enhance its storage stability, the optode (as a 1/4" paper circle) was sealed into a Scotch thermal laminating pouch by a laminator. Although hue of an unlaminated dry optode increases by >5 after only one day of dark storage at room temperature, a negligible hue change (< 1) of the sealed optode has been observed after 3 months under the same storage conditions. To load samples into the sealed optode, one or more holes could be made in the pouch and aqueous sample will spread across the entire optode paper. Indeed, strong wicking ability is a unique advantage of paper

Figure 4. Pictures of water drops on various paper surfaces at different time points after drop addition.



compared to commonly used plasticized PVC. Figure 4 shows the water contact angles on different paper-based surfaces. The modification of paper with the hydrophobic sensing ingredients made the surface more hydrophobic, but water could still spread. Secondary modification with buffer yields an even more hydrophilic surface than the base optode, which further facilitates sample spreading. Within the laminate shown in Figure 5, the optode paper was fully wetted within 10 s by simply immersing the optode end of the laminate into aqueous samples. Figure 5 shows the calibration curve toward F^- in DI water obtained with the final optode system, which was used for

Figure 5. Calibration curve of F^- in DI water obtained on the final optode modified with c (10 min (see Fig. 5)).



testing of tap water samples in Ann Arbor, Michigan. The F^- concentrations measured by the color change agree well with those measured by a standard lanthanum fluoride-based ISE method (within 10% error; see Table S1). Such an analytical system is well-suited for detection of F^- in various drinking water supplies by non-professional personnel or in resource-limited settings. This is because of the following features: i) the users do not need to pre-treat the sample, which is advantageous compared to commercial optical fluoride strips/kits (requires addition of 1 M HCl) and ISE (requires addition of ionic strength adjuster); ii) a smartphone with a color analysis app is more reliable than the naked eye, but does not bring extra cost of a photometer or a high impedance pH/mV meter; and iii) the total cost for such strip is ~ \$0.012 (Table S2).

The paper-based plasticizer-free optode also works for sensing levels of other anions using different metal-based Lewis acid ionophores such as In(III) octaethylporphyrin^[16] and [9]-mercuracarborand-3^[17] (data not shown). Another mechanism

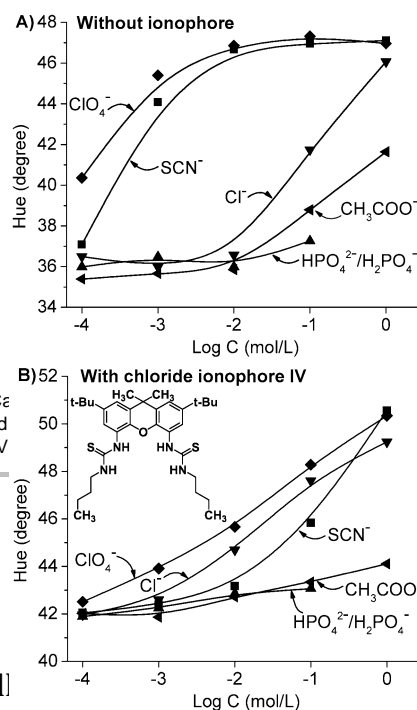


Figure 6. $C_6 7.5$ obtained with ionophore IV

of anion recognition for creating ISOs is hydrogen bonding. Therefore, a preliminary experiment has been conducted for a known hydrogen bonding-based ionophore. Both, tridodecylmethylammonium chloride (TDMAC), chromoionophore IV (ChIV), and a chloride ionophore IV (a thiourea type hydrogen bonding-based receptor; for Figure 6B only) were printed onto filter paper and colorimetric analysis was performed by the same smartphone system as used for fluoride detection. As shown in Figure 6, the addition of ionophore increased the logarithmic selectivity coefficients^[3a] toward Cl⁻ over hydrophobic SCN⁻ and ClO₄⁻ by at least 2 orders of magnitude, and over hydrophilic acetate anion by ~ 1 order of magnitude. This confirms that hydrogen bonding recognition used in plasticizer-based ion-selective sensors could be extended to our paper-based optode configuration as well.

In conclusion, we have demonstrated that cellulose paper as a solid substrate is able to replace plasticized polymer films to create anion-selective optodes. With advantages of easy printability, low cost, low toxicity, good compatibility with pH adjuster and microfluidics, paper-based ISOs hold great promise for anion analysis of aqueous samples. In future work, we will not only examine more sensing compounds developed for traditional ISOs but also design new compounds to enhance their interaction with cellulose and/or tune the dielectric properties of the adsorption layer, which may result in improved and flexible analytical performances.

Acknowledgements

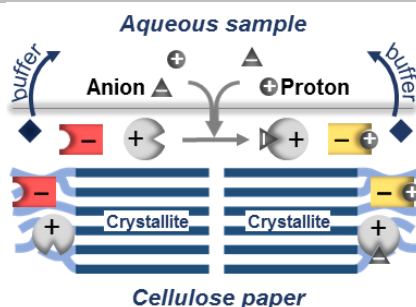
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Keywords: analytical methods • sensors • cellulose paper • anion-selective optode • inkjet printing

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COMMUNICATION

Cellulose paper as a sole substrate allows adsorption of a lipophilic anion ionophore and pH-sensitive indicator dye to enable heterogeneous anion sensing via an anion-proton co-extraction mechanism. This platform also enables adsorption of a buffer salt as the sample pH adjuster to prevent pH dependence of the optical anion response. The adsorption takes place on the surface of both crystalline and non-crystalline domains, and the bulk of only loosely packed non-crystalline domains.



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