The Effect of Paper on the Detection Limit of Paper-Based Potentiometric Chloride Sensors

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Abstract

While paper is an excellent material for use in many other portable sensors, potentiometric paper-based sensors have been reported to perform worse than conventional rod-shaped electrodes, in particular in view of limits of detection (LODs). Reported here is an in-depth study of the lower LOD for Cl^- measurements with paper-based devices comprising AgCl/Ag transducers. Contamination by Cl^- from two commonly used device materials—a AgCl/Ag ink and so-called ashless filter paper—was found to increase the concentration of Cl^- in paper-contained samples far above than what is expected for the spontaneous dissolution of the transducer's AgCl, thereby worsening lower LODs. In addition, for the case of Ag^+, the commonly hypothesized adsorption of metal cations onto filter paper was found not to significantly affect the performance of AgCl/Ag transducers. We note that in the context of chemical analysis, metal impurities of paper are often mentioned in the literature, but Cl^- contamination of paper has been overlooked.

High mechanical stability, biodegradability and a low cost make paper an attractive material for the design and fabrication of analytical devices.[1,2] Filter paper in particular has long been used in chemical analysis due to its ability to wick solutions through capillary action, whether for the urine test strips developed first in 1880,[3–7] for the pioneering work with paper chromatography in the early 1900s,[8] or for the electroanalytical Kodak Ektachem slides of the 1980s.[9] The current popularity of paper-based devices resulted to a large extent from the simple and affordable wax printing and melting techniques used to create hydrophobic barriers and control liquid flow, as introduced by the Whitesides and Lin groups in 2009.[2,4] The term "paper-based device" does not have a single definition, though. Rather, it has been used in a generic manner to describe any device in which at least one component is made of paper.[10] The most common role of paper is sample collection and wicking, with sensing components either attached to, printed on top of, or integrated into the paper itself.

In the field of potentiometric sensors, paper has been used either to hold the sample (such as in strip-type sensors dipped into sample solutions to collect samples),[11–13] to wick samples to conventional rod-shaped electrodes,[14,15] or to serve as a platform substrate onto which the sensing components are placed.[16–18] Although many paper-based devices of this type respond linearly in the clinically relevant range, many have either lower LODs worse than conventional rod-shaped ion-selective electrodes (ISEs) or exhibit non-Nernstian response slopes. For sandwich-based designs, in which the sensing membrane is placed between two pieces of filter paper, a worse LOD was found for both a divalent anion (bilirubin)[19] and a monovalent cation (K^+)[20]. For more integrated setups, in which membranes are embedded into the filter paper, an order of magnitude or more worsening of LOD was observed for Cl^- and K^+.[16,17,21] Nanomolar

lower LODs have been achieved with strip-type paper-based sensors for Cd^{2+}, Ag^{+}, and K^{+}, but only when the paper components of those devices were coated with carbon nanotubes and were not in direct contact with the samples.[22] A worsened lower LOD has been reported even when paper is only the sample holder to measure Cd^{2+}, Pb^{2+}, Ag^{+}, and K^{+} with large rod-shaped ISEs.[14,23–25] Paper sampling was also found to result in super-Nernstian responses when detecting Cd^{2+} and Pb^{2+} with solid-state and solid-contact ISEs, respectively.[23] Such deviations from the theoretically predicted response slope are indicative of interactions between paper and the target ion or kinetically limited processes, restricting device reproducibility. While pre-treating paper with inorganic salts of the target ions resulted in Nernstian response slopes, the lower LODs of such devices were still worse than for conventional potentiometric sensors.[23]

We describe here limitations of paper-based potentiometric sensors with different types of AgCl/Ag and AgBr/Ag transducers. In an ideal system, the lower LOD of these solid-state ISEs is determined by dissolution of the silver halide into the aqueous sample, which is controlled by the very low solubility product of the silver halide.[26] However, we observed higher than expected LODs for Cl^{-} sensing. We show here that Cl^{-} contamination from AgCl/Ag ink is a key factor in worsening of the lower LOD, as is, surprisingly, Cl^{-} contamination of the paper.

We wondered whether Cl^{-} contamination of samples also results from interactions of Ag^{+} with paper, lowering the activity of free Ag^{+} and increasing solubility of AgCl. Indeed, as early as 1960, Pickering and co-workers reported adsorption capacities of Whatman filter paper for Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} on the order of 4 μg/g paper, values that are reduced in acidic solutions and solutions with high backgrounds of K^{+} or Mg^{2+} salts.[27] They also noted an increased concentration of Mg^{2+} and Ca^{2+} in solutions after exposure to paper. This led them to conclude that adsorption

of heavy metal ions to paper results from ion exchange with H^+, Mg^{2+} and Ca^{2+}, all ions that are probably present in filter paper mostly as counter ions to the many carboxylate groups of the cellulose polymer chains.[27] More recent work showed that treatment of paper pulp with peroxide and alkaline solutions increases the adsorption of divalent cations to paper, both treatments that increase the number of carboxylate groups.[28,29] Consistent with these observations, Ota et al. showed that the transport of cations through cellulose-based materials with negatively charged surfaces is significantly decreased as compared to other types of paper.[30] While evidence for the interactions of various cations with filter paper is plenty, only few equilibrium parameters, such as binding constants describing cations to paper, have been reported. Metal adsorption onto cellulose nanomaterials has been quantified with a view to water remediation, but such studies reported adsorption to functionalized rather than non-functionalized cellulose such as filter paper.[31–33] To this end, we also quantified binding of Ag^+ to paper.

Experimental Section

Materials. Reagents were purchased from the following sources: KCl, $AgNO_3$, citric acid, and Whatman grade 1 filter paper from Sigma-Aldrich (St. Louis, MO, USA); sodium citrate dihydrate from Mallinckrodt (St. Louis, MO, USA); unflavored gelatin from Kraft (Chicago, IL, USA); AgCl/Ag ink (AGCL-675; consisting, according to the supplier's material data safety sheet, of 40–60% silver, 10–25% AgCl, 25–50% γ-butyrolactone, and 5–15% urethane acrylate oligomer) from Nayaku Advanced Materials (Westborough, MA, USA); Polx1200 polyester continuous knit filament cleanroom wipes from Berkshire Corporation (Great Barrington, MA, USA); and gold disk electrodes (2 mm diameter; embedded into an inert Kel-F polymer shaft) from CH Instruments

(Austin, TX, USA). In-house deionized water was purified to a resistivity of 18.2 MΩ/cm with a Milli-Q PLUS reagent-grade water system (Millipore, Bedford MA, USA) and used for all experiments involving water.

Paper Adsorption Studies. Six stock solutions of $AgNO_3$ in the range from 10^{-5} to 10^{-2} M were prepared. For both paper and textile analysis, 6 g of material was separately added to 25 mL of each $AgNO_3$ solution. After 2 min, an aliquot of the solution was removed with a plastic syringe, and the concentration of Ag in these samples was determined with an iCAP 7600 inductively coupled plasma optical emission spectrometer (ICP-OES; ThermoFisher, Waltham MA, USA).

Fabrication of Paper-Based Sensors. Microfluidic zone barriers were hand-drawn in matching patterns on both sides of paper or textile substrates using China Wax pencils (Sharpie brand, Atlanta, GA, USA). For each of the two electrical connections, AgCl/Ag ink was applied to one side of the paper or textile using a rubber-tipped paintbrush (Royal Sovereign, UK) to achieve uniform coverage. This was followed by curing for 10 min at 100 °C in ambient atmosphere to both melt the wax and allow it to permeate through the thickness of the substrate as well as to let evaporate the solvent contained in the AgCl/Ag ink. For devices comprising AgCl or AgBr coated Ag wires and AgCl coated Ag plates, no AgCl/Ag ink was applied.

Ink-Coated Gold Electrodes. The 2 mm diameter gold disk electrodes were polished over polishing cloths with aqueous dispersions of alumina (0.3 and 0.05 µm, Buehler, Lake Bluff, IL, USA). They were then cleaned in piranha solution (concentrated sulfuric acid and 30% hydrogen peroxide solution in a 3:1 ratio). *Caution: piranha solution is a strong oxidizing reagent, is highly corrosive, and should be handled with care*. The electrodes were then cleaned by ultrasonication

in water and ethanol and dried with a flow of nitrogen. A continuous coating of the AgCl/Ag ink was applied using a rubber-tipped sculpting brush and allowed to dry overnight. A double-junction type external reference electrode (DX200, Mettler Toledo, Switzerland; 3.0 M KCl saturated with AgCl as inner filling solution and 1.0 M LiOAc as bridge electrolyte) was used for measurements with this type of electrodes.

Potentiometric Measurements. Electrode potentials were measured using an EMF 16 high-impedance voltmeter (input impedance 10 TΩ) controlled by EMF Suite 1.03 software (Lawson Labs, Malvern PA, USA). For devices with a AgCl or AgBr coated Ag wire as transducer, small holes were made in the reference and sample zones of the paper and the wires were inserted through these holes (Figure S1, Supporting Information). When using a AgCl/Ag plate, the plate was held flush against the paper with binder clips. (See the Supporting Information for the preparation of AgCl coated Ag wires and plates.) Two alligator clips were used to connect the devices to the voltmeter, and all devices were placed on top of a sheet of PVC and held in place with binder clips for stability. Both the plates and wires were stored in 0.1 M KCl solution saturated with AgCl when not in use and washed with H_2O before each use and between uses. For each device, 20 µL each of the aqueous sample and reference solution were simultaneously deposited into the respective zones. It took approximately 30 s for the solutions to fully wet the paper; the recorded response was the average EMF over the following 30 s. Activity coefficients were calculated according to a two-parameter Debye-Hückel approximation,[34] and EMF values were corrected for liquid-junction potentials with the Henderson equation.[26]

For measurements with acidic sample solutions, 0.55 M citrate buffer (pH 2.4) was used. This concentration was chosen to ensure that the paper would not affect the pH, as the

concentrations of acidic groups on cellulose nanofibers and pulp have been reported as 100 µmol/g cellulose[32] and 50-200 µmol/g pulp,[29,35–37] respectively (approximately equivalent to a 0.1 M concentration when a 20 µL droplet of sample is placed onto a 2 cm^2 section of paper, as typical for the work described here).

Leaching of Cl^- from Paper and Textile. To determine its Cl^- content, 2.3 g filter paper was dry ashed, dissolved in 10 mL 10 wt % HNO_3, and analyzed with the mercury(II) thiocyanate method, using a Lachat QuikChem8500 flow injection analyzer.[38] To assess the possibility of Cl^- contamination of aqueous samples upon brief contact with filter paper or pieces of textile, 35 mL of H_2O was exposed to 10 g of paper or textile. After 5 min, a water sample was removed using a separate plastic syringe and analyzed for Cl^-.

Leaching of Cl^- from Ink. To assess leaching of Cl^- from AgCl/Ag ink, 6 glass vials were coated on their inner walls with AgCl/Ag ink and dried overnight under vacuum. Then, 4 mL water was added to each vial and stirred with a magnetic stir bar for 5 min. Then, the solution was removed and analyzed potentiometrically for Cl^- using a AgCl/Ag coated Ag wire as ISE and a capillary reference electrode (AgCl/Ag, 3.0 M KCl inner filling solution).[39]

Results and Discussion

Performance of AgCl/Ag Ink Transducers in Absence of Paper. For this work, a commercial AgCl/Ag ink was initially used to prepare Cl^- sensors because such inks are commonly used as ion-to-electron transducers in paper-based electrochemical devices.[2,16,17,19,20,40] Besides Ag nanoparticles and AgCl, this ink also contained according to the supplier a urethane acrylate oligomer and γ-butyrolactone. We first examined the performance of the AgCl/Ag ink using 100

mL beakers to contain the samples. For this purpose, commercial rod-shaped electrodes were coated with a layer of the AgCl/Ag ink, the solvent was allowed to evaporate, and potentiometric measurements with the thus prepared ISEs were performed with respect to a conventional double-junction reference electrode. Lower LODs were determined by extrapolation of the linear EMF response to the value observed when no target ion was added to the sample, as this is standard practice in the ISE literature.[41]

In this type of electrochemical cell, the only phase boundary potential expected to depend on the sample is at the interface of the sample and the AgCl.[26] Consequently, the measured electromotive force (EMF) depends on the activity of Cl^- ions according to the Nernst equation, $EMF = E° - 58.2\ \mathrm{mV} \log a_{Cl^-}$ (response slope for room temperature). The lower LOD is expected for both Cl^- and Ag^+ to be $10^{-4.9}$ M, as given by the square root of the solubility product of AgCl ($1.6 \times 10^{-10}\ M^2$).[26]

The ink-coated Au electrodes responded to Cl^- with a lower LOD of $10^{-4.8 \pm 0.1}$ M Cl^- (n=3), and to Ag^+ with a lower LOD of $10^{-5.0 \pm 0.1}$ M Ag^+ (n=4), as shown in Figure S1. These values are within error of what theory predicts, confirming that in the absence of paper these ISEs perform in an ideal manner. Therefore, we proceeded to use paper-based devices using the same AgCl/Ag ink.

Performance of Paper-Based Devices with AgCl/Ag Ink Transducers. A dumbbell-shaped wax pattern was drawn onto rectangular pieces of ashless filter paper to define separate reference and sample zones as well as a central contact area (see Figure 1A and Figure S2 of the Supporting Information), as introduced by Lan et al.[42] For potentiometric measurements, 20 µL 0.1 M KCl reference solution was applied to the rectangular area in contact with the reference

electrode, and 20 µL sample solution was applied to the rectangular zone in contact with the indicator electrode. The solutions were then allowed to wick into the diamond-shaped contact area to contact one another (see Figure 1A). This procedure results in three phase boundary potentials affected by the deposited solutions. The size of the liquid junction potential that arises at the phase boundary between the 0.1 M KCl reference solution and the sample solution can be predicted using the Henderson equation and is kept small by use of a highly concentrated solution of KCl.[26] The phase boundary potential at the reference solution/AgCl/Ag interface is determined by the concentration of the reference solution, and is, therefore, independent of the sample. Consequently, the measured EMF (which comprises the sum of all phase boundary potentials) only varies with the phase boundary at the sample/AgCl/Ag interface, which depends upon the Cl^- concentration in the sample as predicted by the Nernst equation.

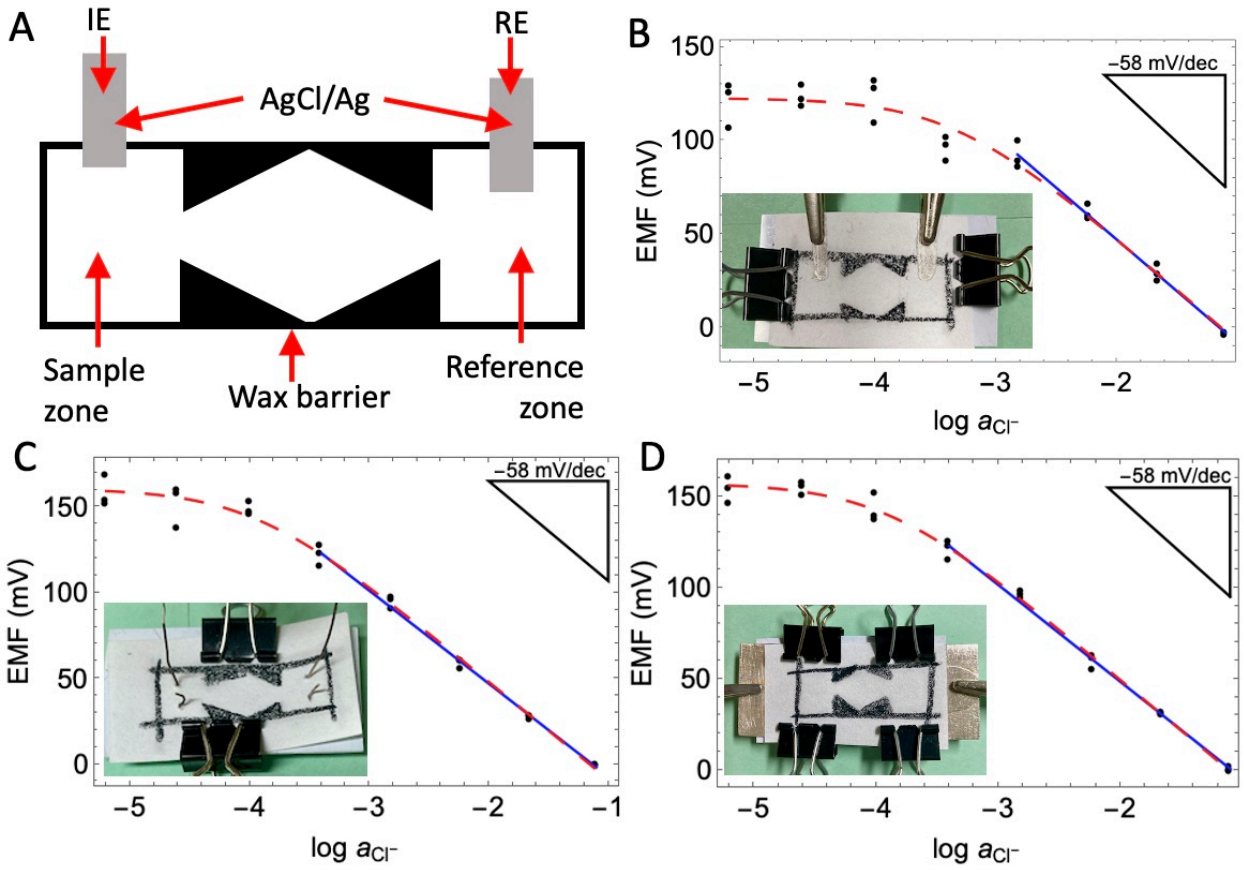

Figure 1. (A) Schematic of paper-based devices used (RE: reference electrode, IE: indicator electrode). Potentiometric responses to KCl solutions as measured with Cl^- sensing devices with (B) ink, (C) wire, and (D) plates as AgCl/Ag transducers; insets show photographs of the corresponding device setups. Blue solid lines are fits of the linear portions of the response curve, and red dashed lines are non-linear fits of all data using a modified Nicolskii–Eisenman equation accounting for a lower LOD (i.e., $EMF = E° - 58.2$ mV log (a_{Cl^-} + LOD), where a_{Cl^-} is the activity of Cl^- in the sample).

The potentiometric responses to Cl^- of paper-based devices with AgCl/Ag ink transducers are shown in Figure 1B–D and Table 1. Each concentration was measured with 3 different devices.

Because these are single-use devices, a total of 24 devices contributed to each calibration curve. Use of these paper-based devices with AgCl/Ag ink directly applied to the paper resulted in a lower LOD of $10^{-3.4 \pm 0.3}$ M Cl^-, as shown in Figure 1B. This LOD is significantly higher than the value of $10^{-4.9}$ M, as expected from the solubility of AgCl and as indeed observed in the beaker-based measurements. However, it is consistent with detection limits previously reported for paper-based potentiometric devices comprising electrodes prepared with a AgCl/Ag ink.[20]

Table 1. Potentiometric responses to Cl^- of devices with different types of AgCl/Ag transducers (see Figure 1).

AgCl/Ag Form	Setup	Slope (mV/decade)	LOD (M)
Ink	Paper-based	–55.3 ± 4.0	$10^{-3.4 \pm 0.3}$
Ink	Textile-based[43]	–56.8 ± 1.3	$10^{-4.1 \pm 0.1}$
Ink	Paper-based (acidic solutions)	–63.4 ± 2.5	$10^{-3.3 \pm 0.2}$
Ink	Textile-based (acidic solutions)	–61.1 ± 5.0	$10^{-3.9 \pm 0.3}$
Ink	Au electrodes in beaker	–54.9 ± 0.5	$10^{-4.8 \pm 0.1}$
Ink*	Au electrodes in beaker*	+54.4 ± 1.4	$10^{-5.0 \pm 0.1}$
Wire	Paper-based	–54.2 ± 2.0	$10^{-4.0 \pm 0.2}$
Wire	Paper-based (0.01 wt % gelatin)	–53.4 ± 2.4	$10^{-3.5 \pm 0.2}$
Wire	Textile-based	–55.0 ± 1.0	$10^{-4.4 \pm 0.1}$
Wire	Beaker	–57.5 ± 0.4	$10^{-4.9 \pm 0.1}$
Wire	Beaker*	+55.7 ± 0.8	$10^{-4.8 \pm 0.1}$
Plate	Paper-based	–54.2 ± 2.0	$10^{-3.9 \pm 0.1}$
Plate	Beaker	–58.6 ± 1.2	$10^{-4.8 \pm 0.1}$

*Data corresponds to Ag^+ sensing

Characterization of the AgCl/Ag Ink. Given the discrepancy in the results from the experiments with, on one hand, the beakers and, on the other hand, the paper-based devices, we characterized the ink in view of impurities. For that purpose, 4 mL water was added to reaction vials previously coated on their inside walls with AgCl/Ag ink, and, after 5 min of stirring, those water samples were transferred into other vials for potentiometric determination of the Cl^- concentration using a AgCl/Ag wire transducer as indicator electrode. Water stored for 5 min in non-coated control vials did not contain any measurable Cl^-, but water stored for 5 min in the ink-coated vials contained $10^{-3.7\pm 0.1}$ M Cl^- (n=3). This corresponds to 90 ± 30 ppm leachable Cl^- in the ink, and we note that there may well be more Cl^- leaching into samples if the leaching time were longer. Because the Cl^- concentration in the aqueous sample exceeded the value predicted by the solubility of AgCl considerably, it follows that most of this leachable Cl^- is present in the ink in a form other than AgCl. To the best of our knowledge, there are no previous reports of Cl^- contaminants in commercial AgCl/Ag inks with which to compare this value.

These result show that while ppm level reagent impurities in the AgCl/Ag ink do not affect measurements in large samples, they cannot be ignored for measurements with microfluidic devices and even for sample volumes of a few milliliters. Assuming an extent of mass transfer comparable to that observed in the experiments with the internally coated vials, leaching of Cl^- from the 3 mg of ink on a paper-based device into a 20 µL droplet of pure H_2O deposited would result in a $10^{-3.5}$ M Cl^- concentration. This value is very close to the observed lower LOD for paper-based devices with transducers prepared from AgCl/Ag ink. It follows that using a commercial AgCl/Ag ink such as the one used here can significantly worsen the lower LOD for Cl^-.

Performance of Cl^- Sensing Devices with Alternative AgCl/Ag Transducers. To determine whether the observed worsening of the lower LOD in paper-based devices is unique to the AgCl/Ag ink we used, or whether it occurs also for other types of AgCl/Ag electrodes integrated into paper-based devices, we used Ag wires and plates, both electrochemically AgCl coated and differing from one another only in their area of contact with paper (see insets of Figures 1C and 1D). Specifically, the wires and plates contacted approximately 0.1 and 2.0 cm^2 of paper, respectively.

As for the AgCl/Ag ink transducers, the performance of the AgCl/Ag wires and plates was first measured in 100 mL sample volumes with a conventional double-junction reference electrode. The lower LODs and slopes for both Cl^- and Ag^+ were all within error consistent with theory (see Table 1), showing no evidence for sample contamination by the transducers.

The potentiometric responses of paper-based devices with a AgCl/Ag wire or AgCl/Ag plate transducer to varying concentrations of KCl are shown in Figure 1C-D. Devices using a AgCl/Ag wire or plate gave a lower LOD of $10^{-4.0 \pm 0.2}$ M Cl^- and $10^{-3.9 \pm 0.1}$ M Cl^-, respectively, both worse than expected based on the solubility product of AgCl. While these two transducers differ in the contact area between electrode and paper by a factor of 20, the lower LODs were within error of one another, suggesting that the paper-induced worsening of the LOD is not affected by the area of the filter paper/electrode interface.

While typically AgCl coated Ag wires were stored in KCl solution, control experiments were also performed by immersing these transducers into deionized water for 1 h before and after each measurement to rule out contamination of samples from KCl solution adhering to these transducers. However, results thus obtained provided no evidence for such a mode of

sample contamination (for details, see the Supporting information). It was also hypothesized that convection in samples might affect the lower LOD, influencing how quickly contaminants might be dissolved into the samples closeby to the transducers. However, experiments with paper-based devices and samples that contained in addition to KCl also 0.01 wt % gelatin gave LODs that did not differ from experiments without gelatin (see the Supporting Information for full experimental details). As gelatin is well known to suppress convection,[44] we conclude that convection is not relevant in this system.

It is interesting to note that for all transducers tested, the standard deviation of the measured EMF was invariably larger at Cl^- concentrations below the devices' LOD, as seen in Figure 1B-D. This has not been previously reported and suggests that the interference introduced by the filter paper depends on parameters that are of lesser importance to the EMF response at higher Cl^- concentrations. This and the observed increase in response time at the lower LOD may be related to a slow rate of leaching of Cl^- out of the paper substrate.

Performance of Br^- Sensing Devices with AgBr/Ag Transducers. Clearly, while use of the AgCl/Ag ink resulted in the most pronounced worsening of the lower LOD for Cl^-, some LOD worsening is also observed for paper-based devices with AgCl/Ag wire and plate transducers. This raised the question whether the worsening of the lower LOD for paper-based devices is unique to AgCl/Ag transducers, or whether it is also observed for other silver halide transducers. To address this question, we used AgBr coated Ag wires with KBr solutions as samples, as shown in Figure S4 and Table S1. Analogous to the observations with AgCl/Ag transducers for the detection of Cl^-, paper-based devices measuring Br^- also had a lower LOD ($10^{-5.5 \pm 0.3}$ M) worse than what is expected from the square root of the AgBr solubility product (5.35×10^{-13} M^2), i.e., $10^{-6.1}$ M.

However, the extent to which paper worsened the LOD for Br^- was less pronounced than for Cl^- (see the Supporting Information for full data). Indeed, the slight worsening of the lower LOD for Br^- sensing with AgBr/Ag transducers appears to be the result of the same Cl^- contamination that also affects Cl^- sensing, as discussed further below.

Adsorption of Ag^+ to Filter Paper. All the results described above are consistent with a lower LOD affected by a Cl^- concentration in samples higher than what is predicted by the solubility of AgCl. We wondered whether that may be explained by the adsorption of Ag^+ to filter paper, increasing the overall solubility of AgCl in a medium comprising paper. Indeed, adsorption of metal cations onto negatively charged cellulose is often posited as an explanation for the performance limitations of paper-based devices.[4,14,23]

To investigate this hypothesis, filter paper was suspended in aqueous $AgNO_3$ solutions of varying concentrations, and aliquots of the equilibrated solutions were then removed and analyzed for silver by ICP-OES. From this data, the extent of Ag^+ adsorption to the paper was determined by difference. The amount of Ag^+ adsorbed onto filter paper as a function of the equilibrium concentration of $AgNO_3$ is shown in Figure 2, with a fit based on Langmuir adsorption theory. The fit gives a maximum surface coverage of $9.0 \times 10^{-4} \pm 1.7 \times 10^{-4}$ g Ag/g filter paper (8.3 µmol Ag/g filter paper), with an equilibrium constant for adsorption of 500 ± 300 M^{-1}.

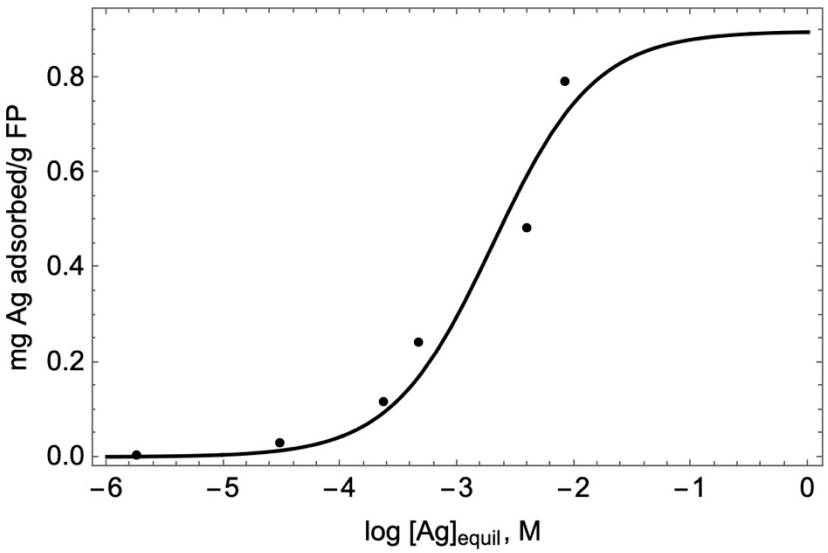

Figure 2. Adsorbed amount of Ag^+ per g filter paper (FP) versus logarithm of the equilibrium concentration of Ag (black dots), as determined by ICP-OES, along with a fit based on Langmuir adsorption theory (solid black line) for adsorption of Ag^+ onto filter paper.

While there are not enough points in the higher concentration range of the adsorption data shown in Figure 2 to conclusively distinguish between a Langmuir or Frumkin type adsorption process, the relevant Ag^+ concentration range for understanding lower LOD interferences in biological samples using paper-based $Cl^–$ sensors with AgCl transducers is well below 1 mM Ag^+. This readily follows from the solubility product of AgCl ($1.6 \times 10^{–10}$ M^2), which at the surface of a AgCl transducer predicts concentrations of Ag^+ below 1 mM for any sample that contains more than $10^{–6.8}$ M $Cl^–$.

Given the weak affinity of Ag^+ for paper as evident from Figure 2, the purity of the filter paper, and the reported concentration of carboxyl groups of 50-100 µmol/g filter paper,[29,31,35,37] it appears likely that this type of Ag^+ adsorption is related to carboxylic acid groups of cellulose.

Pickering attributed the adsorption of divalent cations to filter paper to an ion exchange with metals on the paper,[27] but the concentrations of metals in ashless filter paper as reported by commercial suppliers are only in the low ppm range.[45] Therefore, in a system with ashless filter paper, the cations that exchange with Ag^+ are more likely hydronium ions formed by deprotonation of carboxylic acid groups, which, based on their similarity to gluconic acid, may be estimated to have pK_a values of about 4 and are, therefore, expected to dissociate readily. The equilibrium constant for Ag^+ adsorption to filter paper, as evident from Figure 2, is very small, but it is larger than the extremely small formation constant of the 1:1 complex of Ag^+ and acetate of only 0.73 M^{-1}.[46] Given the relatively high concentration of carboxyl groups in paper of 50-100 µmol/g, it is possible that adsorbed Ag^+ ions electrostatically interact with more than one carboxylate group.

As can be seen from Figure 2, there is less than 0.1 mg Ag^+ adsorbed per gram filter paper for any concentration of Ag^+ in the sample lower than 1 mM. A fit of the equilibrium concentration of Ag^+ versus the total Ag^+ concentration in the system for data below 1 mM results in a slope of 0.67 ± 0.04 (see Figure S5). This predicts a decrease in the equilibrium concentration of Ag^+ by 0.2 logarithmic units, which is expected to increase the concentration of Cl^- in equilibrium with a AgCl transducer in an otherwise Cl^- free sample by an analogous 0.2 logarithmic units. However, we see experimentally an increase in the lower Cl^- LOD in paper-based devices that is much more significant (see Table 1 and discussion above). This shows that while there is some very weak Ag^+ adsorption, this effect is far too weak to explain the worsened lower LOD that we observed in paper-based devices.

The weakness in binding of Ag^+ to cellulose needs to be understood not only by the weak affinity of Ag^+ for carboxylate groups as ligands but also as the result of competition with hydronium ions. We observed that solutions of 1 x 10^{-5} to 1 x 10^{-2} M $AgNO_3$ had an average pH of 6.0 ± 0.3 before exposure to filter paper and 5.2 ± 0.2 after addition of filter paper. A moderate level of acidity of these solutions is expected also as the result of equilibration with the atmosphere, as at 25 °C and 1 atm the solubility of CO_2 in water is 0.57 mg/L,[47] which with the pK_a of carbonic acid[46] of 6.352 results in a pH of 5.6. The very slight acidification of the $AgNO_3$ solutions upon addition of filter paper is nevertheless noteworthy and is consistent with Ag^+ versus hydronium ion exchange.

Weak binding of Ag^+ to cellulose and competition for adsorption with hydronium ions is also consistent with the observed effect of the pH on the Cl^- response. This is shown by the performance of paper-based devices with AgCl/Ag ink, which were also tested with reference and sample solutions prepared in 0.55 M citrate buffer at pH 2.4. These devices had a lower LOD of $10^{-3.3 \pm 0.1}$ M Cl^-, a slope of –63.4 ± 2.5 mV / decade. There was no significant change in lower LODs using paper substrates when samples of lower pH were measured. If adsorption onto a negatively charged substrate were to explain the observed lower LODs, then an improved lower LOD would be expected. Therefore, these findings indicate that the interaction of carboxylate groups with Ag^+ does not explain the worsened lower LODs for Cl^- of paper-based interferences.

Chloride Impurities in Filter Paper. As we determined that Ag^+ adsorption to paper does not facilitate the dissolution of AgCl from AgCl/Ag transducers, we further examined the paper substrate itself, again looking for impurities. While filter paper is often referred to as a pristine material, it is not free from impurities. Previous studies of various industrial filter papers have

detected metal ions as well as anionic impurities such as Cl^-, SO_4^{2-}, and NO_3^-, species that may have already been present in the raw cellulose or were introduced in the manufacturing process.[48–50] So-called ashless filter paper for analytical chemistry purposes is typically characterized by suppliers in view of alkali, earth alkali, and heavy metal ion content, but halide concentrations are not normally reported. Therefore, we analyzed filter paper using dry-ashing followed by detection with the mercury(II) thiocyanate method, giving the Cl^- content of filter paper as 20.1 ± 2.2 µg Cl^-/g (n=3). While the supplier's documentation for the Whatman grade 1 filter paper used in this work does not mention chlorine as a possible contaminant, the current value is close to the value of 11.55 µg Cl/g filter paper reported many years ago for Whatman 41 filter paper, which is also cellulose based.[50]

To confirm that the thus detected Cl^- in paper can be leached into aqueous samples, filter paper was immersed in H_2O for 5 min. This relatively short time period was chosen because, when using paper-based devices, measurements are typically taken within 1 min of deposition of the sample onto the device, as evaporation of water begins right away and eventually starts to affect sample concentrations; typically, after 5 min almost all solution has evaporated.[43] Analysis of purified water revealed no measurable Cl^- prior to contact with filter paper but after exposure to filter paper for 5 min, a Cl^-concentration of 14.6 ± 0.6 µg Cl^-/g filter paper was found (n=3).

The source of this Cl^- may be related to the bleaching process that is used in papermaking to clean pulps, remove lignin, and increase both the absorbency and brightness of paper.[51] While the Hg^{2+} used in the mercury(II) thiocyanate method for Cl^- analysis may bind anionic oxygenated chlorine species, the solubilities of Ag^+ species such as perchlorate and hypochlorite are very high,[46] making the precipitation of such salts—and, thereby, interference with the silver halide

transducer—an unlikely cause for the observed worsening of the lower LOD for Cl^-. We also note that if impurities of oxygenated chlorine species were present to a significant degree so as to oxidize silver metal and, thereby, raise the Ag^+ concentration, this would lower rather than raise the LOD for Cl^-. This suggests that Cl^- contamination is indeed the main cause of LOD deterioration.

Using the value of 14.6 ± 0.6 μg Cl^-/g filter paper found by soaking of paper in H_2O for 5 min, leaching of Cl^- from paper would lead to a concentration of $10^{-3.4 \pm 0.1}$ M Cl^- in a 20 μL droplet of pure H_2O in the sample zone of 2 cm^2 (see SI for calculations). As this matches the experimentally found lower LOD for paper-based devices with AgCl/Ag ink, it appears likely that the contamination of paper with Cl^- is the dominant cause for observed lower LOD in Cl^- sensing.

To determine whether leachable Cl^- ions can be removed from filter paper by simple rinsing, paper was submerged in H_2O for 5 min, removed from the suspension, rinsed with H_2O, and submerged again in H_2O for a total of three 5-min soaks, dried overnight, and then used for sensor fabrication as normal. This pretreatment did not improve the lower LOD as compared to untreated paper, indicating that the three 5-min soaks are not sufficient to remove all leachable Cl^-. More vigorous attempts to remove Cl^- ions from the filter paper were hindered by disintegration of the paper.

The contamination of samples by Cl^- leaching from the paper substrate also affected the Br^- measurements with AgBr/Ag transducers described above. For the Br^- measurements shown in Figure S4, Cl^- is an interfering ion that contaminates the sample, worsening not only the lower LOD for Br^- but, as a result of the kinetics of leaching, also reducing the reproducibility of the measured EMF near and below the lower LOD. Given a selectivity coefficient of $\log K^{\mathrm{pot}}_{\mathrm{Br,Cl}}$ of –2.5,

as it can be obtained from the ratio of the solubility products of AgBr and AgCl,[26] a detection limit of $10^{-5.9 \pm 0.1}$ M Br^- is estimated for samples contaminated with $10^{-3.4 \pm 0.1}$ M Cl^-. The experimentally observed lower LOD of $10^{-5.5 \pm 0.3}$ M for Br^- is slightly worse, but this difference appears well within the range of error often caused by the well-documented Hulanicki effect[52] (that is, a deviation of the EMF of silver halide ISEs from the thermodynamically predicted value at the lower LOD).

Whatman 1, the paper used in this work, has been used in a number of studies on paper-based ISEs. To explore whether there is something unique to Whatman 1, we also fabricated devices with three other types of filter paper previously reported for use in paper-based ISEs. However, there was no improvement in the LOD for chloride with any of these alternative devices (see the Supporting Information for full results). Indeed, one of the three alternative filter papers provided a slightly worsened LOD for chloride. This suggests that low level chloride contamination of filter paper is quite common.

Considering the possibility of a different supporting substrate, we also performed experiments using both AgCl/Ag ink and AgCl coated Ag wires as transducers using the textile fabric Polx1200 as device substrate instead of the filter paper. Polx1200 is a knitted sample-wicking polyester textile designed for use as a cleanroom wipe that was previously used as a supporting substrate for ISEs.[43] Textile-based devices using AgCl/Ag transducers exhibited lower LODs of $10^{-4.1 \pm 0.1}$ and $10^{-4.4 \pm 0.1}$ for ink and wire transducers, respectively. This is still not as low as predicted by the solubility product of AgCl in water, but it is an improvement over paper-based devices (see the Supporting Information for full discussion of results). Noting this favorable property of Polx1200, we also considered the wax barriers as a possible source of contamination. However, using textile-based devices without wax barriers, we saw no improvement in device performance (see the Supporting Information for details), confirming that the wax barriers do not negatively affect the LOD for chloride.

Conclusions

Our results show that Cl^- contamination of samples from both a commercial AgCl/Ag ink and filter paper explains the sub-optimal performance of paper-based potentiometric Cl^- sensors. While both AgCl/Ag ink and so-called ashless filter paper have sufficiently high enough purities for use with large sample volumes, leaching of Cl^- significantly worsens the LOD in devices with sample sizes on the order of microliters and even a few milliliters. Improved lower LODs for Cl^- may be obtained by use of device substrates with a higher purity, such as synthetic textiles, and the preparation of AgCl coated Ag electrodes directly by oxidation of Ag. More careful formulation of AgCl/Ag inks may also be considered. Notably, adsorption of Ag^+ onto negatively charged cellulose was confirmed to occur at very high Ag^+ concentrations but has only a minimal effect on potentiometric Cl^- sensing. This work demonstrates the sensitivity of small-volume potentiometric devices both to sample depletion by interaction with the platform substrate and to contamination from impurities of the sensing membrane and the platform substrate. These are problems that can, however, be avoided if materials are thoughtfully selected, for example, by the replacement of paper by a textile as the platform substrate[43] or cautious selection of silver inks of higher purity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information includes pictures of device setups; the preparation of AgCl coated Ag wires and Ag plates; potentiometric responses of Cl^- sensing devices in 0.01 wt % gelatin solution, of Br^- sensing devices, of alternative filter paper-based devices, and of textile-based devices;

calculations of Cl^- concentration in a droplet. This material is available free of charge on the ACS Publications website at DOI: (to be added by editor).

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Author Contributions

B.K.T. contributed with the design and fabrication of capillary reference electrodes. E.J.H. performed all the other experimental work. E.J.H. and P.B. performed the data interpretation and prepared the manuscript.

Notes

The authors declare no competing financial interest.

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