All-Solid-State Reference Electrodes Based on Colloid-Imprinted Mesoporous Carbon and Their Application in Disposable Paper-Based Potentiometric Sensing Devices.

6 AUTHORS, INCLUDING:

William H Smyrl
University of Minnesota Twin Cities
252 PUBLICATIONS **5,213** CITATIONS

Philippe Buhlmann
University of Minnesota Twin Cities
155 PUBLICATIONS **7,950** CITATIONS

All-Solid-State Reference Electrodes Based on Colloid-Imprinted Mesoporous Carbon and Their Application in Disposable Paper-based Potentiometric Sensing Devices

Jinbo Hu,[†] Kieu T. Ho,[†] Xu U. Zou,[†] William H. Smyrl,[‡] Andreas Stein,*,[†] and Philippe Bühlmann*,[†]

[†]Department of Chemistry, University of Minnesota, 207 Pleasant Street SE, Minneapolis, Minnesota 55455, United States
[‡]Department of Chemical Engineering and Materials Science, University of Minnesota, 421 Washington Avenue SE, Minneapolis, Minnesota 55455, United States

S *Supporting Information*

ABSTRACT: Reference electrodes are used in almost every electroanalytical measurement. Here, all-solid-state reference electrodes are described that employ colloid-imprinted mesoporous (CIM) carbon as solid contact and a poly(vinyl chloride) reference membrane to contact the sample. Such a reference membrane is doped with a moderately hydrophilic ionic liquid and a hydrophobic redox couple, leading to well-defined constant potentials at the interfaces of this membrane to the sample and to the solid contact, respectively. Due to the intrinsic properties of CIM carbon, reference electrodes with a CIM carbon solid contact exhibit excellent resistance to common interfering agents such as light and O_2, with outstanding potential stability in continuous potentiometric measurements. The potential drift of CIM carbon-based reference electrodes without redox couple is as low as 1.7 μV/h over 110 h, making them the most stable all-solid-state reference electrodes reported so far. To demonstrate the compatibility of CIM carbon-based reference electrodes with miniaturized potentiometric systems, these reference electrodes were integrated into paper-based potentiometric sensing devices, successfully replacing the conventional reference electrode with its reference electrolyte solution. As a proof of concept, disposable paper-based Cl^- sensing devices that contain stencil-printed Ag/AgCl-based Cl^- selective electrodes and CIM carbon-based reference electrodes were constructed. These sensing devices are inexpensive, easy to use, and offer highly reproducible Cl^- measurements with sample volumes as low as 10 μL.
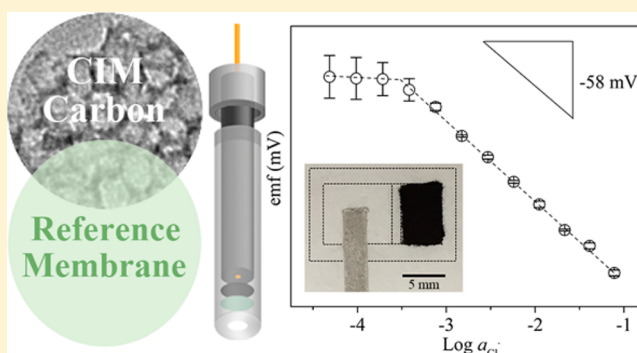
Ion-selective electrodes (ISEs) are widely used in clinical analysis, process control, and environmental monitoring.[1−5] In view of the need for affordable and portable analytical devices for small sample volumes, miniaturizable all-solid-state ISEs are highly desirable but are only meaningful if the reference electrode is also miniaturized. While the development of all-solid-state ISEs represents a highly active research field,[5−7] much less work has been performed to develop all-solid-state reference electrodes,[8] even though the accuracy of potentiometric measurements relies equally on both the reference and measurement half cells.

Conventional reference electrodes are typically Ag/AgCl or Hg/Hg_2Cl_2 half cells and are connected to the sample through a salt bridge. The latter usually contains an aqueous solution of an equitransferent salt that minimizes the liquid junction potential at the interface of the bridge electrolyte and the sample.[9] Although very stable and reliable, such reference electrodes exhibit disadvantages owing to the presence of the salt bridge, such as the need for frequent maintenance, a large size, and the mutual contamination of the bridge electrolyte and sample.[10,11]

A recent example that demonstrates the difficulty of integrating conventional reference electrodes into affordable and disposable sensing devices is that of the paper-based ion sensors that were recently developed for the analysis of Cl^-, K^+, Na^+, and Ca^{2+}.[12] Different from other paper-based potentiometric sensors where paper was used either as a substrate to mechanically support the solid contact[13−16] or as a microfluidic sampling tool,[17] wax-imprinted paper used in this design served as a substrate to support the electrodes, the reference electrolyte, and the sample. Although highly miniaturized, these ion sensors still employed a conventional reference system that contained a stencil-printed Ag/AgCl electrode coupled with a KCl solution as the reference electrolyte.[12] This conventional reference system requires the manual application of the reference electrolyte and, therefore, still relies on transport and storage of supply reagents. This demonstrates the need for replacing conventional designs.

One of the key components of an all-solid-state reference electrode is a hydrophobic reference membrane attached to an electronically conducting solid contact, thus resembling the setup of an all-solid-state ISE. The reference membranes are usually doped with ions that can leach into the samples on a slow but continuous basis so that the phase boundary potentials at the reference membrane/sample interfaces are sample-independent and defined by the interfacial distribution of the doping ions. Several ions have been doped into reference membranes, including polyions,[18] ionic liquids,[19−21] and lipophilic and hydrophilic salts.[22−24] At the reference membrane/solid contact interface, the interfacial potential can be stabilized by employing various solid contacts that are also used in all-solid-state ISEs. The most common solid contacts include conducting polymers[5,22,23,25,26] and nanostructured carbon materials, such as three-dimensionally ordered macroporous (3DOM) carbon,[27,28] carbon nanotubes,[13,29,30] and graphene.[15,31] Several miniaturized potentiometric cells into which an all-solid-state ISE and reference electrode are integrated have been proposed.[14−17]

The electrochemical performance of these all-solid-state reference systems is highly dependent on the solid contact between the reference membrane and the underlying electron conductor. We demonstrated previously that a highly capacitive interface between the ISE membrane and the solid contact provides for a high potential stability of all-solid-state ISEs,[27] and we have shown that the same is also true for reference electrodes with a reference membrane/solid contact interface.[28] In particular, 3DOM carbon-based reference electrodes were found to exhibit a potential drift as low as 42 μV/h over 26 days, which is very favorable in terms of continuous long-term use.[28] However, in regard to device-to-device reproducibility, it is a disadvantage that the 3DOM carbon derived from resorcinol and formaldehyde contains some redox-active surface functional groups that can affect the reproducibility of the calibration curve intercept, $E°$.[32] Moreover, the monolithic nature of 3DOM carbon is not optimal for mass production and integration into miniaturized potentiometric sensing devices.

To address these issues, we report here the use of colloid-imprinted mesoporous (CIM) carbon as a solid contact to construct all-solid-state reference electrodes. CIM carbon was recently introduced as a solid contact material to fabricate all-solid-state ISEs.[33] It consists of open and interconnected mesopores that can be filled with a polymeric sensing phase to provide a bicontinuous arrangement of carbon and polymer phases.[34] It has a very high level of purity because it is prepared from a pure hydrocarbon mesophase-pitch precursor. The use of CIM carbon combined with the hydrophobic redox buffer consisting of cobalt(II) and cobalt(III) tris(4,4′-dinonyl-2,2′-bipyridyl) ($[Co(C_9,C_9\text{-bipy})_3]^{2+/3+}$) has enabled the construction of all-solid-state ISEs with a standard deviation of $E°$ as low as 0.7 mV.[33] Benefiting from the high double-layer capacitance provided by its interconnected mesopores, the emf drift of these all-solid-state ISEs is 1.3 μV/h over 70 h. Moreover, the synthesis of CIM carbon can be easily scaled up, and CIM carbon is prepared in powder form and, therefore, can be made into thin films, suspensions, or potentially carbon inks for production and fabrication on an industrial level.

Herein, we use CIM carbon with 24 nm diameter mesopores to fabricate all-solid-state reference electrodes. The reference membranes are doped with the ionic liquid 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_8\text{min}^+]$-$[C_1C_1N^−]$) to define the potential at the reference membrane/sample interface,[19] as well as a hydrophobic redox buffer consisting of $[Co(C_9,C_9\text{-bipy})_3](TPFPB)_2$ and $[Co(C_9,C_9\text{-bipy})_3](TPFPB)_3$ to define the potential at the CIM carbon/reference membrane interface.[35] Benefiting from the high capacitance of the interface between the CIM carbon and the reference membrane, outstanding potential stability is observed, with a potential drift as low as 1.7 μV/h over 110 h, making CIM carbon-based reference electrodes the most stable all-solid-state reference electrodes reported so far. To demonstrate their compatibility with miniaturized potentiometric systems, CIM carbon-based reference electrodes are integrated into disposable paper-based Cl⁻ sensing devices to replace the conventional Ag/AgCl reference electrodes, eliminating the reference electrolyte and the associated liquid junction potentials.

■ EXPERIMENTAL SECTION

Materials. Reagents were obtained from the following sources: mesophase pitch from Mitsubishi Gas Chemicals (Tokyo, Japan), Ludox AS-40 colloidal silica from Sigma-Aldrich (St. Louis, MO), o-nitrophenyl octyl ether (o-NPOE) and high molecular weight poly(vinyl chloride) (PVC) from Fluka (Buchs, Switzerland), ionic liquid $[C_8\text{min}^+][C_1C_1N^−]$ from IOLITEC (Tuscaloosa, AL), Tecoflex SG-80A polyurethane from Thermedic Polymer Products (Woburn, MA), and AGCL-675 Ag/AgCl ink from Conductive Compounds (Hudson, NH). All chemicals were used as received without further purification. Deionized water was purified to a resistivity of 18.2 MΩ/cm with a Milli-Q PLUS reagent-grade water system (Millipore, Bedford, MA). CIM carbon and the redox couple consisting of $[Co(C_9,C_9\text{-bipy})_3](TPFPB)_2$ and $[Co-(C_9,C_9\text{-bipy})_3](TPFPB)_3$ were prepared as previously reported.[33−35]

Electrode Fabrication. The 2 mm diameter gold disk electrodes (CH Instruments, Austin, TX) were polished over polishing cloths with aqueous dispersions of alumina (0.3 and 0.05 μm, Buehler, Lake Bluff, IL). They were cleaned by ultrasonication in water and ethanol and dried with a flow of argon. CIM carbon powder was manually ground for 5 min using a pestle. The CIM carbon suspension was prepared by ultrasonicating 47.5 mg of CIM carbon and 2.5 mg of PVC as a binder in 1 mL of freshly distilled tetrahydrofuran (THF) for 30 min. An amount of 30 μL of the CIM carbon suspension was drop-cast onto the gold electrodes and left to dry, forming CIM carbon films with a thickness of approximately 200 μm.

Precursor solutions for reference membranes were prepared by dissolving in 2 mL of freshly distilled THF 60 mg the ionic liquid $[C_8\text{min}^+][C_1C_1N^−]$, 120 mg of PVC as polymeric matrix, and 120 mg of o-NPOE as the plasticizer, as described by Zhang et al.[28] Moreover, 1.4 mmol/kg each of $[Co(C_9,C_9\text{-bipy})_3](TPFPB)_2$ and $[Co(C_9,C_9\text{-bipy})_3](TPFPB)_3$ was added to this solution as an internal reference. The precursor solutions were stirred for 2 h to ensure complete dissolution.

To form reference membranes with a thickness of approximately 100 μm, two portions of the precursor solution (20 μL, followed by 30 μL after 1 min) were drop-cast onto the CIM carbon layer on a gold electrode. The coated electrode was then mounted into a cylindrical body with a screw cap at the opposite end, as previously reported[33] (see the Supporting Information, Figure S1). Prior to measurements, these electrodes were conditioned in a 1.0 mM NaCl solution for 1 h.[35]

Fabrication of Paper-based Cl⁻ Sensing Devices.
Paper-based sample zones and microfluidic channels were
defined by patterning polyurethane lines that penetrated
through the whole thickness of ashless filter papers (Whatman
Grade 589/2 white ribbon). Approximately 2.5 g of polyur-
ethane was dissolved in 40 mL of THF, and this solution was
then applied to both sides of the paper using a capillary,
forming polyurethane lines approximately 2 mm in width. The
Ag/AgCl electrodes were patterned on paper by stencil
printing. A hand-cut Frisket Film (low tack, Grafix, Maple
Heights, OH) was used as the stencil, and Ag/AgCl ink was
applied to the openings of the stencil using a rubber brush,
followed by a curing process at 100 °C for 15 min.

To form 2 mm wide reference membranes, a 5 μL
microcapillary was used to apply the precursor solution onto
paper. To ensure the full penetration of the membrane
components through the entire thickness of the paper, the
precursor solution was applied on both sides of the paper four
times with a 1 min time interval between applications to allow
THF to evaporate. A CIM carbon−reference membrane
suspension was prepared by ultrasonicating for 30 min 60 mg
of CIM carbon in 1 mL of the solution containing the reference
membrane components. The resulting suspension was then
applied onto the paper using a capillary to form a homogeneous
mixture of CIM carbon and reference membrane, with an effort
to maximize the contact area between this homogeneous
mixture and the reference membrane, which was applied
separately.

Potentiometric Measurements. Electrode potentials
were measured with an EMF 16 potentiometer (input
impedance 10 TΩ) controlled by EMF Suite 1.03 software
(Lawson Labs, Malvern, PA). To test the electrochemical
performance of the CIM carbon-based all-solid-state reference
electrodes, a conventional double-junction 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. To test the response of paper-
based Cl⁻ sensing devices, two copper alligator clips were used
to connect the Ag/AgCl and CIM carbon electrodes to a
potentiometer. All of the paper-based Cl⁻ sensing devices were
used without preconditioning, i.e., without exposure of the Ag/
AgCl electrode and the reference membrane to aqueous
solutions prior to measurements. Activity coefficients were
calculated according to a two-parameter Debye−Hückel
approximation.[36]

RESULTS AND DISCUSSION

**Ionic Response of CIM Carbon-based Reference
Electrodes.** A good reference electrode should not respond
to any sample species. To test whether this was also true for all-
solid-state reference electrodes consisting of a gold electrode
coated with a CIM carbon layer and a reference membrane
doped with the hydrophobic redox buffer $[Co(C_9,C_9\text{-}$
$bipy)_3]^{2+/3+}$ and the ionic liquid $[C_8min^+][C_1C_1N^-]$, the
ionic responses of such electrodes were measured against a
conventional double-junction reference electrode. The resulting
emf responses are shown in Figure 1. Overall, the obtained
calibration curve intercept, $E°$, is −120.6 ± 3.7 mV for three
electrodes, and for a given electrode, the $E°$ is −120.8 ± 8.0 mV
in six electrolytes. For each electrolyte, there is only a very small
emf response of the CIM carbon-based reference electrodes in
the concentration range from 10^{-7} M to 10^{-1} M, demonstrating
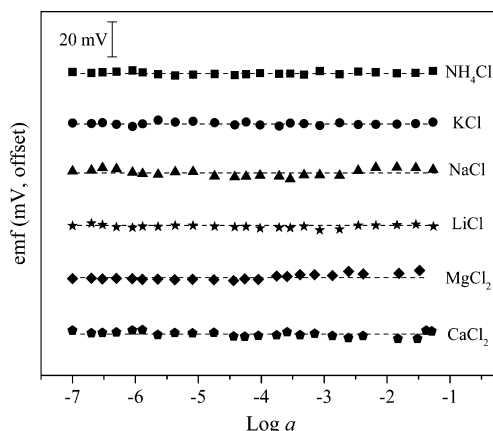the low potential variability of CIM carbon-based reference

Figure 1. Potentiometric responses of gold/CIM carbon/reference
membrane electrodes in aqueous solutions of NH_4Cl, KCl, NaCl, LiCl,
$MgCl_2$, and $CaCl_2$ in the concentration range from 10^{-7} to 10^{-1} M.
The responses were measured by addition of aliquots of concentrated
salt solutions under continuous stirring. The response curves have
been shifted vertically for clarity.

electrodes to ions with different charges and hydrophilicities.
The change in emf over the entire range of activities is 1.9 ± 0.8
mV/decade for NH_4^+, 1.1 ± 0.6 mV/decade for K^+, 0.9 ± 0.5
mV/decade for Na^+, 0.9 ± 0.6 mV/decade for Li^+, 1.1 ± 1.0
mV/decade for Mg^{2+}, and 0.5 ± 0.3 mV/decade for Ca^{2+} (n =
3). This low dependence of the emf on the concentrations of
ions is consistent with the phase boundary potential at the
reference membrane/sample interface being defined by
partitioning of the ionic liquid between the hydrophobic
reference membrane phase and the aqueous sample phase.
Unlike in the case of an ISE membrane, transfer of sample ions
into the reference membrane is not occurring to an extent that
it affects the phase boundary potential.

**Use of CIM Carbon-based Reference Electrodes to
Measure Cl⁻ Responses of a Ag/AgCl ISE.** To assess the
suitability and reproducibility of CIM carbon-based reference
electrodes in ion-selective potentiometry, Cl⁻ measurements
were performed with a AgCl-coated Ag wire as the ISE and two
different reference electrode assemblies (i.e., gold/CIM
carbon/reference membrane with or without redox couple).
For comparison, a conventional double-junction reference
electrode was also employed. The Cl⁻ responses were
measured by successive dilution of a 0.1 M NaCl solution
while monitoring the emf. The corresponding potentiometric
Cl⁻ response characteristics are shown in Figure 2 and
summarized in Table 1.

As Table 1 shows, CIM carbon-based reference electrodes
both with and without the hydrophobic redox couple yield
Nernstian responses (i.e., −55.5 ± 1.0 mV/decade with a
reference membrane with the redox couple and −57.7 ± 2.3
mV/decade when no redox couple was used), with values that
are within error indistinguishable from the response slope
obtained with a conventional double-junction reference
electrode (i.e., −55.9 mV/decade). The detection limits
obtained from these different reference electrodes were all
approximately $10^{-4.4}$ M. These values are close to the intrinsic
detection limit for Cl⁻ using a Ag/AgCl ISE (i.e., $10^{-4.9}$ M),
which is determined by the solubility of AgCl.

Because there is no internal reference for the gold/CIM
carbon/reference membrane electrodes without redox couple,
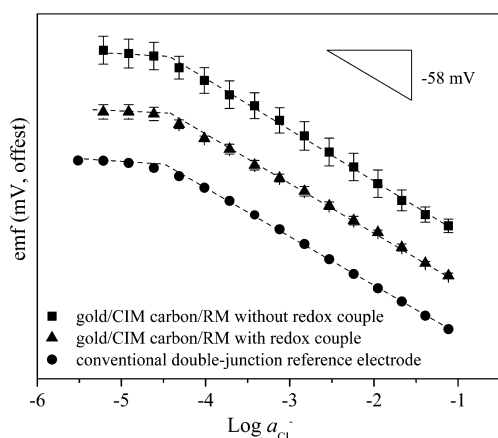the standard deviation of $E°$ (12.9 mV) is relatively large. This

Figure 2. Potentiometric responses to Cl^- of a Ag/AgCl ISE against reference electrodes with different electrode configurations, i.e., gold/CIM carbon/reference membrane (RM) without redox couple, gold/CIM carbon/reference membrane with redox couple, and a commercial double-junction reference electrode. The response curves have been shifted vertically for clarity.

Table 1. Potentiometric Cl^- Responses of a Ag/AgCl ISE vs Reference Electrode Assemblies with a Reference Membrane (RM) or a Conventional Double Junction[a]

reference electrode	slope (mV/decade)	$E°$ (mV)	detection limit (M)
gold/CIM carbon/RM without redox couple	−57.7 ± 2.3	122.9 ± 12.9	$10^{-4.4}$
gold/CIM carbon/RM with redox couple	−55.5 ± 1.0	82.8 ± 2.8	$10^{-4.4}$
conventional double-junction	−55.9	−12.1	$10^{-4.5}$

[a]Means and standard deviations for five separate measurements with one Ag/AgCl ISE and five different reference electrodes. The $E°$ values were obtained by extrapolation of the linear section of the emf response to a Cl^- activity of 1.0 M.

value is comparable to what has been observed in the past for many solid contact ISEs and is not necessarily problematic if devices are properly calibrated, but it is unsuitable for calibration-free measurements with disposable miniaturized sensing devices. The poor electrode-to-electrode repeatability can be significantly improved by doping the reference membrane with the hydrophobic redox couple $[Co(C_9,C_9\text{-}bipy)_3]^{2+/3+}$, reducing the standard deviation of $E°$ to 2.8 mV.

Effects of Light, Oxygen, and Carbon Dioxide. Light, O_2, and CO_2 have been reported to interfere with the response of some all-solid-state ISEs,[8] and the possibility of such interferences should also be considered for all-solid-state reference electrodes. Generally, photosensitivity can be observed with conducting polymer and semiconductor solid contacts that have a suitable band gap.[7] Interference caused by CO_2 can be attributed to changes in the pH of a water layer formed at the solid contact/membrane interface,[37] and O_2 can interfere by forming an irreversible O_2 half-cell at the surface of the underlying electron conductor or by oxidizing functional groups on organic conductors.[38]

The effect of light on gold/CIM carbon/reference membrane electrodes was investigated by continuously recording their emf values versus a conventional double-junction electrode while switching off and on the fluorescent tube lights in the laboratory. The effects of O_2 or CO_2 were tested by bubbling

these gases into 1.0 mM NaCl sample solutions, followed by purging with Ar to remove those gases again. As shown in the top two traces of Figure 3, no significant effects of light and O_2
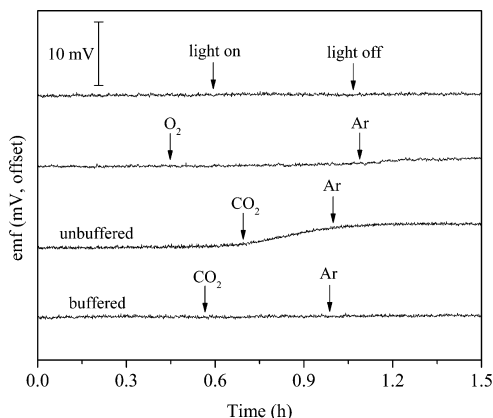

Figure 3. Effects of light, O_2, and CO_2 on the potential stability of gold/CIM carbon/reference membrane electrodes. The effect of CO_2 was studied both in an unbuffered 1.0 mM NaCl solution and a 0.1 M phosphate buffer solution (pH 7.5). The response curves have been shifted vertically for clarity.

were observed. While the insensitivity to light is due to the absence of a band gap of CIM carbon in the visible range, the excellent resistance to O_2 can be attributed to the low amounts of redox-active impurities and the absence of functional groups on the surface of CIM carbon.

When CIM carbon-based reference electrodes were exposed to CO_2 in an unbuffered solution, however, a potential drift of 9.5 mV/h is observed (Figure 3, third trace from the top). This drift can be attributed to a decrease of the solution pH, promoting the co-ion extraction of H^+ and the ionic liquid anion, $[C_1C_1N^-]$, into the reference membrane. This affects the phase boundary potential at the reference membrane/sample interface, as we have reported previously.[28] This effect, combined with fluctuations in the stir rate, may have affected the noise level of the data shown in Figure 1. To eliminate this effect and make it possible to study the influence of CO_2 on the CIM carbon/reference membrane interface, a phosphate buffer (pH = 7.5) was used, stabilizing the pH of the sample solution. As shown in the bottom trace of Figure 3, no significant effect of CO_2 is observed with this pH buffered sample system, demonstrating the excellent resistance of the CIM carbon/reference membrane interface to CO_2. Use of an ionic liquid that is less subject to protonation would enable the construction of CIM carbon-based reference electrodes with resistance to CO_2 in pH unbuffered solutions.

Long-Term Potential Stability. Potential stability is another important aspect for all-solid-state ISEs and reference electrodes, especially when they are used to continuously monitor the concentration of an analyte over an extended period of time. In this study, the potential stability of CIM carbon-based reference electrodes was assessed by monitoring the potentials of these references electrodes continuously for 110 h in a 1.0 mM NaCl solution at a constant temperature of 25 °C (see Figure S2 of the Supporting Information). To avoid leaching of the redox couple from the reference membrane into the sample as a cause of potential drifts, gold/CIM carbon/reference membrane electrodes without redox couple were used. Due to the large double-layer capacitance of the CIM

carbon layer, the potential drift of these reference electrodes was as low as 1.7 ± 1.2 μV/h ($n = 3$), which is on the same level as that for CIM carbon-based ISEs (i.e., 1.3 ± 0.3 μV/h).[33] For measurements that require long-term potential stability with high $E°$ reproducibility, a more hydrophobic redox couple or a redox couple covalently attached to the polymer backbone could be employed.

Design of Disposable Paper-based Potentiometric Cl⁻ Sensing Devices. Because they combine affordability, scalability, simplicity, and flexibility, paper-based analytical devices have recently attracted much attention.[39−41] In particular, miniaturized potentiometric Cl⁻ sensing devices were fabricated on wax-printed paper, with two stencil-printed Ag/AgCl electrodes serving as the ISE and the reference electrode. These devices resembled the one shown in Figure 4a
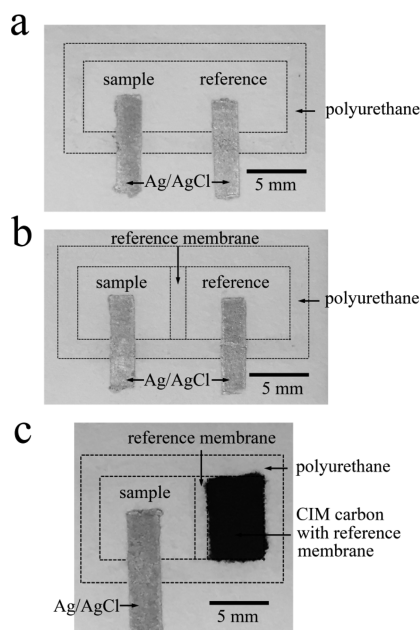

Figure 4. Photographs of paper-based potentiometric Cl⁻ sensing devices with different designs. (a) Device with a Ag/AgCl ISE and a Ag/AgCl reference electrode. (b) Device with a Ag/AgCl ISE and a reference electrode with a reference membrane. (c) Device containing a Ag/AgCl ISE and a CIM carbon-based reference electrode with a reference membrane.

but had a wax, rather than a polyurethane, barrier to contain aqueous solutions. For measurements, one droplet each of the sample and a reference electrolyte solution had to be applied onto the paper close to the corresponding electrodes, resulting in spontaneous wicking of the two liquids into the central contacting area to complete the electric circuit.[12] Although these Cl⁻ sensing devices have the advantage of being simple to use, they are subject to sample dependent liquid junction potentials at the sample/reference electrolyte interface and require the manual application of not only the sample but also a reference electrolyte solution.

To further simplify these paper-based potentiometric sensing devices and improve their accuracy, CIM carbon-based reference electrodes were used in this work to replace the conventional Ag/AgCl reference electrodes. Three designs were tested in a step-by-step approach to the final device. To start, a design similar to the one used for the previously reported paper-based Cl⁻ sensing devices, i.e., with two stencil-printed

Ag/AgCl electrodes, was used (Figure 4a). However, instead of printed wax,[12] polyurethane was used to form the hydrophobic barriers that define the microfluidic channels. Polyurethane was chosen not only because it is inexpensive, readily commercially available, and can be inkjet-printed for mass fabrication,[42] but also because its use avoids the melting process that is required to fabricate wax-printed paper devices.[12,43] In a second step, to eliminate the liquid junction potentials at the sample/reference electrolyte interface, a reference membrane was integrated into the central zone of the device, as shown in Figure 4b. Finally, a CIM carbon−reference membrane reference system was used to replace the conventional Ag/AgCl reference electrode (Figure 4c).

Use of a Reference Membrane to Eliminate Liquid Junction Potentials in Paper-based Potentiometric Cl⁻ Sensing Devices. To assess the effectiveness of reference membranes to eliminate the liquid junction potentials in paper-based potentiometric Cl⁻ sensing devices, Cl⁻ measurements were performed using a 1.0 M LiCl reference electrolyte and sample solutions containing different LiCl concentrations. LiCl was chosen here on purpose because of the large difference in the ionic mobilities of Li⁺ and Cl⁻, which results in liquid junction potentials as large as tens of millivolts. This offered the advantage that pinholes through the paper-supported reference membrane, which would have compromised the intended use of the reference membranes, would have been readily recognized by the occurrence of large liquid junction potentials within such pinholes.

As shown in the lower trace of Figure 5 (open circles), the Cl⁻ sensing devices without reference membranes (as shown in
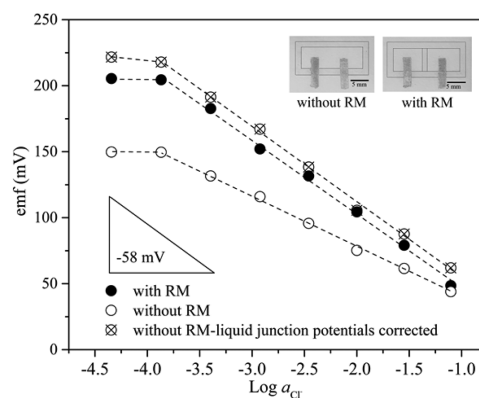

Figure 5. Comparison of the response to LiCl of paper-based potentiometric Cl⁻ sensing devices with and without reference membranes (RMs). The open and crossed circles represent the emf of the paper-based potentiometric Cl⁻ sensing device without a reference membrane before (open circles) and after (crossed circles) subtraction of the calculated liquid junction potential. The solid circles represent the emf of the paper-based potentiometric Cl⁻ sensing device with a reference membrane without any mathematical manipulation. Photographs of the two types of devices are shown on the top right.

Figure 4a) exhibit a sub-Nernstian response with a slope of -38.8 ± 1.3 mV/decade, whereas their counterparts with reference membranes (Figure 4b) yielded a Nernstian response with a slope of -57.1 ± 1.5 mV/decade in the range from $10^{-1.0}$ to $10^{-3.5}$ M (solid circles). The inferior response slope of the Cl⁻ sensing devices without reference membranes could be improved by mathematically correcting liquid junction potentials using the Henderson equation, as illustrated in the

top trace in Figure 5 (crossed circles).[44] The corrected response slope of −57.0 ± 1.3 mV/decade matches with the results for the devices with reference membranes, which demonstrates that the behavior of the system is well understood. However, corrections of liquid junction potentials are not readily possible for real samples with complex and unknown compositions.[45]

Integration of a CIM Carbon–Reference Membrane Reference System into Paper-based Potentiometric Cl⁻ Sensing Devices. The use of these disposable paper-based Cl⁻ sensing devices can be further simplified by employing an all-solid-state CIM carbon-based reference electrode to replace the conventional Ag/AgCl reference electrode, eliminating the reference electrolyte (Figure 4c). To test their electrochemical performance, 10 μL aliquots of sample solution containing different concentrations of NaCl were applied to the area around the Ag/AgCl ISE. The resulting Cl⁻ response curve is shown in Figure 6. In this potentiometric cell, all phase
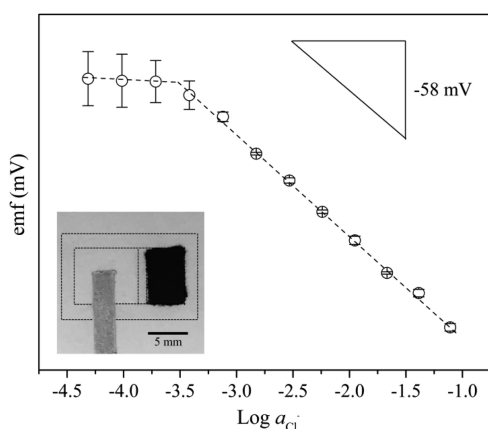

Figure 6. Potentiometric Cl⁻ response curve of paper-based potentiometric Cl⁻ sensing devices containing a Ag/AgCl ISE and a CIM carbon-based reference electrode with a reference membrane. A photograph of the device is shown on the bottom left. The average and standard deviation of each data point is based on measurements with three individual devices.

boundary potentials are well-defined. Specifically, the phase boundary potential between the sample and Ag/AgCl is defined by the redox reaction $AgCl(s) + e^- \rightleftarrows Ag(s) + Cl^-(aq)$, the phase boundary potential at the sample/reference membrane interface is defined by the ionic liquid, and the phase boundary potential at the reference membrane/CIM carbon interface is defined by the redox couple. As a result, these paper-based Cl⁻ sensing devices exhibit a highly reproducible Nernstian response with a slope of −59.8 ± 0.9 mV/decade and a $E°$ of 19.8 ± 2.1 mV over the range from $10^{-1.0}$ to $10^{-3.5}$ M. This demonstrates that CIM carbon-based reference systems can be successfully integrated into miniaturized potentiometric systems based on paper.

CONCLUSIONS

This work has demonstrated that CIM carbon can be used as a solid contact material to fabricate all-solid-state reference electrodes. This permits the construction of high-performance all-solid-state potentiometric ISEs and reference electrodes with the same type of solid contact, polymer matrix, fabrication process, and electrode configuration. CIM carbon-based reference electrodes exhibit a very low dependence of the

half-cell potential in solutions of various electrolytes with concentrations in a wide range. Due to the low amounts of redox-active impurities on the surface of CIM carbon, phase boundary potentials at the membrane/CIM carbon interfaces can be defined well by the $[Co(C_9,C_9\text{-bipy})_3]^{2+/3+}$ redox couple, permitting a high electrode-to-electrode reproducibility of $E°$. The interconnected mesopores of CIM carbon offer the added advantage of a high double-layer capacitance, resulting in potential drifts as low as 1.7 μV/h and making these electrodes the most stable all-solid-state reference electrodes reported so far.

We also demonstrated that the CIM carbon-based reference system can be integrated into a disposable paper-based potentiometric Cl⁻ sensing device, replacing the conventional Ag/AgCl reference electrode, eliminating the need for reference electrolyte, and eliminating liquid junction potentials. These miniaturized Cl⁻ sensing devices with CIM carbon-based reference systems are inexpensive, easy to handle, and offer very reproducible Cl⁻ measurements with sample volumes as low as 10 μL. Ongoing research involves the development of paper-based potentiometric sensing devices for other ions employing the same CIM carbon-based reference system, and the development of even more hydrophobic redox buffers to extend the calibration-free character of these devices to long-term measurements.

ASSOCIATED CONTENT

⑤ Supporting Information

Schematic of an all-solid-state reference electrode based on CIM carbon and an emf trace showing long-term stability. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors
*A. Stein. E-mail: a-stein@umn.edu.
*P. Bühlmann. E-mail: buhlmann@umn.edu.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Bakker, E.; Bühlmann, P.; Pretsch, E. *Chem. Rev.* **1997**, *97*, 3083–3132.
(2) Johnson, R. D.; Bachas, L. *Anal. Bioanal. Chem.* **2003**, *376*, 328–341.
(3) Amemiya, S. In *Handbook of Electrochemistry*; Zoski, C. G., Ed.; Elsevier: Amsterdam, 2007.
(4) Bühlmann, P.; Chen, L. D. In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Jonathan, W. Steed, P. A. G., Eds.; John Wiley & Sons, Ltd: New York, NY, 2012.
(5) Bobacka, J.; Ivaska, A.; Lewenstam, A. *Chem. Rev.* **2008**, *108*, 329–351.
(6) Bakker, E.; Pretsch, E. *Angew. Chem., Int. Ed.* **2007**, *46*, 5660–5668.

(7) Lindner, E.; Gyurcsányi, R. E. *J. Solid State Electrochem.* **2009**, *13*, 51−68.

(8) Michalska, A. *Electroanalysis* **2012**, *24*, 1253−1265.

(9) Dohner, R. E.; Wegmann, D.; Morf, W. E.; Simon, W. *Anal. Chem.* **1986**, *58*, 2585−2589.

(10) Payne, R. B.; Buckley, B. M.; Rawson, K. M. *Ann. Clin. Biochem.* **1991**, *28*, 68−72.

(11) D'Orazio, P.; Verghese, D. *Ann. Clin. Biochem.* **1991**, *28*, 628−629.

(12) Lan, W.-J.; Zou, X. U.; Hamedi, M. M.; Hu, J.; Parolo, C.; Maxwell, E. J.; Bühlmann, P.; Whitesides, G. M. *Anal. Chem.* **2014**, *86*, 9548−9553.

(13) Rius-Ruiz, F. X.; Bejarano-Nosas, D.; Blondeau, P.; Riu, J.; Rius, F. X. *Anal. Chem.* **2011**, *83*, 5783−5788.

(14) Rius-Ruiz, F. X.; Crespo, G. A.; Bejarano-Nosas, D.; Blondeau, P.; Riu, J.; Rius, F. X. *Anal. Chem.* **2011**, *83*, 8810−8815.

(15) Ping, J.; Wang, Y.; Fan, K.; Tang, W.; Wu, J.; Ying, Y. *J. Mater. Chem. B* **2013**, *1*, 4781−4791.

(16) Novell, M.; Guinovart, T.; Blondeau, P.; Rius, F. X.; Andrade, F. J. *Lab Chip* **2014**, *14*, 1308−1314.

(17) Cui, J.; Lisak, G.; Strzalkowska, S.; Bobacka, J. *Analyst* **2014**, *139*, 2133−2136.

(18) Mi, Y.; Mathison, S.; Bakker, E. *Electrochem. Solid-State Lett.* **1999**, *2*, 198−200.

(19) Kakiuchi, T.; Yoshimatsu, T. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1017−1024.

(20) Shibata, M.; Sakaida, H.; Kakiuchi, T. *Anal. Chem.* **2011**, *83*, 164−168.

(21) Cicmil, D.; Anastasova, S.; Kavanagh, A.; Diamond, D.; Mattinen, U.; Bobacka, J.; Lewenstam, A.; Radu, A. *Electroanalysis* **2011**, *23*, 1881−1890.

(22) Kisiel, A.; Marcisz, H.; Michalska, A.; Maksymiuk, K. *Analyst* **2005**, *130*, 1655−1662.

(23) Kisiel, A.; Michalska, A.; Maksymiuk, K.; Hall, E. A. H. *Electroanalysis* **2008**, *20*, 318−323.

(24) Mousavi, Z.; Granholm, K.; Sokalski, T.; Lewenstam, A. *Analyst* **2013**, *138*, 5216−5220.

(25) Cadogan, A.; Gao, Z.; Lewenstam, A.; Ivaska, A.; Diamond, D. *Anal. Chem.* **1992**, *64*, 2496−2501.

(26) Zuliani, C.; Matzeu, G.; Diamond, D. *Talanta* **2014**, *125*, 58−64.

(27) Lai, C.-Z.; Fierke, M. A.; Stein, A.; Bühlmann, P. *Anal. Chem.* **2007**, *79*, 4621−4626.

(28) Zhang, T.; Lai, C.-Z.; Fierke, M. A.; Stein, A.; Bühlmann, P. *Anal. Chem.* **2012**, *84*, 7771−7778.

(29) Crespo, G. A.; Macho, S.; Rius, F. X. *Anal. Chem.* **2008**, *80*, 1316−1322.

(30) Rius-Ruiz, F. X.; Kisiel, A.; Michalska, A.; Maksymiuk, K.; Riu, J.; Rius, F. X. *Anal. Bioanal. Chem.* **2011**, *399*, 3613−3622.

(31) Ping, J.; Wang, Y.; Wu, J.; Ying, Y. *Electrochem. Commun.* **2011**, *13*, 1529−1532.

(32) Fierke, M. A.; Lai, C.-Z.; Bühlmann, P.; Stein, A. *Anal. Chem.* **2010**, *82*, 680−688.

(33) Hu, J.; Zou, X. U.; Stein, A.; Bühlmann, P. *Anal. Chem.* **2014**, *86*, 7111−7118.

(34) Li, Z.; Jaroniec, M. *J. Am. Chem. Soc.* **2001**, *123*, 9208−9209.

(35) Zou, X. U.; Zhen, X. V.; Cheong, J. H.; Bühlmann, P. *Anal. Chem.* **2014**, *86*, 8687−8692.

(36) Meier, P. C. *Anal. Chim. Acta* **1982**, *136*, 363−368.

(37) Fibbioli, M.; Morf, W. E.; Badertscher, M.; de Rooij, N. F.; Pretsch, E. *Electroanalysis* **2000**, *12*, 1286−1292.

(38) Vázquez, M.; Bobacka, J.; Ivaska, A.; Lewenstam, A. *Sens. Actuators, B* **2002**, *82*, 7−13.

(39) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. *Anal. Chem.* **2009**, *82*, 3−10.

(40) Maxwell, E. J.; Mazzeo, A. D.; Whitesides, G. M. *MRS Bull.* **2013**, *38*, 309−314.

(41) Cate, D. M.; Adkins, J. A.; Mettakoonpitak, J.; Henry, C. S. *Anal. Chem.* **2014**, *87*, 19−41.

(42) van den Berg, A. M. J.; Smith, P. J.; Perelaer, J.; Schrof, W.; Koltzenburg, S.; Schubert, U. S. *Soft Matter* **2007**, *3*, 238−243.

(43) Carrilho, E.; Martinez, A. W.; Whitesides, G. M. *Anal. Chem.* **2009**, *81*, 7091−7095.

(44) Morf, W. E. *The Principles of Ion-Selective Electrodes and of Membrane Transport*; Elsevier: New York, NY, 1981.

(45) Upreti, P.; Metzger, L. E.; Bühlmann, P. *Talanta* **2004**, *63*, 139−148.