Easy-to-Make Capillary-Based Reference Electrodes with Controlled, Pressure-Driven Electrolyte Flow

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ABSTRACT

As solid-contact potentiometric sensors based on novel materials have reached exceptional stabilities with drifts in the low μV/h range and long-term, and calibration-free potentiometric measurements gain more and more attention, reference electrode designs that used to be satisfactory for most users do not satisfy the needs of new challenging applications. It is important that the interface between a reference electrode and the sample, often provided by a salt bridge, remains constant in ion composition over time. Excessive restriction of the flow of bridge electrolyte, e.g., by use of a nanoporous frits or gelled reference electrolyte solutions, can result in contamination of the salt bridge with sample components and depletion of the reference electrolyte by diffusion into samples. This can be avoided by use of salt bridges that flow freely into the sample. However, commonly used reference electrodes with free-flowing junctions often suffer either from experimental difficulties in assuring a minimum flow rate or from excessive flow rates that require frequent replenishing of bridge electrolyte. To this end, we developed a reference electrode that contains a concentrated electrolyte contacting samples through a 10.2 μm capillary. By applying a minimal pressure of 10.0 kPa, a flow rate of 100 nL/h is achieved. This maintains a constant liquid junction potential at the interface with the sample and avoids contamination of the reference electrode, as evidenced by a potential stability of 6 ± 3 μV/h over 21 days. With such a minimal flow rate, there is no need to refill the reference electrode electrolyte for years.

Keywords: Reference Electrodes, Salt Bridges, Capillary, Charge Screening, Liquid Junction, Potential Stability

Reference electrodes are designed and fabricated to provide reproducible and stable half-cell potentials in a wide variety of sample solutions.[1] Free-flowing reference electrodes achieve this by interfacing concentrated reference electrolyte solutions with the sample, providing both physical and electrical contact.[1-2] A liquid junction potential forms where the salt bridge and sample meet. By choosing electrolyte solutions with cations and anions that have matched mobilities (e.g., K^+ and Cl^-),[3] changes in liquid junction potentials with sample composition can be minimized.[3-7] In addition, the bridge electrolyte in these free-flowing electrodes continuously flows into the sample, which ensures that concentrations in the salt bridge do not change due to diffusion of ions into or out of the salt bridge. The constant ionic composition of the liquid–liquid interface ensures a stable liquid-junction potential and, thereby, a stable reference potential.[2] However, while reference electrodes with a free-flowing salt bridge provide the most reliable reference potentials, significant volumetric flow rates necessitate frequent replenishing of the bridge electrolyte solution. Moreover, the flow rates out of many of these electrodes changes throughout experiments as the height of electrolyte solution in the salt bridge and, therefore, pressure changes, which may impact long term measurements.

To reduce such complications, porous frits, cracked glass junctions, fiber plugs, gels, or other methods of flow restriction can be used to inhibit the convective flow of reference electrolyte into samples.[1, 8-16] Indeed, for reference electrodes that comprise a nanoporous glass frit (4–100 nm pores), convective ion transport is a less efficient mode of mass transport than diffusion, resulting in depletion of electrolyte within the frit and sample components fouling the salt bridge.[17-20] Such low flow rates and the ensuing contamination limit the use of low-flow salt bridges for long term measurements. Evidently, the same problem also exists in the limiting case when the electrode design does not permit for solution flow at all, as it is the case for gel-filled systems.[8, 21-

[23] To the contrary, increases in pore size to 500 nm and larger result in large flow rates that require frequent replenishment of the bridge electrolyte and result in significant contamination of the samples with bridge electrolyte.[17]

Nanoporous glass frits used to limit convective flow also have another disadvantage. Due to negative charges on the surface of glass, they bias the reference potential by charge screening. when the Debye length in intermediate or low ionic strength samples approaches or exceeds the diameter of the pores of the frit.[17-18] It appears that this problem is avoided in the reference electrodes developed by Gao and co-workers, who used arrays of channels through a glass plate to interface samples with a salt bridge.[24] Exact channel diameters were not reported but the comment that those channels were smaller than 500 nm suggests that they were probably large enough to avoid charge screening. The reported net standard deviation over 6 months of ±0.30 mV looks very promising, but unfortunately linear flow rates of solution within the channels and long term EMF drift values were not reported. Pressure was provided with a pressurized gas (345 kPa) to give a volumetric flow rate of 360 nL/h. In other work, pressure to drive the flow of bridge electrolyte into samples was obtained by taking advantage of the vapor pressure of organic solvents or using weighted pistons.[24-30]

Here we report on an easy-to-make reference electrode that contains only a single 10.2 μm diameter capillary as the connection between a KCl reference electrolyte solution and the sample. By controlling the pressure of the reference electrolyte solution to the value of 10.0 kPa, a linear flow rate of 1.2 m/h and volumetric flow rate of 100 nL/h is achieved that avoids diffusion of sample components into the salt bridge. The liquid junction potential at the interface of the electrolyte-filled capillary with samples can be predicted with the Henderson equation[31] and is not affected by charge screening at the capillary walls. By minimizing the contact area of the salt

bridge with samples through use of a capillary, a minimal flow rate can be achieved that makes these electrodes well-suited for long-term measurements where constant flow rates and minimal drift are essential.

EXPERIMENTAL SECTION

Materials

Solutions were prepared using deionized and charcoal-treated water (>18.2 MΩ cm specific resistance) obtained with a Milli-Q PLUS reagent grade water system (Millipore, Bedford, MA). AgCl, KCl, $FeCl_3$, NaCl, Na_2HPO_4, NaH_2PO_4, methylene blue, and 1.0 M HCl were purchased from Sigma-Aldrich, Ag wires (0.5 mm diameter, ≥ 99.9% purity) from Alfa Aesar, tee connectors with compression screw-on straight connectors for 6.35 mm outer diameter (O.D.) tubing (part number 50775K395), backflow-prevention (one-way) valves with barbed fittings for 4.76 mm I.D. tubing (part number 7757K42), tubing supports (6.35 mm O.D.; 4.76 mm I.D.; part number 50775K403), high accuracy, low-pressure gauge for the pressure range 0–60 inches of H_2O (0–15 kPa; part number 4026K17), Tygon tubing (12.7 mm O.D.; 9.52 mm I.D) from McMaster-Carr, and Tygon tubing (6.35 mm O.D.; 4.32 mm I.D.) from Home Depot (Minneapolis, MN, USA) 10.2 μm I.D. fused silica capillary coated with polyimide (part number 1068150005) from Molex (Lisle, IL, USA), and two-part epoxy glue (extra working time) from Loctite (Westlake, OH, USA). Ketchup, chicken broth, and kefir were purchased at a local supermarket and used as received, while the unfiltered beer was a home-brewed Dark Mild Ale brewed 3 months prior to measurements and stored in a fridge. All chemicals were used as received.

Reference Electrode Fabrication

A 10 cm section of capillary was inserted into a 3 cm section of Tygon tubing with an inserted nylon tubing support (Figure S1). The space between the tubing and capillary support and

between the tubing support and capillary was filled with well-mixed two-part epoxy glue, which was then allowed to cure for at least 24 h. In a similar manner, 5 cm AgCl-coated Ag wire (preparation reported previously[19]) was inserted into a 3 cm section of Tygon tubing with an inserted tubing support. To secure the wire in place and seal the tubing, the tube and support were then filled in with well-mixed two-part epoxy glue, which was allowed to cure for at least 24 h (Figure S1). The capillary contained within the Tygon tubing was trimmed to a total length of 5 cm, mounted into a screw cap, and attached to a tee connector (see Figures 1, S2, and S3). Likewise, a 10 cm section of Tygon tubing was attached to the same tee connector using a screw cap (Figure 1). The electrode body was then filled completely with either AgCl-saturated 3.0 M KCl solution (prior to electrochemical measurements) or 50.8 mM methylene blue solution (prior to flow rate experiments) so that a small amount of solution overflow entered the open-ended tubing protruding from the third opening of the tee connector (~ 5 mL solution was required in either case). The AgCl-coated Ag wire contained within the Tygon tubing was then attached to the third inlet of the tee connector using a screw cap (Figures 1 and S4). The 10 cm of tubing from the tee connector was attached to a 3-way y-shaped connector. Tygon tubing (12.7 mm O.D.) was then used to attach one remaining branch of the 3 way-connector to a high-accuracy, low pressure gauge for monitoring the applied pressure. The other branch of the 3-way connector was connected with Tygon tubing to a barbed one-way backflow prevention valve, which allowed gas to be added to the system (Figure S5). Pressure was applied to the electrode through the one-way valve using house nitrogen. A very low stream of nitrogen was applied to the one-way valve until the pressure gauge read 40 inches of H_2O (10 kPa). Pressure loss to the system was monitored with the attached pressure gauge, and more nitrogen was added in the same manner when needed. Alternatively, pressure could be applied through the one-way valve using a large pipet bulb.

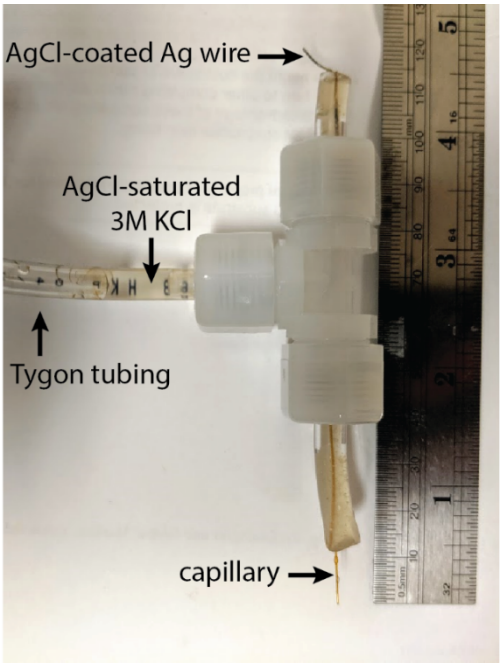

Figure 1. Image of a fully assembled capillary-reference electrode. See Figure S5 of the Supporting Information for photographs of the assembled capillary-based reference electrode.

Reference Electrode Flow Rates

The reference electrode flow rate was measured using electrodes filled with 50.8 mM methylene blue and pressurized to 10.0 kPa with N_2. The capillary of the reference electrodes was placed into a 20 mL vial filled with a known mass of H_2O. The vial was sealed using parafilm to prevent evaporation (with a 0.5 mm hole to prevent pressurization). Reference electrodes were removed after the initially colorless H_2O was visually colored blue after 2 – 3 days. An example of a solution after this time is shown in Figure S6. The absorbance of the resulting solutions was measured using UV–vis spectroscopy at 664 nm. A calibration curve was measured using 10.1, 5.07, 1.52, and 0.812 μM methylene blue solutions. Each measurement was performed using a 1 cm pathlength quartz cuvette. A representative calibration curve is shown in Figure S7. Note that the experimentally found flow rate of 1.2 m/h results in a mass transport rate exceeding diffusion by many orders of magnitude (e.g., diffusion of Fe^{3+} in bulk water over 1 h has been estimated as

2.1 mm),[19] confirming the assumption that diffusive mass transport of methylene blue into the sample did not affect these measurements significantly.

Potential Measurements

All potentials (E) were measured with an EMF 16 potentiometer (Lawson Labs, Malvern, PA) controlled with EMF Suite 1.02 software.

Reproducibility of Reference Potential Measurements

The potentials of capillary-based reference electrodes were measured relative to both a free-flowing double-junction reference electrode with a 3.0 M KCl bridge electrolyte and AgCl-saturated 3.5 M KCl reference electrolyte (DX200, Mettler Toledo, Switzerland)[2] and a AgCl-coated Ag wire. Initially, each electrode was placed into 200 mL of deionized and charcoal-treated water before KCl was added by additions from 0.010 and 1.0 M KCl solutions. Activity coefficients were calculated using a two-parameter Debye–Hückel approximation,[32] and potential measurements of the AgCl-coated Ag wire were corrected for liquid junction potentials using the Henderson equation[31] for the interface of the KCl-containing sample with the capillary based (3.0 M KCl) or free-flowing (3.0 M KCl) reference electrode (i.e., the same liquid junction calculation in each case).

Reference Potential Stability Measurements

Potentials of three capillary-based reference electrodes were measured in stirred AgCl-saturated 100 mM KCl at 32 °C after being in contact with the solution for 48 h for temperature equilibration. The experimental temperature was controlled using a water-jacketed sample beaker with a water-recirculating bath and a temperature-regulated dark Faraday cage (Watlow EZ-Zone Temperature Controller, Brooklyn Park, MN, USA). The jacketed sample beaker was sealed on the top using parafilm to prevent evaporation of the solution. Potentials of each electrode were

measured relative to a AgCl coated Ag wire for 21 days in the Faraday cage. The applied pressure decreased approximately 125 Pa each day, and N_2 was added daily to maintain a constant 10.0 kPa. In addition, using an identical setup to that above, the reference potentials of the capillary-based reference electrodes were monitored as the applied pressure was varied from 1.2 to 12.4 kPa.

Potentials of three capillary-based reference electrodes were also measured in complex food samples, including ketchup, chicken broth, green fruit smoothie, and unfiltered home-brewed beer. Electrodes were equilibrated in 100 mM KCl held at 32 °C in a temperature-controlled Faraday cage for at least one hour before switching the electrodes to a food product that was also equilibrated for at least one hour in the same cage. Potentials were recorded every 30 seconds for 24 h in unstirred food samples versus a pH half-cell electrode (HI2111B, Hanna Instruments).

The potential of one capillary-based reference electrode was measured over several days versus a pH half-cell electrode in artificial blood serum solution using a flow-through system constructed of PVC piping and tee fittings. A water-recirculating bath was used to drive flow of the solution through the PVC pipe at the fast rate of 12 cm/s.

Resistance Measurements

The resistance of the capillary-based reference electrode was measured using the known shunt method.[33] The potentials, E_1, of three reference electrodes were first measured in stirred 100 mM KCl at room temperature versus a AgCl-coated Ag wire. The potentials, E_2, of each electrode were measured a second time after the electrodes were individually shorted to the AgCl-coated Ag wire through a 10 MΩ resistor. Resistances were calculated as $10\ \text{M}\Omega \times (E_1 - E_2)/E_2$.

RESULTS AND DISCUSSION

Reference Electrode Flow Rate and Electrical Resistance

In order for a capillary-based reference electrode to be useful for electrochemical measurements, its flow rate must be small enough so that sample solutions are not significantly contaminated with bridge electrolyte. Moreover, the flow rate must be large enough so that components of the sample solution cannot diffuse against the fluid flow into the bridge electrolyte, as discussed previously.[19,20] The volume of solution that flows through a capillary over time (V/t) can be described with the following equation:[34]

$$V/t = p \pi r^4 / 8 \eta L \quad (1)$$

where V is volume, t is time, p is pressure, r is radius, η is viscosity, and L is the length of the capillary. Substituting into eqn 1 the parameters of the reference electrode capillaries, viscosity of 3 M KCl,[35] and pressure used in this work ($\eta = 1.012 \times 10^{-3}$ Pa s, p = 10,000 Pa, L = 0.05 m, and $r = 5.1 \times 10^{-6}$ m) gives $V/t = 5.25\ 10^{-14}$ m^3/s or 189 nL/h. Flow rates experimentally determined with 50.8 mM methylene blue solutions were within 100 ± 10 nL/h (n = 3) somewhat lower than the theoretically predicted ones but notwithstanding of similar magnitude. Importantly, these flow rates are over three orders of magnitude lower than the flow rate through porous polyethylene frits with pore diameters of ≈ 10 μm (318 ± 279 μL/h) and less than two orders of magnitude larger than that of a reference electrode with a porous Vycor frit (0.004 ± 0.002 μL/h).[17] With this flow rate, a capillary-based reference electrode would not need to be refilled for years, as it contains ~5 mL of electrolyte solution, and contamination from inner filling solution flowing into the sample is very small. For example, in a 10 h experiment with a reference electrode comprising 3 M KCl inner filling solution and a 1 mL sample, the addition in KCl concentration would be 3 mM. If this value is still too large, a different electrolyte salt may be chosen. Alternatively, the diameter of the

capillary may be reduced as long as the accompanying increase in electrical resistance is acceptable.

Importantly, the experimental flow rate for our capillary-based reference electrodes can be converted to a linear flow rate at which the electrolyte solution passes through the end of the capillary (1×10^{-10} m^3/h divided by $(5.1 \times 10^{-6}$ m$)^2 \times 3.14$), resulting in 1.2 m/h. This rate of flow is sufficient to prevent effectively the diffusion of electrically neutral compounds and ions into the reference electrode (K^+ and Cl^- diffuse approximately 3 mm over one hour), unlike is the case for reference electrodes utilizing nanoporous frits.[19] The small size of the contact area between the sample and the capillary that results from the use of a capillary gives rise to a resistance of 32 ± 3 MΩ, which is slightly smaller in magnitude than the resistance of a typical pH glass electrode and is, therefore, fully compatible with the input impedance of modern potentiostats.[36] Because the resistance relevant to a potentiometric is the one of the total electrochemical cell, the resistance of the reference electrode is only of substantial relevance when it exceeds the resistance of the measuring electrode. We also note that should there be a need for a smaller electrical resistance, the length of the capillary could be reduced substantially.

Reference Electrode Potential Reproducibility and Stability

Capillary-based reference electrode potentials were monitored in solutions containing KCl at various concentrations relative to a AgCl-coated Ag wire as well as relative to a free-flowing double junction reference electrode. It was expected that that the potential of the AgCl-coated Ag wire would vary according to the Nernst equation[31] for Cl^- activity, and the half-cell potentials of both the capillary-based and the free-flowing double junction reference electrode would vary only with the concentration of KCl in the sample solution as predicted by theory for liquid junction potentials.[3] Figure 2 shows the potential of a AgCl-coated Ag wire as Cl^- sensor in a sample

solution of varying Cl activity ($\log a_{Cl}$ = –6.7 to –1.7), measured once relative to a capillary-based and once relative to a free-flowing double junction reference electrode (experiments repeated three times with newly prepared capillary-based reference electrodes each time). After correction for the liquid junction potential, the average slope of the linear region ($\log a_{Cl}$ = -3.7 to -1.7) of the Cl^- response was identical for the capillary-based and free-flowing reference electrode, -56.3 ± 0.2 mV/decade.

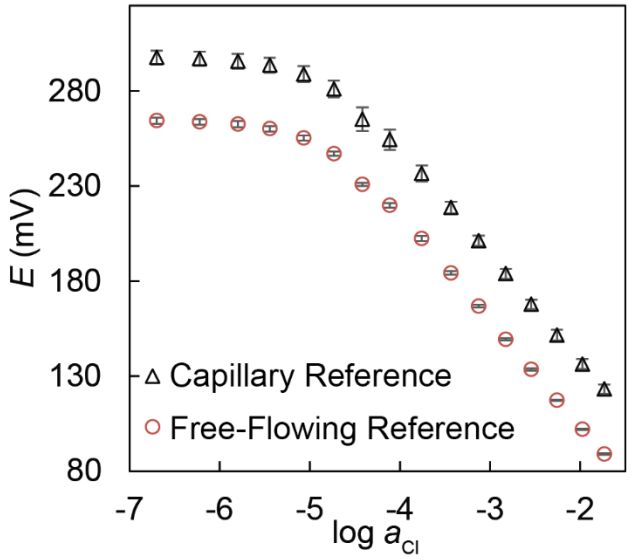

Figure 2. Average potentials of a AgCl-coated Ag wire measured relative to a capillary-based and free-flowing reference electrode as a function of the Cl^- activity of a KCl solution (experiment repeated three times). *E* values are corrected for liquid junction potentials.[18] Error bars are the standard deviation for the average of three separate measurements. The two calibration curves are offset from one another because the inner filling solutions of the free-flowing and the capillary-based reference electrode were 3.5 M and 3.0 M KCl, respectively.

In addition, the potential of the capillary-based reference electrode was measured relative to the free-flowing double junction reference electrode. Each reference electrode interfaced the

sample with a 3.0 M KCl bridge electrolyte. After correction for a constant offset caused by the use of a free-flowing reference electrode with a 3.5 M KCl bridge electrolyte and a capillary-based reference electrode with a 3.0 M KCl bridge electrolyte, the average potentials for three separate experiments (with three newly prepared capillary-based reference electrodes and a single free-flowing reference electrode) did not vary statistically from 0 mV over the activity range studied (log a_{Cl} = –6.7 to –1.7), which indicates that the capillary-based reference electrode performs in an identical manner to the free-flowing reference electrode with a sleeve junction (Figure 3).

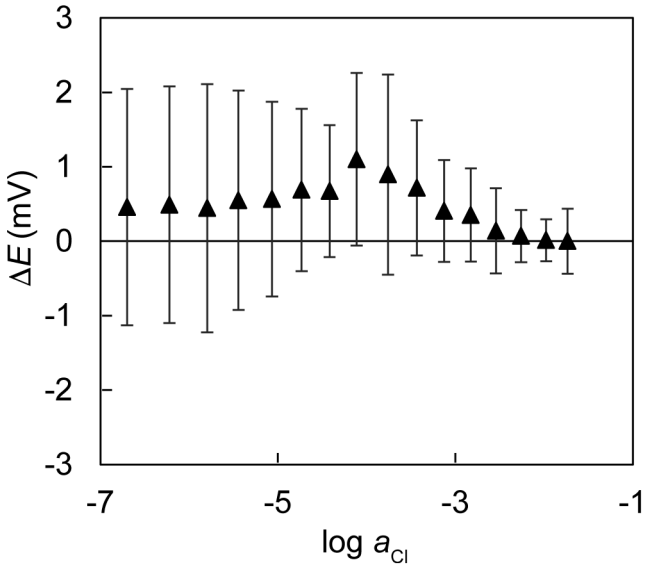

Figure 3. Average potentials, *E*, of capillary-based reference electrodes measured relative to a free-flowing double-junction reference electrode with a 3.0 M KCl bridge electrolyte as a function of the Cl^- activity of KCl in the sample. *E* values of the capillary-based and free-flowing reference electrodes are not corrected for liquid junction potentials. Error bars are the standard deviation for the average of three separate measurements with three newly prepared capillary-based reference electrodes. Note that ΔE was adjusted to account for the difference in inner filling solutions of the free-flowing reference electrode (3.5 M KCl) and capillary-based reference electrode (3.0 M KCl).

Potentials of the capillary-based reference electrodes were monitored continuously for 21 days to determine the stability of the reference potential (see Figure 4 with data for 3 electrodes). Note that in this case potentials were measured relative to a AgCl-coated Ag wire to avoid artifacts from potential liquid junction fluctuations at the salt bridge interface of free-flowing sleeve-junction electrodes, which in our experience can be significant over periods of days when bridge electrolyte is not replenished regularly to avoid substantial changes in the filling height of the salt bridge electrolyte (see Figure S8 of the Supporting Information).

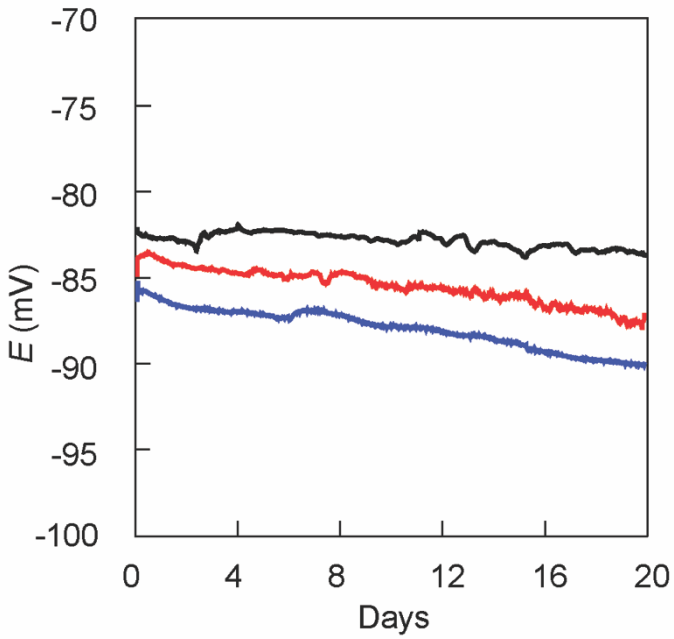

Figure 4. Individual potentials of three capillary based reference electrodes measured relative to a AgCl coated Ag wire in a AgCl-saturated 100 mM KCl solution at 32 °C, after 48 h initial equilibration.

The thus determined drift of the capillary-based reference electrodes was 6 ± 3 µV/h (n=3), with a standard deviation of the measured potential of 2 mV (n=3). This drift is comparable to the

lowest drifts reported for solid-contact reference electrodes.[37] Notably, initial experiments in which three capillary-reference electrodes were immersed into a sample in a temperature-controlled water-jacketed beaker showed a strong correlation of the three simultaneously measured potentials (Figure S9). This is believed to be due to temperature dependence of the response of the AgCl-coated silver wire and the laboratory's temperature fluctuations that influence the parts of the reference electrodes not immersed into the sample solution. Therefore, a temperature-regulated Faraday cage was used to further minimize temperature fluctuations. Potentials were also measured as a function of the pressure applied (1.2 to 12.4 kPa) to the reference electrode. Data for four capillary-based reference electrodes do not indicate a dependence of the reference potential on the applied pressure (Figure S10).

Over the course of 21 days in 100 mM KCl (Figure 4), the three capillary-based reference electrodes show a very low drift of about 0.14 mV per day and required no additional inner filling solution. This makes them well suited for use in long term electrochemical experiments. Because preliminary experiments suggest that the AgCl-coated silver wires cause much of the remaining small drift, we are currently exploring how the AgCl/Ag reference half cell can be optimized to reduce the residual EMF drift even further.

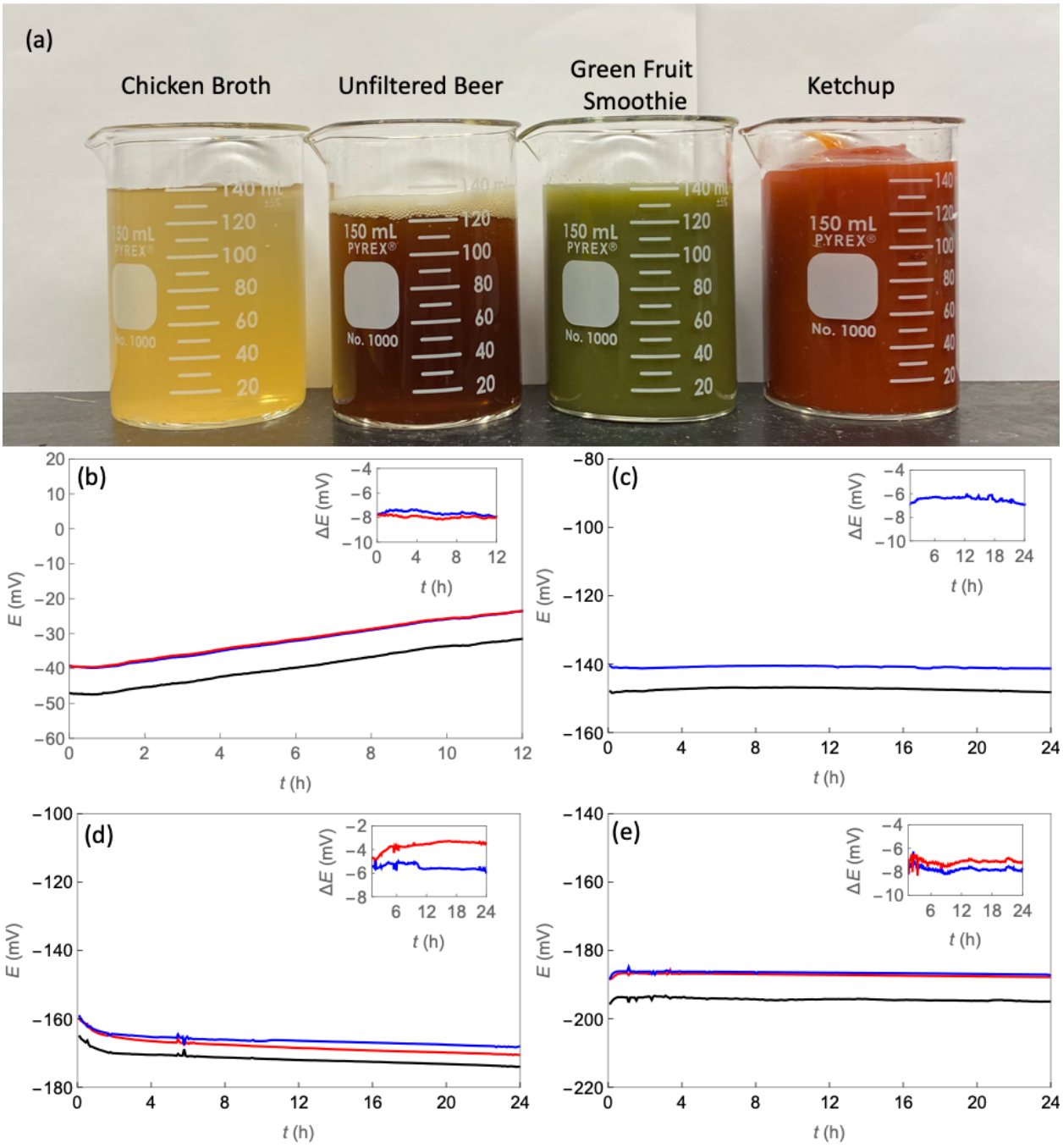

Figure 5. (a) Food samples used in long-term stability studies, with the individual potentials of separate capillary-based reference electrodes measured over 12 or 24 h versus a pH half-cell electrode in (b) chicken broth (n=3), (c) unfiltered homebrewed beer (n=2), (d) green fruit smoothie (n=3), and (e) ketchup (n=3). Insets represent the difference in potential between two

separate capillary-based reference electrodes in each sample. All experiments were carried out in a temperature-controlled Faraday cage at 32 ^{o}C.

The drifts of capillary-based reference electrodes versus a pH half-cell electrode were also measured in a 32 ^{o}C Faraday cage in four different food samples, that is, chicken broth, unfiltered beer, green fruit smoothie, and ketchup, as shown in Figure 5a. These samples varied not only in chemical makeup but also opacity (ketchup and the smoothie being completely opaque) and viscosity. As shown by Figure 5b, the potential drift in chicken broth was larger than for the other three samples, which this is most likely due to the chicken broth spoiling throughout the duration of the 12 h experiment, causing a change in the pH over time. However, comparison of the three capillary-based electrodes in the experiment shows exceptional electrode-to-electrode performance. For each experiment, the potentials of Electrodes 2 and 3 were subtracted from that of Electrode 1 to determine how similar the drift and overall performance was (see insets in Figure 5 and Figure S11). While the average EMF drifts in each food sample were larger than that in KCl solution, the main cause of this drift is change in pH. The drifts of the potential difference plots are quite minimal. showing that the capillary-based reference electrodes behaved well even in these complex samples. In chicken broth, the drift of the difference plots for Electrodes 2 and 3 were -7 and -33 μV/h. In unfiltered beer (Figure 5c), the EMF of two capillary based reference electrodes exhibited a drift of -19 ± 5 μV/h (n=2) over 24 h while the drift in the potential difference plot was only -5 μV/h. In the green fruit smoothie (Figure 5d), some spoilage was also observed (separation of sample into layers and a pungent rotting fruit smell), resulting in a somewhat larger EMF drifts, with an average of -214 ± 39 μV/h (n=3). In this case the difference plots also showed somewhat larger drifts of 26 and 23 μV/h. This could be due to the need for a longer temperature equilibration time as the smoothie may not have fully come to 32 ^{o}C within the hour resulting in a

steep decrease in potential over the first 2 h. In ketchup (Figure 5e), the average drift was -41 ± 4 μV/h (n=3), with the difference plots having very minimal slopes of 1.1 and 6.4 μV/h. Again, as ketchup came from the fridge and was a rather viscous sample, more temperature equilibration time could have been used to improve these drifts as there was a sharp increase in the potential over the first hour of the experiment. The difference plots between electrodes do show exceptional electrode-to-electrode performance, making these electrodes viable options in almost any type of sample, from those that are chemically complex to those that have a range of viscosities.

Additionally, a capillary-based electrode was successfully integrated into a flow-through system. The potential in circulating solution comprising 4.1 mM KCl, 99.6 mM NaCl, 0.78 mM Na_2HPO_4, and 0.22 mM NaH_2PO_4 (pH 7.4), mimicking the electrolyte composition of blood serum, versus a pH half-cell electrode was only 3 μV/h over 50 h (Figure S12). This minimal drift does indicate that these electrodes are good candidates for use in long-term flow through measurements.

CONCLUSIONS

Recent developments in the field of solid-contact ion-selective electrodes have pushed indicator electrode drifts into the low μV/h range, bringing us close to calibration-free long-term monitoring. However, conventional reference electrode designs that used to be satisfactory for most users no longer satisfy the needs of such challenging applications. Indeed, we believe that some of the recently published reports on solid-contact ion-selective electrodes erroneously interpret contributions of the reference half-cell potential to the total EMF drift as originating from the sensing electrode. To this end, we developed a capillary-based reference electrode with electrolyte flow into the sample controlled by an externally applied pressure. This reference electrode provides (i) a volumetric flow rate of 100 ± 10 nL/h, (ii) potential stability of 6 ± 3 μV/h,

(iii) electrical resistance of 32 ± 3 MΩ, and (iv) reference potentials that vary according to the theoretically predicted liquid junction potential, without any interference from charge screening by charges on the capillary wall. By controlling the flow of reference electrolyte through the capillary using externally applied pressure, flow and not diffusion dominates the transport of ions through the capillary. This ensures that the interface of the electrolyte-filled capillary with sample solutions remains constant and clean over time, which is essential for a stable and reproducible liquid junction potential. Unlike in the case of free-flowing reference electrodes that rely on the height of their inner filling solution to define the flow rate of electrolyte, the capillary-based reference electrode only exhibits minimal changes in flow rate over time. In addition, by decreasing the contact area of the salt bridge with the sample by use of a capillary as opposed to a porous frit or sleeve-junction, the volumetric flow rate is greatly reduced while still keeping the linear flow rate high. Each of these characteristics makes these capillary-based reference electrodes well suited for electrochemical measurements in solutions of various ionic strength over short and or long periods of time. Applications in which these capillary-based reference electrodes will be particularly useful include long term measurements with minimal maintenance as well as situations in which frequent recalibration is difficult, contamination of the reference electrode with sample components must be avoided, or the consumption of large volumes of flowing bridge electrolyte is not acceptable.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information includes images of a tubing support, Tygon tubing, epoxy-sealed capillary, epoxy-sealed AgCl-coated Ag wire, reference-electrode tee connector, fully assembled

capillary-based reference electrode, free-flowing reference electrode with a sleeve-junction, and coloration of an initially colorless sample solution after flow of methylene blue (50.8 mM) through the capillary of a capillary-based reference electrode. It also includes figures of a representative calibration curve of methylene blue, and potentials of three capillary-based reference electrodes, measured relative to a AgCl-coated Ag wire and as a function of the applied pressure, as well as the potentials of three electrodes in different food samples relative to a pH electrode, and one electrode in a flow-through system relative to a pH electrode. This material is available free of charge on the ACS Publications website at DOI: *(to be added by editor)*.

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Notes

The authors declare no competing financial interest.

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REFERENCES

1. Inzelt, G.; Lewenstam, A.; Scholz, F., *Handbook of Reference Electrodes*. Springer: Heidelberg, Germany, 2013.

2. Dohner, R. E.; Wegmann, D.; Morf, W. E.; Simon, W., Reference electrode with free-flowing free-diffusion liquid junction. *Anal. Chem.* **1986,** *58* , 2585-2589.

3. Morf, W. E., *The Principles of Ion-Selective Electrodes and of Membrane Transport*. Elsevier: New York, 1981.

4. Harper, H. W., Calculation of liquid junction potentials. *J. Phys. Chem.* **1985,** *89*, 1659-1664.

5. Morf, W. E., Calculation of liquid-junction potentials and membrane potentials on the basis of the Planck theory. *Anal. Chem.* **1977,** *49*, 810-813.

6. Davison, W.; Covington, A. K.; Whalley, P. D., Conventional residual liquid junction potentials in dilute solutions. *Anal. Chim. Acta* **1989,** *223*, 441-447.

7. Gorman, M.; Murphy, S. M. C., Liquid junction potential calculations. *J. Chem. Educ.* **1949,** *26*, 579-580.

8. Victoria, L.; Ortega, M. G.; Ibanez, J. A., Construction and evaluation of an inexpensive reference electrode with internal electrolyte in agar matrix. *J. Chem. Educ.* **1990,** *67*, 179-180.

9. East, G. A.; del Valle, M. A., Easy-to-Make Ag/AgCl Reference Electrode. *J. Chem. Educ.* **2000,** *77*, 97.

10. Giner, J., A Practical Reference Electrode. *J. Electrochem. Soc.* **1964,** *111*, 376-377.

11. Shinwari, M. W.; Zhitomirsky, D.; Deen, I. A.; Selvaganapathy, P. R.; Deen, M. J.; Landheer, D., Microfabricated reference electrodes and their biosensing applications. *Sensors* **2010,** *10*, 1679-715.

12. Suzuki, H.; Shiroishi, H.; Sasaki, S.; Karube, I., Microfabricated Liquid Junction Ag/AgCl Reference Electrode and Its Application to a One-Chip Potentiometric Sensor. *Anal. Chem.* **1999,** *71*, 5069-5075.

13. Suzuki, H.; Hirakawa, T.; Sasaki, S.; Karube, I., Micromachined liquid-junction Ag/AgCl reference electrode. *Sens. Actuators B* **1998,** *46*, 146-154.

14. Suzuki, H.; Taura, T., Thin-Film Ag/AgCl Structure and Operational Modes to Realize Long-Term Storage. *J. Electrochem. Soc.* **2001,** *148*, E468.

15. Suzuki, H.; Hirakawa, T.; Sasaki, S.; Karube, I., An integrated three-electrode system with a micromachined liquid-junction Ag/AgCl reference electrode. *Anal. Chim. Acta* **1999,** *387*, 103-112.

16. Zhou, J.; Ren, K.; Zheng, Y.; Su, J.; Zhao, Y.; Ryan, D.; Wu, H., Fabrication of a microfluidic Ag/AgCl reference electrode and its application for portable and disposable electrochemical microchips. *Electrophoresis* **2010,** *31*, 3083-3089.

17. Mousavi, M. P.; Saba, S. A.; Anderson, E. L.; Hillmyer, M. A.; Bühlmann, P., Avoiding Errors in Electrochemical Measurements: Effect of Frit Material on the Performance of Reference Electrodes with Porous Frit Junctions. *Anal. Chem.* **2016,** *88*, 8706-8713.

18. Mousavi, M. P.; Bühlmann, P., Reference electrodes with salt bridges contained in nanoporous glass: an underappreciated source of error. *Anal. Chem.* **2013,** *85*, 8895-8901.

19. Anderson, E. L.; Lodge, T. P.; Tata, G.; Veglia, G.; Bühlmann, P., More than a Liquid Junction: Effect of Stirring, Flow Rate, and Inward and Outward Electrolyte Diffusion on Reference Electrodes with Salt Bridges Contained in Nanoporous Glass. *Anal. Chem.* **2019**, *91*, 7698-7704.

20. Anderson, E. L., Troudt, Blair K., Bühlmann, P., Critical Comparison of Reference Electrodes with Salt Bridges Contained in Nanoporous Glass with 5, 20, 50, and 100 nm Diameter Pores. *Anal. Sci.* **2020,** *36*, 187-191.

21. Hassel, A. W.; Fushimi, K.; Seo, M., An agar-based silver|silver chloride reference electrode for use in micro-electrochemistry. *Electrochem. Commun.* **1999,** *1*, 180-183.

22. Huang, I. Y.; Huang, R.-S.; Lo, L.-H., Improvement of integrated Ag/AgCl thin-film electrodes by KCl-gel coating for ISFET applications. *Sens. Actuators B* **2003,** *94*, 53-64.

23. Liao, W. Y.; Chou, T. C., Fabrication of a planar-form screen-printed solid electrolyte modified Ag/AgCl reference electrode for application in a potentiometric biosensor. *Anal. Chem.* **2006,** *78*, 4219-23.

24. Gao, F.; Broadley, S.; Chen, T.-Y.; Payne, P. M.; Silverman, H., A Stable Ultra Low Flow Reference Electrode Using a Nanochannel Glass Array Junction. *ECS Trans.* **2006,** *1*, 1-8.

25. Maurer, O.; Thieme, R. Apparatus for producing a pressure on the electrolytes in an electrode. German Patent 1,233,172, Jan. 26, 1967.

26. Yonco, R. M.; Nagy, Z. Long life reference electrode. U.S. Patent 4,818,366, Jul. 30, 1989.

27. Crandall, E. D.; DeLong, J., A pressure- and flow-insensitive reference electrode liquid junction. *J. Appl. Physiol.* **1976,** *41*, 125-128.

28. Koopman, F.; Kunkler, J. L. Reference and glass electrodes capable of withstanding high pressures. U.S. Patent 3463717A, Aug. 26, 1969.

29. Schindler, J. G.; Riemann, W. Reference electrodes for electrochemical analytical instruments. German Patent 2,559,816, Sept. 21, 1978.

30. Sugano, K.; Katayama, T.; Koyama, M. Flow-through reference electrodes. Japanese Patent 61,086,643, May 2, 1986.

31. Bard, A.; Faulkner, L., *Electrochemical Methods: Fundamentals and Applications*. John Wiley and Sons: Hoboken, New Jersey, 2001; Vol. 2.

32. Meier, P. C., 2-Parameter Debye–Hückel approximation for the evaluation of mean activity-coefficients of 109 electrolytes. *Anal. Chim. Acta* **1982,** *136*, 363-368.

33. Oesch, U.; Simon, W., Lifetime of neutral carrier based ion-selective liquid-membrane electrodes. *Anal. Chem.* **1980,** *52*, 692-700.

34. Gupta, S. V., *Viscometry for Liquids, Springer Series in Materials Science 194*. Springer: Switzerland, 2014.

35. *CRC Handbook of Chemistry and Physics*. 99th ed.; CRC Press/Taylor & Francis: Boca Raton, Florida, 2018.

36. Anderson, E. L.; Bühlmann, P., Electrochemical Impedance Spectroscopy of Ion-Selective Membranes: Artifacts in Two-, Three-, and Four-Electrode Measurements. *Anal. Chem.* **2016,** *88*, 9738-9745.

37. Hu, J.; Ho, K. T.; Zou, X. U.; Smyrl, W. H.; Stein, A.; Bühlmann, P., All-solid-state reference electrodes based on colloid-imprinted mesoporous carbon and their application in disposable paper-based potentiometric sensing devices. *Anal. Chem.* **2015,** *87*, 2981-7.

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