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Paper-Based Electroanalytical Devices with an Integrated, Stable Reference Electrode

Wen-Jie Lan[a], E. Jane Maxwell[a], Claudio Parolo[a,b], David K. Bwambok[a], Anand Bala Subramaniam[a], and George M. Whitesides[a,c*]

[a] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

[b] Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia, CIN2, Campus UAB, Barcelona, Spain

[c] Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138

[*] Corresponding author E-mail: gwhitesides@gmwgroup.harvard.edu

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With the rapid development of portable lab-on-a-chip devices, miniaturized electrochemical systems have attracted increasing attention, because they are sensitive, insensitive to light levels, independent of human interpretation of color, easily interfaced with the web, and require only a small volume (several μL) of sample.[1] We and others are exploring Electrochemical Paper-based Analytical Devices (EPADs)[2-6] (see Table S1 for additional references). EPADs often employ a printed Ag/AgCl "pseudo-reference electrode" whose potential ($AgCl(s) + e^- \rightleftarrows Ag(s) + Cl^-(aq)$) depends on the concentration of chloride ions in the sample solution.[7] These devices cannot maintain a stable potential unless a high and stable concentration of soluble chloride salts is added to the sample prior to measurement. This requirement limits their general applicability.

Several commercial hand-held micro-electrochemical diagnostic systems (for example, the i-STAT® clinical analyzer[8]) determine analytes in human blood through amperometry or potentiometry; these systems use miniaturized reference electrodes that resemble scaled-down versions of conventional glass-bodied Ag/AgCl reference electrodes. These three-dimensional miniaturized reference electrodes require sealing of an inner reference solution with a protective layer, and the implementation of a micro-junction between the reference solution and the sample.[9] They may require complicated lithography, or chip-manufacturing techniques.

Paper has been used as an integral component in electrochemical cells (for example, historically as a salt bridge to connect the oxidation and reduction half-cells in conventional galvanic cells, or more recently as a matrix to hold the internal reference solution in a separate reference electrode).[10] An integrated paper-based device that includes an accurate reference electrode would enable the full range of electrochemical measurements (*e.g.*, for voltammetry, and in measurements of pH and concentrations of ions) in a low-cost and disposable format. A
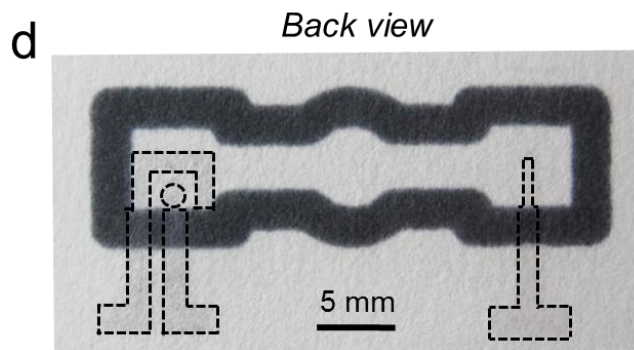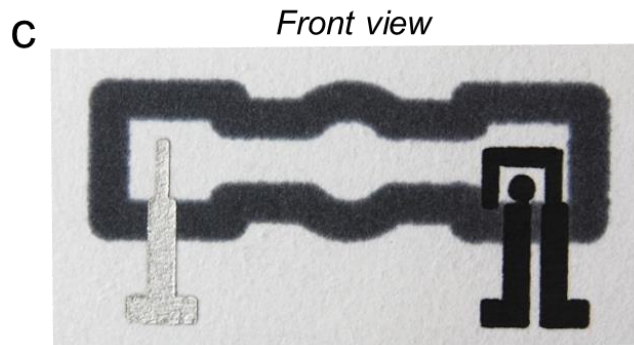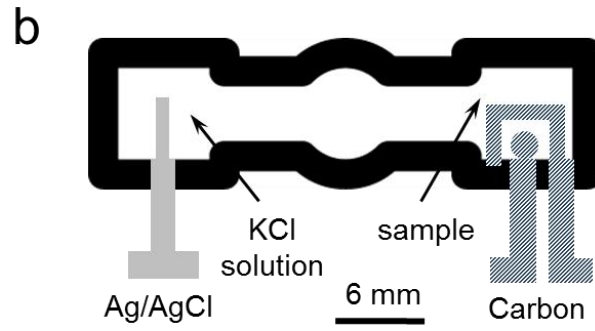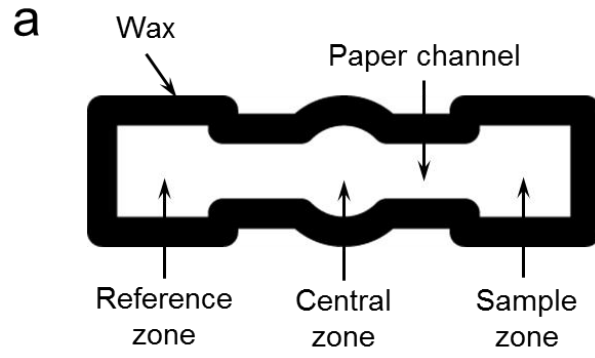
reference electrode of this kind has not yet been described.

Here we report the design of a well referenced Electrochemical Paper-based Analytical Device (herein after referred to as a "rEPAD", Figure 1) comprising a sample zone, a reference zone, and a connecting microfluidic channel that includes a mixing zone. This arrangement allows ionic contact between the sample and the reference solutions, but – due to the slow (diffusive), convection-free transport of ions in the liquid-filled paper channels – prevents interchange of ions between the sample and reference zones. These rEPADs provide a stable and well-defined reference potential for direct and accurate measurements of analytes; their performance is comparable to conventional electrochemical cells that contain commercial glass-bodied Ag/AgCl reference electrodes, and they offer several advantages: (i) they are made of paper, which is inexpensive, lightweight, portable, less fragile than glass, and easily disposable (for single use), (ii) they eliminate the need for storage of the electrode in a solution of KCl, (iii) they are fabricated in a planar structure, and thus appropriate for mass fabrication with roll-to-roll printing, and (iv) they can be easily integrated into systems that permit multiplexed analysis or pipette-free sampling.

Figure 1 shows the geometry of the rEPAD. We defined the microfluidic channels (Figure 1a) by wax printing,[11] and added electrodes to the device by stencil-printing carbon and Ag/AgCl inks in the sample and reference zones,[3] respectively. The resulting devices can be used without further modification (for experiments lasting < 6 min), or can be sealed with tape to minimize evaporation (for longer experiments). The Supporting Information (SI) provides additional details.

When two different solutions are added to the sample and reference zones, either by pipetting (~10 µL, for devices of the type shown in Figure 1) or dipping (for the devices shown

Figure 1. Schematic illustration (a and b) and photographs (c and d) of a referenced Electrochemical Paper-based Analytical Device (rEPAD). The paper was patterned by wax printing to define the sample zone, central contact zone, reference zone, and microfluidic channels. The sample and reference zones include stencil-printed carbon and Ag/AgCl electrodes, respectively. The dashed lines in (d) indicate the approximate boundaries of the carbon and Ag/AgCl ink printed on the back.

a

Wax

Paper channel

Reference zone   Central zone   Sample zone

b

KCl solution   sample

Ag/AgCl   6 mm   Carbon

c

Front view

d

Back view

5 mm

in Figure 2), capillarity pulls the liquids (aqueous solution of electrolytes such as potassium chloride) along the channels to the central zone. Once the liquids meet and completely saturate the hydrophilic network of cellulose fibers, capillary-driven flow and bulk convective transport of liquid stop. Instead, diffusional transport, due to concentration gradients across the interface between the two liquids, dominates the mass transport of ions within the device (subtle differences in the hydrodynamic pressure between the zones might, in principle, result in mass transport by convection, but we have found this contribution to be negligible – see SI).

Because the accuracy of the rEPAD depends on the concentration of the solutions in the reference and sample zones, and not in the zone of contact, we undertook experiments and calculations to determine if the sample and reference have constant concentrations within their respective zones. We sealed the tops and bottoms of the devices with transparent tape to minimize evaporation, and placed a solution of cobalt chloride ($CoCl_2$, pink) in contact with the left inlet of the device, and copper sulfate ($CuSO_4$, blue) with the right inlet. The pink Co^{2+} ions arrived (by wicking) at the zone of contact in less than 15 minutes (Figure 2a). Following this initial rapid transport of liquid, we observed no pink Co^{2+} diffusing into the right zone for 2 hours, and, correspondingly, no blue Cu^{2+} diffusing into the left zone. After 2 hours, the device began to dry by evaporation, as indicated by the color change. The times required for both wicking and evaporation were shorter (<1 min and ~20 min, respectively) in devices that were not sealed with tape (Figure S1 and SI).

We estimated the time required for an ionic species to diffuse from the interface of contact to the sample or reference zone using the Einstein relation (eq 1).[12]

$$\sigma^2 = 2Dt,$$ (1)

Assuming one-dimensional diffusion (a reasonable assumption since the thickness of the paper is

Figure 2. Photographs of a paper-based microfluidic device taken after wicking a pink aqueous solution of $CoCl_2$ and a blue solution of $CuSO_4$ to corresponding zones. The two solutions mixed in the central zone but did not diffuse into the right or left paper zone. The color of the $CoCl_2$ solution became paler after 1 h and blue after 12 h as water in the device gradually evaporated.

a   0 h
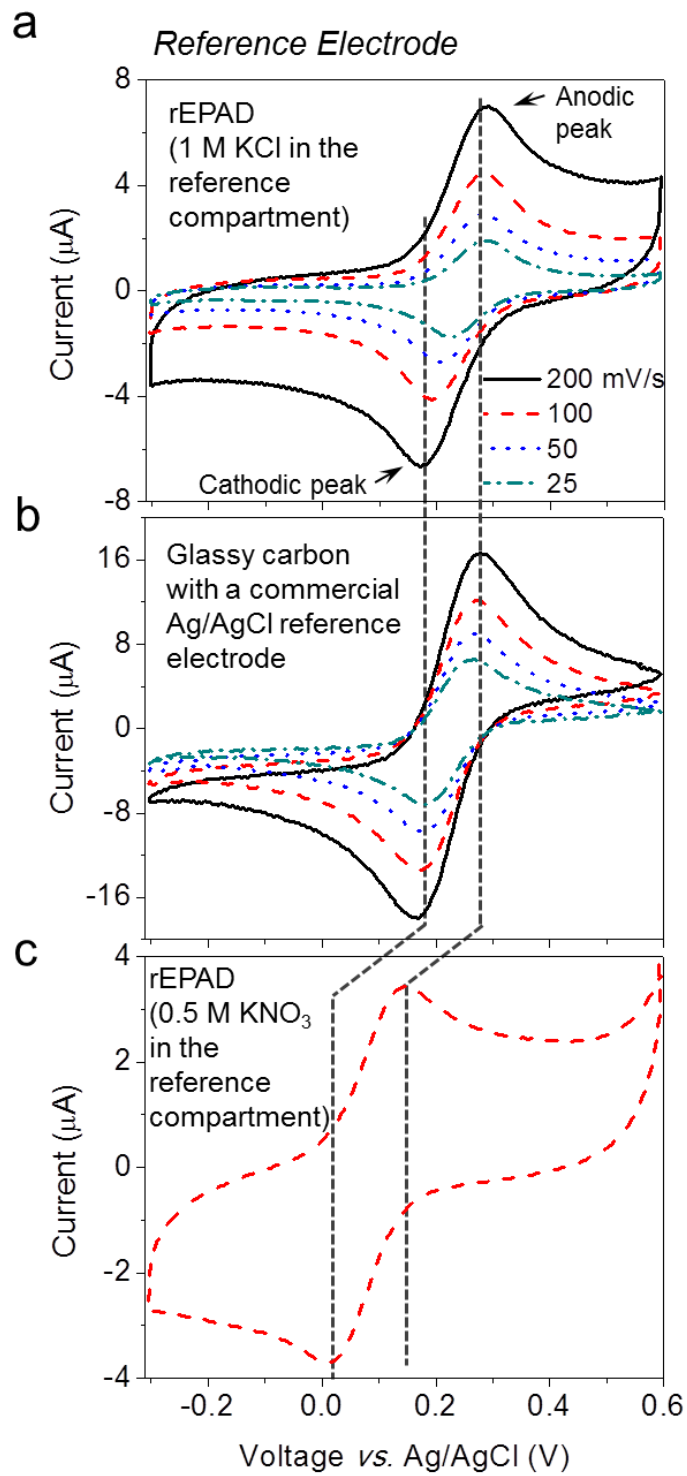
b   1 h

c   2 h

d   6 h

e   12 h

f   24 h

5 mm
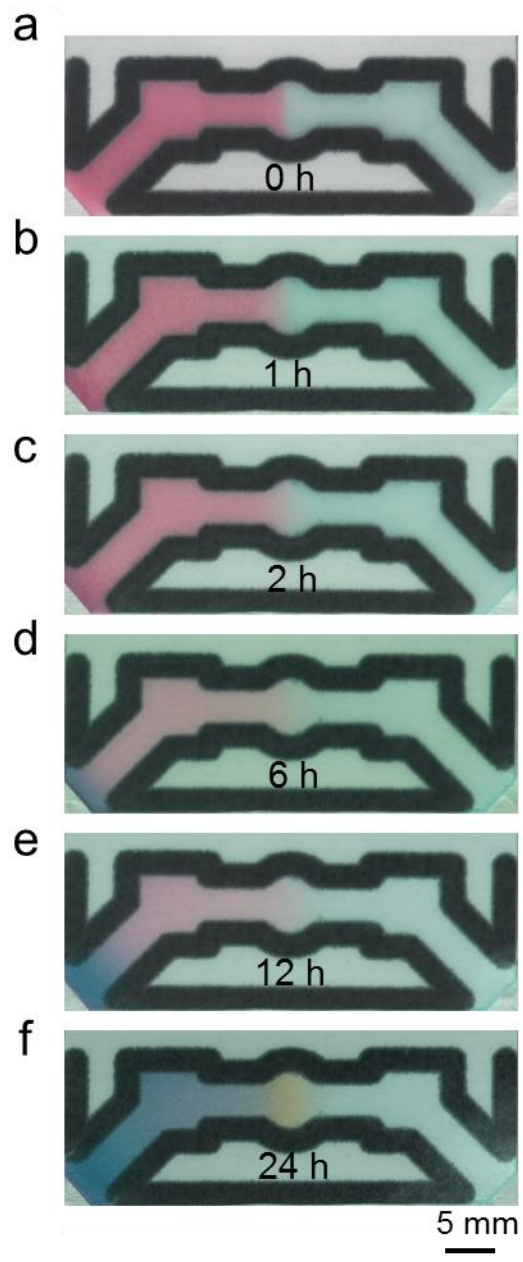
about 200 μm, and the length of the mixing zone is ~5 mm), an ion would diffuse, $\sigma = 5$ mm, during the time period, $t = 10^3 - 10^5$ sec (~20 min - 1 day), for a value of diffusion coefficient (D) expected for an ion (typically 10^{-8} to $10^{-10} m^2/s$).[13] As these times are much longer than the duration of a typical electrochemical measurement (<3 min), we conclude that diffusion is unlikely to appreciably change the concentration of ions in the sample and reference zones of the rEPADs. Theoretically, by lengthening the channel by a factor of ten (plausibly using a serpentine pattern to keep the footprint of the rEPAD small), the times required would become very long (~1 day to ~4 months).

The hydrated rEPAD therefore allows physical contact and ionic conductivity between the reference and working electrodes, while preventing large-scale convection that would alter the concentrations of analyte and chloride ions in their respective zones (and thus shift the potential of the reference electrode). The central zone operates in a manner similar to the porous plug or frit used in glass-bodied reference electrodes (Figure S2, arrow 6). Other parts in the paper device (labeled by the arrows) perform functions similar to those of their glass-bodied counterparts.

Having determined that the ionic constituents in our reference and sample zones were sufficiently isolated for application in a rEPAD, we used cyclic voltammetry, which provides both qualitative and quantitative information (oxidation/reduction potential, half-cell potential, reaction rates, and concentrations),[7] to compare the performance of this device (Figure 1b-d, no sealing tape) with that of a conventional, glass, three-electrode system. Figure 3 shows the effect of the reference solution on the peak potentials for the redox reaction of potassium ferri/ferrocyanide ($Fe(CN)_6{}^{3-}(aq) + e^- \rightleftarrows Fe(CN)_6{}^{4-}(aq)$), as measured by cyclic voltammetry. By using rEPADs and a reference solution of 1 M KCl, we estimate anodic and cathodic peak

Figure 3. Cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ solution at various scan rates obtained from (a) a rEPAD containing a paper-based reference electrode and (b) a commercial electrochemical cell that includes a 3-mm diameter glassy carbon disk working electrode and a conventional Ag/AgCl electrode. 1 M aqueous solutions of KCl and 0.5 M solutions of KNO_3 were used as the internal filling solution of the reference electrode and supporting electrolyte in the sample solution, respectively. (c) Cyclic voltammogram of 1 mM $K_3[Fe(CN)_6]$ solution obtained from a rEPAD using a reference solution of 0.5 M KNO_3 instead of 1M KCl.

a

Reference Electrode

rEPAD
(1 M KCl in the reference compartment)

Anodic peak

Cathodic peak

200 mV/s
100
50
25

b

Glassy carbon with a commercial Ag/AgCl reference electrode

c

rEPAD
(0.5 M KNO₃ in the reference compartment)
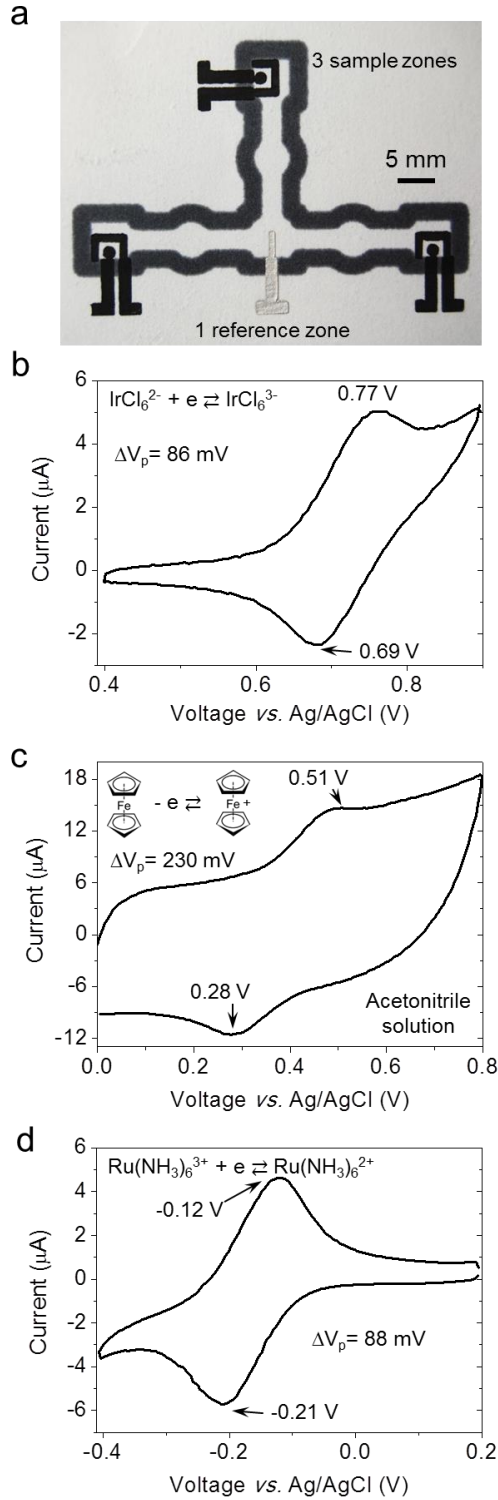
Current (μA)

Voltage *vs.* Ag/AgCl (V)

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potentials (Figure 3a) for the ferri/ferrocyanide couple of 0.28 ±0.01 and 0.19 ±0.02 V,

respectively. These results are in good agreement with the peak potentials (0.28 ±0.02 and 0.17

±0.02 V) obtained using the same sample solution and commercial electrodes (a 3-mm glassy

carbon disk working electrode, a platinum-mesh counter electrode, and a commercial Ag/AgCl

reference electrode).

In contrast, the same rEPADs operating without chloride ions in the reference solution –

an arrangement similar to that of a Ag/AgCl pseudo-reference electrode in direct contact with the

sample solution – showed a large shift (~0.2 V, Figure 3c) in the peak potentials. The peak shift

is due to the ill-defined potential of this quasi-reference electrode, and corresponds to a decrease

of approximately four orders of magnitude in the concentration of chloride ions in the solution

bathing the Ag/AgCl electrode, based on calculations from the Nernst equation (0.059 V per

decade). Under these experimental conditions, the paper-based electrochemical cell is no longer

properly referenced, and the voltage information obtained from the voltammetric curves is not

accurate. The rEPADs exhibit larger capacitive currents than does the glassy carbon electrode,

plausibly because these two systems use different carbon electrodes. The SI provides additional

details on the quantitative performance of the rEPADs.

Having established that the rEPADs exhibit a voltammetric performance similar to that of

commercial reference cells, we evolved the design of the paper-based devices into a multiplexed

system that permits multiple samples to be analyzed simultaneously. Figure 4a shows three

separate electrochemical systems in a single device; the sample zones share the same reference

electrode without cross contamination. This arrangement would be difficult to achieve with

commercial electrochemical cells; an equivalent system would require three separate sample

vials, each containing a working and counter electrode, connected via salt bridges to a fourth vial

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Figure 4. (a) Photograph of a multiplexed rEPAD. Three pairs of carbon working and counter electrodes (located at the top, bottom left, and bottom right of the figure) share the same Ag/AgCl electrode (bottom middle); this design allows electrochemical analysis of three (or by extension of the design, more) different samples simultaneously. (b), (c), and (d) Cyclic voltammograms obtained from a single rEPAD shown in (a) for analysis of three different analytes: 1 mM potassium hexachloroiridate (IV) (K_2IrCl_6) in 0.1 M aqueous KNO_3, 0.5 mM ferrocene ($Fe(C_5H_5)_2$) in acetonitrile solution with 0.1 M $TBAPF_6$, and 1 mM hexaammineruthenium (III) chloride ($Ru(NH_3)_6Cl_3$) in 0.1 M aqueous KNO_3. 10 μL each of the sample and reference solutions were added to the respective zones of a device with no sealing tape. The measurements were performed at a scan rate of 50 mV/s.

a

3 sample zones

5 mm

1 reference zone

b

$IrCl_6^{2-} + e \rightleftarrows IrCl_6^{3-}$

$\Delta V_p = 86$ mV

0.77 V

0.69 V

Current (μA)

Voltage *vs.* Ag/AgCl (V)

c

$- e \rightleftarrows$

Fe   Fe +

$\Delta V_p = 230$ mV

0.51 V

0.28 V

Acetonitrile solution

Current (μA)

Voltage *vs.* Ag/AgCl (V)

d

$Ru(NH_3)_6^{3+} + e \rightleftarrows Ru(NH_3)_6^{2+}$

-0.12 V

$\Delta V_p = 88$ mV

-0.21 V

Current (μA)

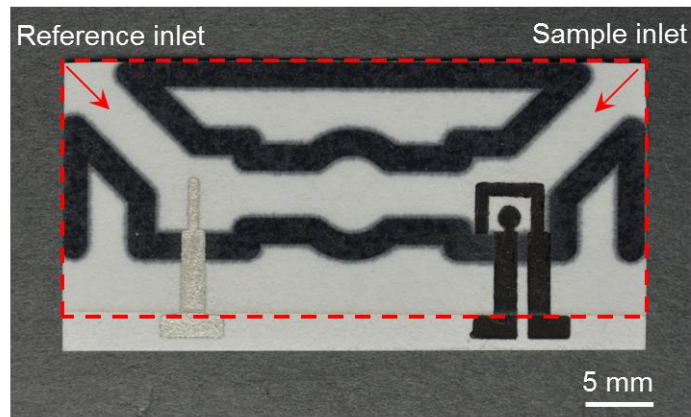Voltage *vs.* Ag/AgCl (V)

13

containing the reference electrode.

We tested the multiplexed rEPADs using three different redox couples, $IrCl_6^{2-/3-}$ (1 mM aqueous solution), ferrocene/ferrocenium (0.5 mM acetonitrile solution), and $Ru(NH_3)_6^{3+/2+}$ (1 mM aqueous solution). We chose these samples because they have very different peak potentials, and because they are widely used as standard redox couples for evaluating the performance of electrochemical devices, and as calibrants for unknown species. Figure 4b-d show that the three samples give the peak potentials expected (~0.7[14], 0.42[15], and -0.12[7] V respectively, *vs.* Ag/AgCl).

The peak currents in Figure 4 are close to each other, a consequence of the similar diffusivities of the redox couples. We emphasize that other electrochemical devices based on a Ag/AgCl pseudo-reference electrode cannot be used to analyze samples prepared in a solvent, such as acetonitrile, that does not dissolve KCl. The SI discusses the compatibility between acetonitrile and the wax barrier and the peak splitting in organic solutions.
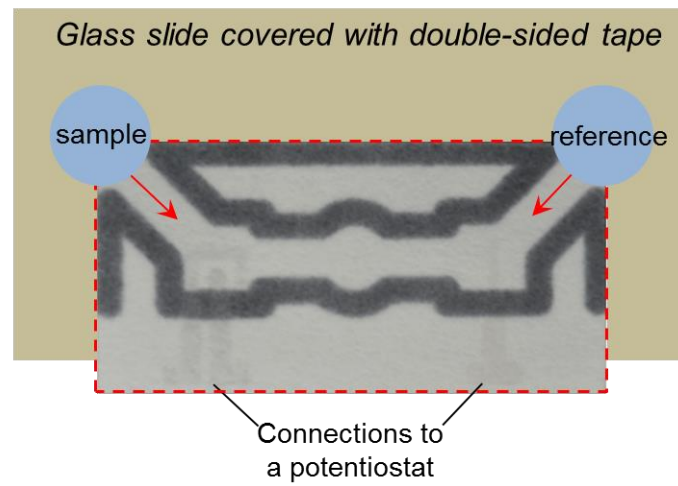
We modified the design of the rEPAD to enable application of the sample and reference solutions without the need for a pipette or injection device; this type of design makes the device suitable for field uses. Figure 5a shows a device that includes inlets at the corners to allow spontaneous wicking of solutions into the relevant zones. We investigated the working lifetime of the rEPADs by performing repeated cyclic voltammetry measurements. The device, which we sealed with tape in order to minimize the rate of solvent evaporation, was functional for approximately 1.5 h (at T~23 °C, RH~15%) after the application of sample (it takes about 15 min for the solutions to wick into the device and mix). Figure 5c shows that the peak potentials remain constant over the interval; this stability indicates a consistent concentration of chloride ions at the reference electrode. The reason for the decrease of the peak current with increasing

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Figure 5. (a) Photograph of a sealed rEPAD with the capability of performing both sample application and on-site sample analysis. Microfluidic channels are defined at the corners of the paper device. The dashed lines indicate the boundaries of the tape covered on top of the device. (b) Schematic illustration of an electrochemical cell containing a sealed rEPAD. The rEPAD is attached to a glass slide by double-sided tape, with the electrode side facing the slide, in order to minimize gravity-driven fluid flow, which might cause the contamination of sample or reference zones. Drops of the sample and reference solutions (<100 μL) were added to the top of the corresponding inlets to allow controlled and continuous wicking into the device. (c) Time-dependent voltammetric curves obtained from a sealed rEPAD shown in (a) with 1 mM $K_3[Fe(CN)_6]$ as the sample and 1M KCl as the reference solution.

a

Reference inlet   Sample inlet

5 mm

b

Glass slide covered with double-sided tape

sample   reference

Connections to
a potentiostat

c

Current (µA) vs Voltage *vs*. 1 M Ag/AgCl (V)

0 min
30
60
90
120

16
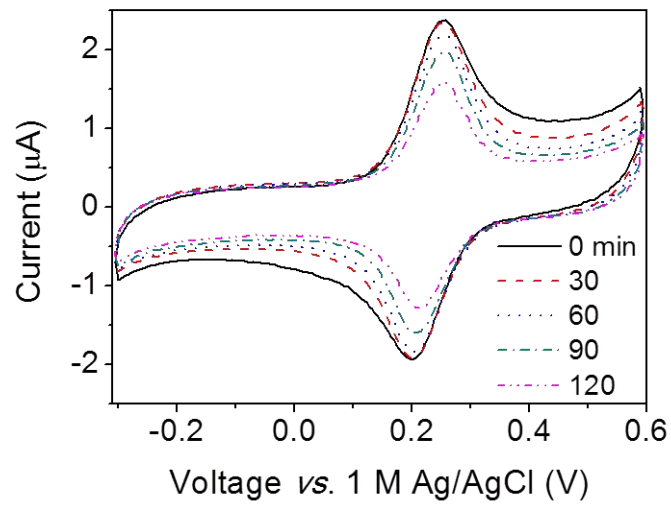
time (>30 min) is not clear at this stage, but may be due to the diffusion of the sample away from the working electrode, adsorption of analyte onto the electrode surface, or reduced ionic conductivity in the device resulting from evaporation of water from the paper channel.

Paper-supported fluids are attractive for electrochemical analyses, and reference electrodes are essential for the development of paper-based electrochemistry. By controlling mass transfer within paper channels (convective during filling; diffusive in use), we have thus designed an electrochemical paper-based analytical device capable of providing accurate voltammetric measurements that are referenced by an electrode with a constant, well-known potential.

The rEPADs have advantages over other miniaturized electrochemical devices: (i) the reference electrode is separate and thus provides a well-defined potential, (ii) the layout can be easily modified, depending on the intended analytical purpose, (iii) the fabrication process does not require complicated thin-film microfabrication processes, and (iv) the device is compatible with samples prepared in some non-aqueous solvents (*e.g.*, CH_3CN). These rEPADs have a simpler structure and a more fully integrated electrochemical reference potential than other paper-based reference electrodes.[10]

These devices are particularly suitable for single-use applications that require a separate reference electrode or an accurate potential, or in cases where chloride ion may interfere with the electrochemical experiment. Combining rEPADs with other, previously demonstrated functions of paper-based devices, including valving, sample pre-concentration, and storage or immobilization of reagents for (bio)chemical assays,[16] should enable more advanced forms of electrochemical analysis in rEPAD-based systems. The concept may also be extended to the

construction of electrochemical devices based on other low-cost materials such as nitrocellulose, cloth, Nylon, cotton string, or silk.

Acknowledgements

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Paper-Based Electroanalytical Devices with an Integrated, Stable Reference Electrode

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[a] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

[b] Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia, CIN2, Campus UAB, Barcelona, Spain

[c] Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138

[*] Corresponding author E-mail: gwhitesides@gmwgroup.harvard.edu

Supporting Information

Experimental

Chemicals: Carbon ink (E3456) and Ag/AgCl ink (E2414) were purchased from Ercon Inc. (Wareham, MA). Potassium nitrate (KNO_3) was purchased from Alfa Aesar. Potassium chloride (KCl), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium hexachloroiridate (IV) (K_2IrCl_6), ferrocene ($Fe(C_5H_5)_2$), hexaammineruthenium (III) chloride ($Ru(NH_3)_6Cl_3$), tetrabutylammonium hexafluorophosphate ($TBAPF_6$), and acetonitrile were purchased from Sigma-Aldrich and used as received.

Electrochemical Supplies: Glassy carbon disk working electrodes (3-mm diameter, part # CHI 104) and Ag/AgCl reference electrodes with 1 M KCl internal filling solution (Part # CHI 111) were purchased from CH Instruments, Inc. A platinum gauze (Stock # 10283, Alfa Aesar) was used as the counter electrode. The working electrodes were polished before voltammetric experiments using a polishing kit (CHI 120) from CH Instruments.

Fabrication and Use of the Device: Paper-based zones and microfluidic channels were fabricated by patterning chromatography paper (Whatman 1 Chr) by wax printing. We fabricated electrochemical analytical devices by stencil-printing carbon ink or Ag/AgCl ink on the wax-printed paper. We generated a stencil for printing by designing patterns of electrodes using AutoCAD® 2012, and cut the pattern into frisket film (Grafix, low tack) using a laser-cutter (VersaLASER VLS3.50, Universal Laser Systems Inc.). We adhered the stencil on top of the paper, and filled the openings of the stencil with ink. We cured the ink by baking the electrodes in an oven at ~100 ℃ for 15-20 min. After the ink dried, we removed the film carefully. The reference and sample solutions were added to the corresponding zones using a pipette. For the

S2

sealed devices, we cut and attached Fellowes® self-adhesive sheet (Staples) to the top and bottom of the device to minimize the effect of evaporation.

Device Geometry: The carbon working electrode is a 1.5-mm diameter disk electrode, while the carbon counter electrode has a larger surface area and surrounds the working electrode (Figure 1). For voltammetric applications, we designed rEPADs such that the carbon working and counter electrodes were stencil-printed on the right side of the device and the Ag/AgCl reference electrode (Ag/AgCl ink associated with the KCl solution) on the left side.

Cyclic Voltammetry: We performed cyclic voltammetry (CV) with paper-based devices (or a commercial electrochemical cell) and a potentiostat (Pine Instrument Co., AFCBP1) interfaced to a computer through a PCI-MIO-16E-4 data acquisition board (National Instruments) for potential and current measurements. Voltammetric data were recorded using in-house virtual instrumentation Pinechem 2.7.9a (Pine Instruments).

Convection in the Paper-Based Devices

Because we pipetted the same small amount of the solution (10 μL) to each zone, the mass transfer in the fluid-filled channel due to convection is negligible, *i.e.*, the convection is not strong enough to drive the solution from one side of the device to the other. In fact, even when 10 μL was applied only to the left zone, the solution stopped at the right-hand part of the microfluidic channel and did not wick into the right zone. Applications of convective flow in paper-based devices are detailed elsewhere (see reference 1).

Lifetime of Paper-Based Devices

We investigated the effect of sealing the devices with tape on the working time of the rEPADs without printed electrodes. Sealed devices require much longer times for the solutions to wick into the central mixing zone, compared to uncovered (Figure S1) devices (~15 min *vs.* ~0.5 min), because the air that initially occupies the central channel must diffuse through the tape or through the sides of the device as it is displaced by the sample and reference solutions. The uncovered devices dried completely within 20 minutes, while the sealed devices remained wet for more than 12 h. The color of the paper with $CoCl_2$ solution changed from pink to blue after the water had evaporated. In Figure 2f, the yellow color in the central contact zone might be due to the precipitation of various crystals including $CuCl_2$, $CoSO_4$, $CoCl_2$, and $CuSO_4$.

The lifetime for the rEPADs with printed electrodes is significantly shorter than the drying time for the unprinted devices because the electrodes creates a gap between the paper layer and the tape layer that allows water vapor to escape. This gap, however, can be filled by inserting a layer of parafilm or other filler materials, if longer device lifetime is desired.

Cost of Voltammetric rEPADs

We estimate the cost of materials of rEPADs to be ~$0.06 per device. Ag/AgCl ink costs $1 per gram, and one gram of ink is sufficient to produce thirty devices by manual stencil printing ($0.033 per device). Graphite ink costs $0.20 per gram, and one gram of graphite ink produces twenty devices by manual stencil printing ($0.01 per device). One page of wax-printed paper (20 cm by 20 cm, 28 devices) costs ~$0.50 ($0.018 per device). Thus, one device costs $0.061 in total. We estimate the cost of one device could be as low as $0.04 using a mixed

Ag/AgCl/graphite ink as the reference electrode material. The estimation above is based on the price of commercial products.

Quantitative Measurements by Cyclic Voltammetry

The stability of the voltage measurement provided by the rEPAD allows us to use cyclic voltammetry to obtain quantitative information about the concentration of analyte. Figure S3a demonstrates a linear relationship between the peak current, i_p, at 0.19 V (*vs*. Ag/AgCl) and the concentration of $K_3[Fe(CN)_6]$ in the range of 1 to 10 mM (a typical range for cyclic voltammetry), in agreement with the Randles-Sevcik equation (eq S1).

$$i_p = (2.69 \times 10^5) n^{3/2} A D_o^{1/2} C_o^* v^{1/2}$$

(S1)

In this equation, n is the number of electrons transferred per analyte, A is the electrode area, D_o is the diffusion coefficient of the analyte, C_o^* is the bulk concentration of the analyte, and v is the scan rate of the applied potential (V/s).

Cyclic voltammetry is not an ideal method for accurate quantitation of electroactive species, because the correction for the non-Faradaic charging current is typically uncertain. When properly referenced, the method does, however, provide an estimate of the analyte concentration; more importantly, the peak potentials, and the shape of the voltammetric curves, provide a qualitative study of redox processes and an understanding of the electrochemical mechanism of the system.

Figure S3b shows that the cathodic peak current is linearly proportional to the square root of the scan rate ($v^{1/2}$) in the rEPAD; this result indicates a reversible wave and diffusion-controlled mass transfer towards the working electrode, in accordance with the expected characteristics of the ferri/ferrocyanide redox couple.

rEPADs for multiplexed voltammetry

The potential differences between the anodic and cathodic peak potentials (ΔE_p) of Ru(NH$_3$)$_6^{3+/2+}$ and IrCl$_6^{2-/3-}$ were 88.4 and 86.4 mV, respectively (Figure 4). These values are slightly higher than the theoretical value for an ideal reversible one-electron redox system (59 mV at 25 °C), and may result from the ohmic resistance of the solution.[2]

Acetonitrile was used to dissolve the ferrocene and tetrabutyl ammonium hexafluorophosphate (TBAPF$_6$). The wax boundaries contained the acetonitrile within the hydrophilic region as long as only a small volume of solution (several μL) was used. Other cross-linked polymers that define channels—*e.g.*, SU-8[3]—would resist dissolution for longer, but were not required here. The applicability of this system is currently limited by evaporation, and the long-term incompatibility of electrochemical organic solvents with the wax barrier and the adhesive tape, but could be improved with further engineering and careful choice of materials.

We speculate that the relatively large capacitive current for the ferrocene/ferrocenium redox couple in acetonitrile (Figure 4c) is due to the interaction between the rough surface of the carbon electrode and the organic solvents used in the paper-based device. The peak splitting (~0.2 V) is often observed as a consequence of the sluggish, heterogeneous kinetics at the surface of the carbon electrodes.[4] Quantitative analysis of samples in organic solvents by rEPADs may be affected by the relatively high instability of the interface between the organic solvent and the aqueous reference solution. The signal/noise ratio, however, can be increased upon reducing the scan rate of the voltage.[5]

Table S1 Applications of paper-based electroanalytical devices

Reference	Species detected/quantified	Electrochemical method
Nie et al. [6,7]	Glucose, lactate, ethanol, and heavy-metal ions	Chronoamperometry, cyclic voltammetry, anodic stripping voltammetry
Carvalhal et al. [8]	ascorbic acid and uric acid	Amperometry
Henry et al. [9]	Glucose, lactate, uric acid, Au, and iron	Chronoamperometry, cyclic voltammetry, square wave voltammetry
Yu et al. [10]	Immunosensor (carcinoembryonic antigen (CEA) et al.)	Differential pulse voltammetry
Lankelma et al. [1]	Glucose	Amperometry
Liu et al. [11]	Adenosine, glucose, H_2O_2	Amperometry, cyclic voltammetry
Shiroma et al. [12]	paracetamol, 4-aminophenol	Amperometry
Knoll et al. [13]	K^+, Na^+	Potentiometry
Novell et al. [14]	NH_4^+, K^+, pH	Potentiometry

Figure S1. Photographs of a paper-based microfluidic device taken after adding distilled water and pink solution of $CoCl_2$ to corresponding zones. The $CoCl_2$ solution mixed with the water in the central zone but did not diffuse into the left paper zone. The color of the $CoCl_2$ solution became paler after 5 min and blue after 10 min as the device gradually dried out because of solvent evaporation. The device was not sealed with tape.
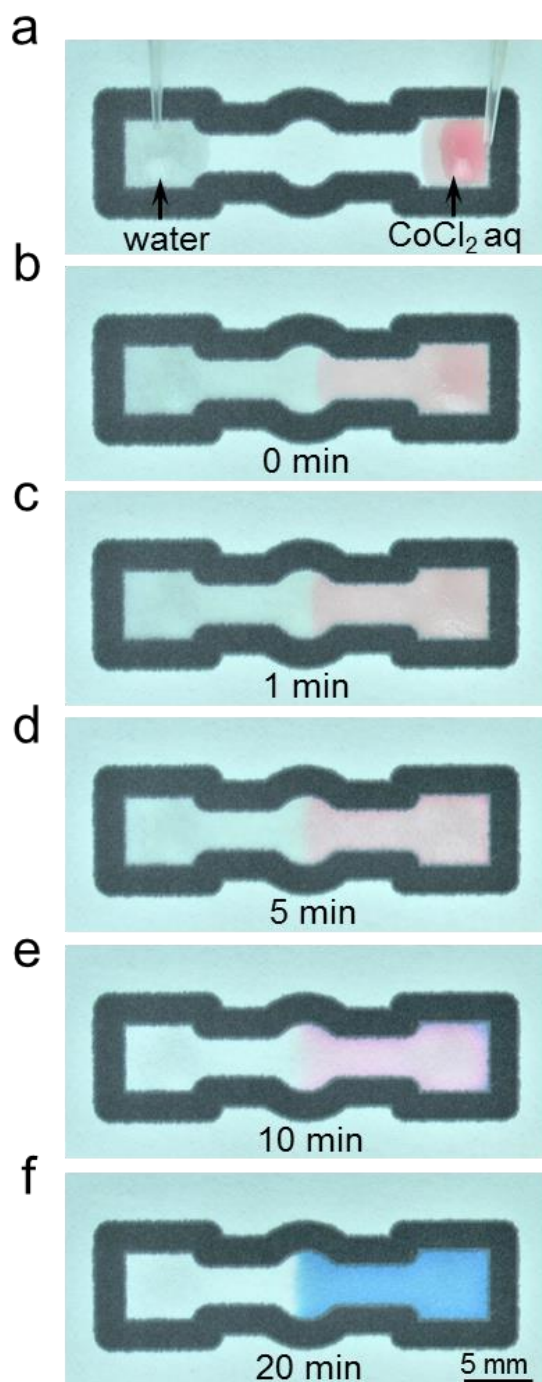
Figure S2. (a) Photograph and schematic illustration of a commercial Ag/AgCl reference electrode. (b) Front view and back view of a paper-based electrochemical device (rEPAD). The paper was patterned by wax printing to define the sample zone, central mixing zone, reference zone, and microfluidic channels. The sample and reference zones include stencil-printed carbon and Ag/AgCl electrodes, respectively. The arrows with the same number as in (a) refer to similar functions. The dashed lines indicate the approximate boundaries of the carbon and Ag/AgCl ink printed on the back.
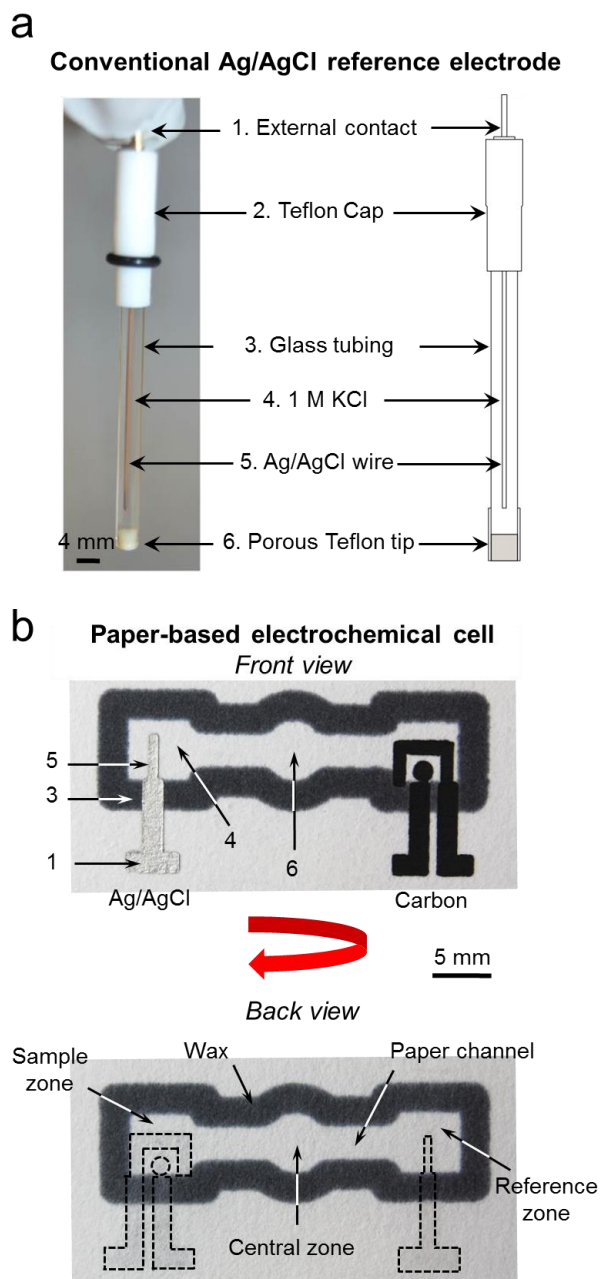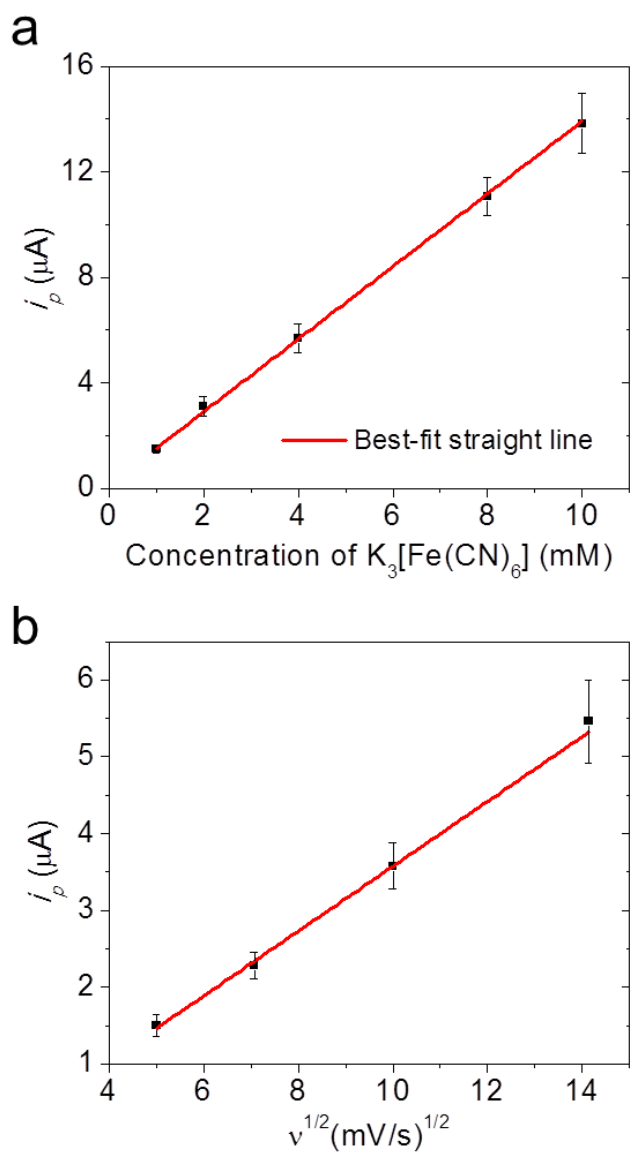
Figure S3. Cathodic peak current (i_p) as a function of (a) the concentration of $K_3[Fe(CN)_6]$ and (b) the square root of the scan rate ($v^{1/2}$) for cyclic voltammetry experiments conducted on rEPADs. The solid lines represent a linear fit to (a) with regression equation: y = 0.16 + 1.4x (R^2 = 0.998, n = 7), and a linear fit to (b) in e with regression equation: y = -0.65 + 0.42x (R^2 = 0.995, n = 7).

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