Long-Term Stability of Screen-Printed Pseudo-Reference Electrodes for Electrochemical Biosensors

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ABSTRACT: Pseudo reference electrodes forms an essential part of electrochemical transducers, where they are used to maintain a reference potential for the system. Yet, little is known about their long-term stability, and any drift may cause undesired changes in the sensor signal. This paper investigates the stability of the reference potential of five material systems of screen-printed reference electrodes. Their potential was continuously monitored for a duration of 40 days in a phosphate buffered solution, and any signal changes were corroborated with EDX elemental analyses, SEM micrographs and cyclic voltammetry. The electrodes were considered to be stable as long as they remained within 30 mV of their initial potential throughout the study. It was found that Ag/AgCl electrodes with a 3:1 atomic ratio kept a stable reference potential (± 2.2 mV), whereas Ag/AgCl electrodes with a 9:1 atomic ratio, despite showing good overall constancy of ± 3.2 mV, seemed to statistically lose stability towards the end of the 40 day trial due to AgCl depletion through dissolution. The daily potential drift for the respective Ag/AgCl electrodes were -0.2 mV (3:1 ratio) and -0.1 mV (9:1 ratio), suggesting a proportionality between the amount of dissociated AgCl close to the electrode surface and the initial AgCl loading. Electrodes consisting of only Ag showed tendencies towards a mixed potential contribution, which reduced the long-term stability (± 24.2 mV) as well as the longevity span (2 days). Ag/Pd electrodes proved to be more unstable (± 29.7 mV), with an average lifetime of around 3.5 days. Pt had the greatest potential instability (± 59.8 mV), rendering its average lifetime to less than a day. It was shown that electrodes which deviates from the Ag/AgCl equilibrium had the greatest potential variation with time.

1. Introduction

The reference electrode provides a stable and known potential towards which the working electrode of an electrochemical cell can be controlled or measured.[1] This is important in potentiometric sensors, where any offset or drift in the reference electrode potential would lead to a direct change in the output signal. For potential-controlled sensors this could result in (i) a change in the electron transfer rate of the targeted reaction, (ii) undesired side reactions of electrolyte constituents, and even of the electrode material itself, or (iii) remain unaffected due to other rate determining processes.[1,2] It is therefore important that the reference electrode potential remains within a limit which mirrors the predefined accuracy of the biosensor throughout its operational lifetime.

Screen-printing is a popular way of making reference electrodes (SPREs). These electrodes have historically been used in one-shot type measurements in disposable low cost consumer diagnostics or therapeutics.[3] Growing interest in uninterrupted gathering of sensor data over longer periods of time sets higher requirements to the stability of the measurement cell. This is especially important for sensors operating in remote or inaccessible locations, where replacement is difficult and costly.[4,5] The properties of electrodes used in electrochemical cells are sensitive to changes in structure and composition on both sides of the electrode-electrolyte-interface. It is evident that a sensor system which is exposed to the environment for a longer period will have an increased probability of succumbing to structural and compositional changes, possibly influencing the electrochemical properties of the system. These may range from oxide

1 layer formation, recrystallization, corrosion, interdiffusion and gas evolution to metal deposition, adsorp-
2 tion and desorption.[6] While SPREs have not been developed for use in long-term applications, their re-
3 producibility[7,8] and low cost makes them desirable candidates.[9]

4 One common type of SPRE consists of solid Ag/AgCl immersed in an electrolyte containing Cl⁻.[10] It is
5 said to be non-polarizable, as the associated redox reaction
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$$AgCl(s) + e^- \rightleftharpoons Ag(s) + Cl^- \qquad (1)$$
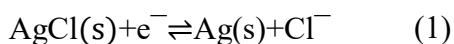
7 operates by fast kinetics. Electrodes which are more polarizable may still be used given that the solution
8 composition remains fairly constant, and the current flowing through it during the measurement period is
9 negligible. In solutions where Cl⁻ is the only halide present, there is a logarithmic (nernstian) relationship
10 between the potential of the Ag/AgCl reference electrode and the Cl⁻ activity. Hence, conventional macro-
11 scale reference electrodes (or reference half-cells) include an internal electrolyte with constant Cl⁻ con-
12 centration. This level of complexity is often not practically feasible without adding significant cost to the
13 biosensor. There have been attempts to develop reference half-cells with reduced size and complexity
14 with varying success. Reported designs with immobilized electrolytes often suffer from one or more of
15 the following: sensitivity to changes in external halide concentration,[11–13] short lifetime[14–16] or long set-
16 up time.[17] A more comprehensive review is given by Sophocleous et al.[18]

17 A simplified design omitting the internal electrolyte of reference half-cells are often referred to as
18 pseudo-reference electrodes (p-REs).[9] These electrodes are directly exposed to the test environment,
19 where a detailed knowledge of the solution medium, or additional calibration steps, are required in order
20 to obtain the desired operating potential. Under these conditions p-REs are often a good choice since they
21 offer simplicity in design, miniaturization feasibility, short conditioning time, no liquid junction poten-
22 tials, and low cost. The blood and interstitial fluid of the human body is one example of an environment
23 where parameters such as salinity and temperature are kept relatively constant.[19] Many electrochemical
24 biosensors are aimed toward such environments, and use phosphate buffered saline (PBS) as a proxy in
25 the development phase.[4,15–17,20] In recent years, the calibration of Ag/AgCl p-REs has received some at-
26 tention,[21,22] enabling correction for changes in Cl⁻ concentration, further extending the use of this cate-
27 gory of reference electrodes. Although p-REs equipped with Ag/AgCl shares the desired qualities of ref-
28 erence half cells with respect to polarizability, there are some sensors that utilize reference electrode ma-
29 terials possessing a less defined interaction with the test solution. Such electrodes are limited to measure-
30 ments which are carried out in such a short time (seconds) that the potential drift becomes insignificant.

31 The scientific literature is lacking in comparative studies of different screen-printed p-RE materials and
32 their potential stability over extended time periods.[4,23,24] In this paper we address the long-term stability
33 of two commercial and three in-house screen-printed p-REs (SPREs) that have been designed for use in
34 biosensor applications. Two Ag/AgCl with different Ag to AgCl atomic ratios of 3:1 and 9:1 respectively,
35 were chosen in order to investigate the longevity of SPREs with well-defined potential determining reac-
36 tions. Note they these come from different suppliers and may differ in composition. These were compared
37 against three SPREs where the potential determining reactions are not that obvious. The first one was
38 made from low temperature cured Ag ink, whereas the second one contained a 2.8:1 Ag/Pd alloy. The
39 paste, which is fired at around 850°C, consists of a porous structure of Ag, Pd and their oxides, and is
40 favored in electronic hybrid circuits due to properties such as chemical inertness and high melting point
41 temperature.[25] The third electrode of this category was made from a Pt paste, following the same process
42 as with the Ag/Pd SPRE. The relevant redox reactions for these three SPRE materials, including also Cl⁻,
43 have standard reduction potentials in the range +0.2223 V to +0.7996 V and is therefore expected to be
44 positive compared to a KCl(4M)/Ag/AgCl half-cell.[26] Ag electrodes are expected to acquire some of their
45 potential from Cl⁻ exchange due to their halide affinity,[27] but may also show mixed potential contributions

1 from reactions involving Ag oxides[28] formed during storage in an oxygen rich environment. Pt and Pt
2 oxides are also known to dissolve in Cl⁻ rich solutions, and may acquire some of its potential from dis-
3 solution reactions.[29,30] The electrodes examined in this paper are listed in Table I and shown in Figure 1.
4 Their potentials were monitored continuously in a PBS solution over a period of 40 days. Their sensitivity
5 towards Cl⁻ in the test solution, changes of surface structure and elemental composition over time, how
6 fast they reach a half-cell potential in physiological buffer solution following immersion, and applicability
7 in cyclic voltammetry have also been investigated.

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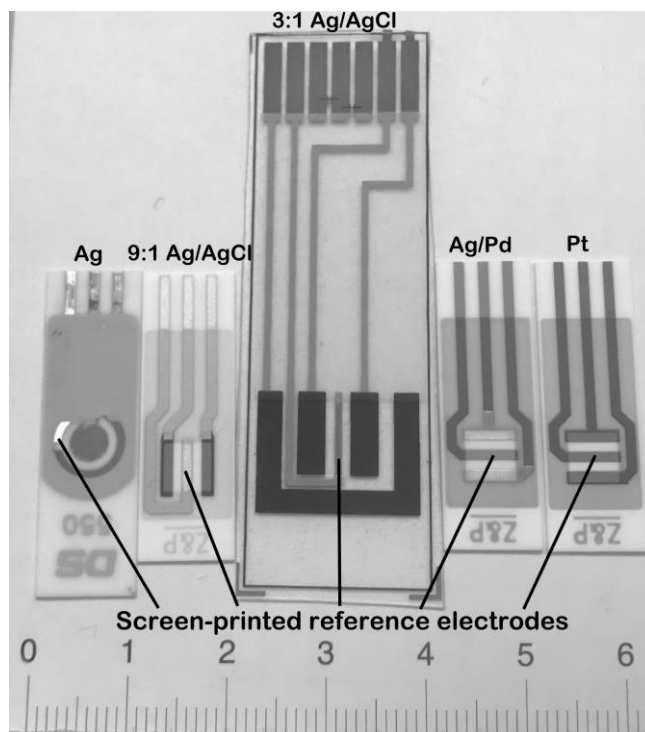
10 Figure 1. Screen-printed electrochemical cells used in this study, depicted next to a centimeter ruler. Each reference electrode location is
11 marked with an arrow and the material composition is listed in Table I.

12 **Table I. Reference electrode materials and supplier (SPREs)**

Material	Electrode Manufacturer	Ink/paste supplier	Ink/paste part num.	Curing temperature
Ag	DropSens, ES	Unknown	Unknown	100 °C
3:1 Ag/AgCl	Microarray Ltd., UK	DuPont Ltd.	5874	100 °C
9:1 Ag/AgCl	In-house	Gwent Electronic Materials Ltd.	C2130916D5	100 °C
Ag/Pd	In-house	Heraeus Precious Metals GmbH & CO	C 2028	850 °C
Pt	In-house	Heraeus Precious Metals GmbH & CO	Cl11-5100	850 °C

13 ## 2. Materials and methods

14 ### 2.1. Reagents and instrumentation

15 Half-cell potentials were measured relative to double junction KCl(4M)/AgCl/Ag (Z113107, Aldrich)
16 electrodes. A solution of 10% w/v KNO_3 was used as the salt-bridge electrolyte. A pH 7.4 PBS solution
17 was made up from 52.8 mM Na_2HPO_4, 13.0 mM NaH_2PO_4 and 137 mM KCl dissolved in de-ionized wa-
18 ter (Custom made, TIG Purita). All reagents were purchased from Sigma Aldrich, and were of reagent
19 grade purity. An electrochemical workstation (EmStat 3 OEM, Palm Instruments BV) was used for elec-
20 trochemical measurements, unless otherwise is stated.

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2.2. Electrode fabrication

3 All SPREs (Table I, Figure 1) were stored at room temperature out of ambient light for at least one month,
4 and were rinsed in de-ionized water before use. The three electrodes made in-house used the same set of
5 screens: (i) One for the reference electrode and conductive tracks, (ii) one for the working and counter
6 electrodes, (iii) and one for the dielectric isolating the conductive tracks from the test solution. Relevant
7 materials and process parameters are listed in Table I. The materials were printed on an Al_2O_3 substrate
8 (A476T, Kyocera) using a semi-automatic screen-printer (Ekra M3, Asys Group) equipped with stainless
9 rubber squeegees for the inks, and steel squeegees for the high temperature cured pastes. The materials
10 were used "as received", and no additional modification such as thinners were added. Each printing step
11 was followed by a curing step at the temperatures listed in Table I. The screens were rinsed in 1-ethoxy-
12 2-pronaol (43306, Alpha Aesar) and dried with line-free cloth to remove any residuals from the previous
13 run.

2.3. Chemical and temperature stability

15 The high temperature cured electrodes pose interesting alternatives as they will be more amenable to high-
16 temperature applications and have a higher degree of chemical resistance. To check their chemical stabil-
17 ity, the five different electrodes were exposed to a strong organic solvent (dimethylformamide) for 5
18 minutes in flow cell, followed by their visual inspection. Their compatibility towards high temperature
19 was tested by contact exposure to a soldering iron holding 350 °C for 1 minute.

2.4. Chloride sensitivity

21 The open-circuit potential (OCP) from the different p-REs was measured against the true reference half-
22 cell in solutions containing different concentrations of KCl in order to assess their potential determining
23 reactions' Cl⁻ dependency.

2.5. Stabilization time

25 The time taken to reach a stable potential following immersion in a test solution is one performance
26 parameter of a reference electrode.[13,17] A timing analysis was performed by measuring the OCP between
27 the SPRE and a true reference half-cell from immersion and until the potential plateaued.

2.6. Long-term stability measurements

29 A multiplexing measurement circuit (Figure S1) was made in order to conduct long-term monitoring of
30 the OCP. The circuit was controlled by a LabVIEW (v15.0f2 32-bit, National Instruments) routine through
31 a DAQ (USB-6009, National Instruments) according to the setup shown in Figure 2. The circuit was
32 programmed to sequentially make connections between a reference half-cell (E0) located in the center
33 and 12 equidistantly surrounding SPREs (E1-E11) through a high impedance (>10^{16} Ω) digital voltmeter
34 (6430, Keithley). The connections were made through an array of mechanical relays controlled by the
35 digital signals S1-S11. The potential of the central reference half-cell was verified versus a lab-master
36 half-cell of identical make before and after the measurement. A preliminary finite element diffusion anal-
37 ysis (COMSOL Multiphysics 5.2a.262, COMSOL AB) estimated that the KCl concentration in the test
38 solution (1 L) should change less than 1 mM during 40 days with this setup, and hence have negligible
39 impact on the electrodes adhering to reaction (1). In contrast, leak rates of 2.1-4.8 µmol h⁻¹ have been
40 reported for single junction half-cells.[16,17] The potential difference between each SPRE and the reference
41 half-cell was sampled once every minute over a period of 40 days. Temperature monitoring was performed
42 in an adjacently placed beaker with an identical volume of the test solution. The analog part of the setup

1 was placed inside a Faraday cage connected to the voltmeter's earth terminal to shield against electro-
2 magnetic noise.

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2.7. Material composition and surface morphology
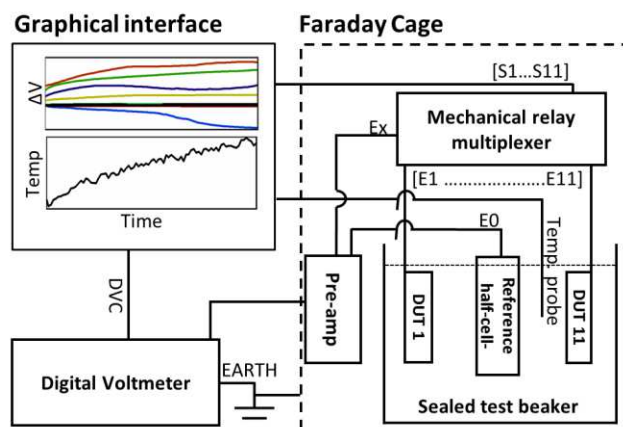
7   Surface structure and chemical composition of the SPREs were analyzed ex-situ immediately before and
8 after the long-term OCP measurements. A scanning electron microscope (SEM) equipped with an energy
9 dispersive x-ray spectroscope (EDX) was used (SU3500, Hitachi).

2.8. Cyclic voltammetry

11 To judge the reference electrodes' suitability in a real application, cyclic voltammetry in the presence of
12 a reversible redox couple (5 mM $[Fe(CN)_6]^{3-/4-}$) in a 1.0 M KCl supporting electrolyte was performed. A
13 chamber with a screen-printed Pt disk (0.503 cm^2) working electrode and a coiled Pt wire counter elec-
14 trode was connected to a reference chamber through a Luggin capillary (Figure S2). The reference cham-
15 ber was used to house the different SPREs. This was performed before and after the long-term OCP meas-
16 urement.

3. Results and Discussion

3.1. Chemical stability and temperature compatibility

19   After exposure to the organic solvent, the low-temperature cured electrode materials had simply vanished
20 from the substrate material. For the high temperature cured materials the electrodes remained in their
21 entirety. Local exposure to high temperature caused the low-temperature cured SPREs to melt away, while
22 the high-temperature cured SPREs remained intact. Hence, high temperature cured Ag/Pd and Pt are the
23 only materials in this study that may be used in chemically aggressive environments and high-temperature
24 applications.

3.2. Chloride sensitivity

26   The pCl ($-\log[Cl^-]$) sensitivity of each SPRE material (n=5) is presented in Figure 3 and Table II. The
27 theoretical response from the Nernst equation, using activity coefficients reported for KCl,[31] is shown for
28 reference. All electrodes exhibit a strong dependency on Cl$^-$ activity, suggesting that Cl$^-$ partakes in their
29 potential determining reactions with the same reaction order. It has been suggested previously that Ag/Pd
30 alloys in Cl$^-$ solutions mainly acquire their potential from reaction (1).[32] It can be seen that the two SPREs
31 made from AgCl-containing materials follow the nernstian relation more closely than the remaining two.
32 The Ag ink exhibits similar Cl$^-$ sensitivity (except at pCl -0.6). This is contradictory to what is reported

by Atkinson et al.[33] They reported that the OCP of a bare Ag SPRE shifted less than 10 mV per pCl. It should be noted that their electrode was accidentally cured at 850°C, potentially creating much more Ag oxides. The Ag/Pd has comparable Cl⁻ sensitivity, but shows a significant standard deviation. This reduced ability to accurately reproduce the potential is a quality which negatively impacts the reproducibility of a biosensor's signal. It is also worth noticing that the standard deviation is increasing with pCl for all electrodes except Ag/Pd and Pt. This mirrors the logarithmic nature of reaction (1) in that the potential is more sensitive to Cl⁻ variation at weaker concentrations. In contrast to the other electrodes, Pt displays a potential inversely proportional to pCl. This implies that the Ag/AgCl equilibrium is not a major contributor to the potential determining reactions, while Pt dissolution in aqueous Cl⁻ is.
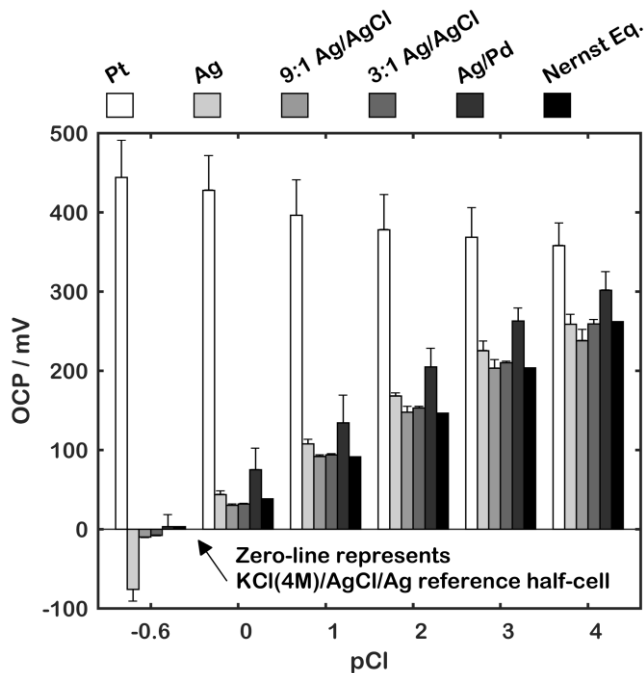
Figure 3. The OCP of five different SPREs (n=5) as a function of Cl⁻ concentrations vs. a KCl(4M)/AgCl/Ag reference half-cell. The error bars represent ±3σ. The theoretical (nernstian) relation for Ag/AgCl electrodes is shown for reference.

Table II. pCl sensitivity for five different SPREs.

| SPRE Material	pCl sensitivity (mV/pCl)
Pt	-17.9
Ag	65.1
9:1 Ag/AgCl	51.9
3:1 Ag/AgCl	55.1
Ag/Pd	60.7
Nernst eq. (23°C)	57.4

3.3. Stabilization time

The timing diagrams for short-term stability of the electrode potential of different SPREs (n=1) are shown in Figure 4. The Ag/AgCl electrodes both settle close to their theoretical value of 83 mV, while the Ag, Ag/Pd and Pt electrodes settle at +10 mV, +70 mV and +240 mV relative to that, respectively. The difference in potential suggests that additional potential determining reactions are involved. All materials had a settling time of less than 10 sec after being immersed in the PBS solution. This can be considered a

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stability point useful in short-term and one-shot type measurements (<60 s). Their ability to quickly stabilize at a definite potential is linked to their direct exposure to the test solution.[9] There are no delaying processes such as wetting of salt matrices, prolonged diffusion paths or mass-transport obstacles.
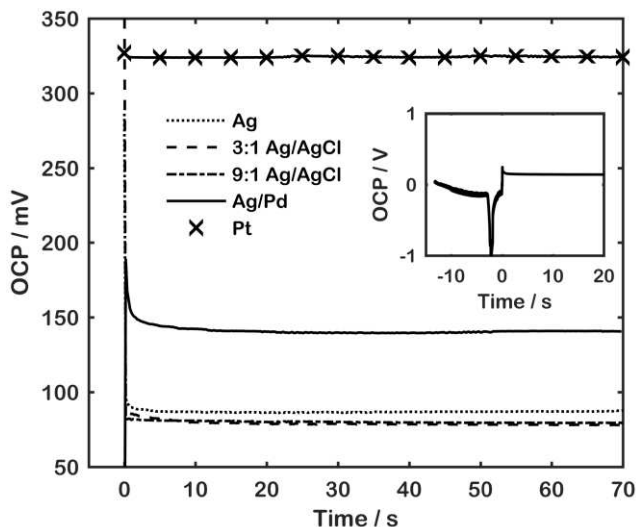

Figure 4. The OCP settling time vs. a KCl(4M)/AgCl/Ag reference half-cell following immersion in a PBS solution at t=0 for different SPRE materials (n=1). The inset shows the typical transition for OCP signals when going from a floating potential (in air) to a distinct potential (immersed).

3.4. Long-term stability measurements

During the long-term stability test of the OCP, the temperature in the solution was measured to 23.4±1.0°C. Cross-correlation between the recorded temperature profile and the OCP profiles did not reveal any significant influence, and was disregarded as a source of error in this study. The OCP evolution profiles of each electrode material ($n \geq 3$) as measured over 40 days is presented in Figure 5, represented as a symmetric standard deviation (shaded) around the average OCP value for each data point. Each individual trace is provided in Figure S3. A stability criterion, defined by Shitanda et al.,[16,17] of a maximum 30 mV change from the initial potential (E(t=0)) was used to quantify the long-term stability of the electrodes. The times at which the electrodes surpassed this requirement on average are indicated in Table III, along with the pooled stability figure.

The OCP profiles of the Ag electrodes show some variability over time (70.0 ± 24.2mV). They generally display two states: (i) Long periods with a stable potential, and (ii) quick and rather large potential changes over a short period of time. Although the formation and consumption of AgCl(s) is likely to contribute to the potential for this electrode, it will only be formed in small amounts and this electrode is therefore more easily influenced by other reactions at the Ag surface. The 9:1 Ag/AgCl electrodes exhibited a low drift (−0.1 mV/day) until about day 30, from which this rate markedly increased, as shown in the inset in Figure 5. The increase is caused by two out of 7 samples drifting off from the main data from this point and onwards. Their pooled stability figure was found to be 85.1 ± 3.2 mV. The 3:1 Ag/AgCl electrodes displayed a steady drift of −0.2 mV/day throughout the 40 day period. It also remained within the 30 mV stability criterion, yielding a stability figure of 82.2 ± 2.2 mV. The different potential drift is owed to the gradual dissolution of AgCl(s) from the electrode surface creating an increase in Cl⁻ concentration in the vicinity of the electrode surface, lowering the potential in accordance with the Nernst equation.[34] The fact that this rate is roughly double for the 3:1 Ag/AgCl electrodes only reflects the larger availability of dissolving species. The transient behavior seen for the Ag/Pd SPREs (Figure S3) is typical for electrodes that do not have a fast charge transfer reaction confining the potential. A small change in current can thus produce a large potential shift on these electrodes. Their long-term stability averages around 3.5 days and

1 yields a pooled stability figure of 66.2 ± 29.7 mV. A higher average potential again suggests that the elec-
2 trodes do not solely acquire their potential from reaction (1). One might have expected an even higher
3 standard deviation from looking at Figure 5. The lower number is a result of the standard deviation for
4 each individual electrode being centered around widely different means. This becomes clearer when look-
5 ing at each individual trace (Figure S3). Pt varies the most in potential (275.4 ± 59.8 mV) and is on aver-
6 age stable for less than a day. Its potential variation may be attributed to the dissolution of Pt and its oxides
7 by way of Cl^-, and its independency upon the Ag/AgCl equilibrium.
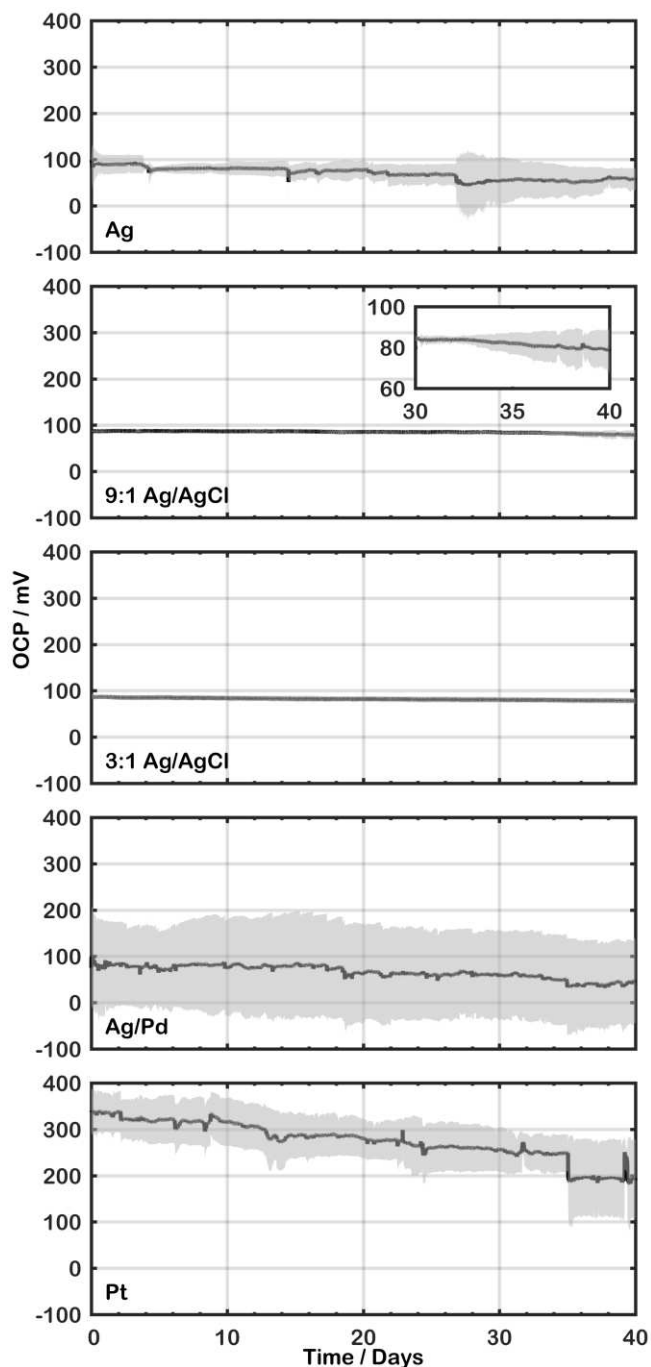
9 Figure 5. The OCP of five different SPREs vs. a KCl(4M)/AgCl/Ag reference half-cell in PBS over 40 days. The solid black lines represent
10 the time-average potential, while the surrounding shaded regions represents a confidence interval of 68.3 %. The inset shows the final 10 days
11 of the 9:1 Ag/AgCl OCP profile in greater detail.

12 **Table III. Stability times for different SPRE materials drifting > 30 mV from the initial OCP, and stability figures denoted as**
13 **grand mean ($\overline{x_p}$) and pooled standard deviation (σ_p).**

	Longevity (days)	Pooled stability $\overline{x_p} \pm \sigma_p$ (mV)	n
Ag	2.1	70.0 ± 24.2	6
9:1 Ag/AgCl	>40	85.1 ± 3.2	7
3:1 Ag/AgCl	>40	82.2 ± 2.2	3
Ag/Pd	3.5	66.2 ± 29.7	9
Pt	0.6	275.4 ± 59.8	4
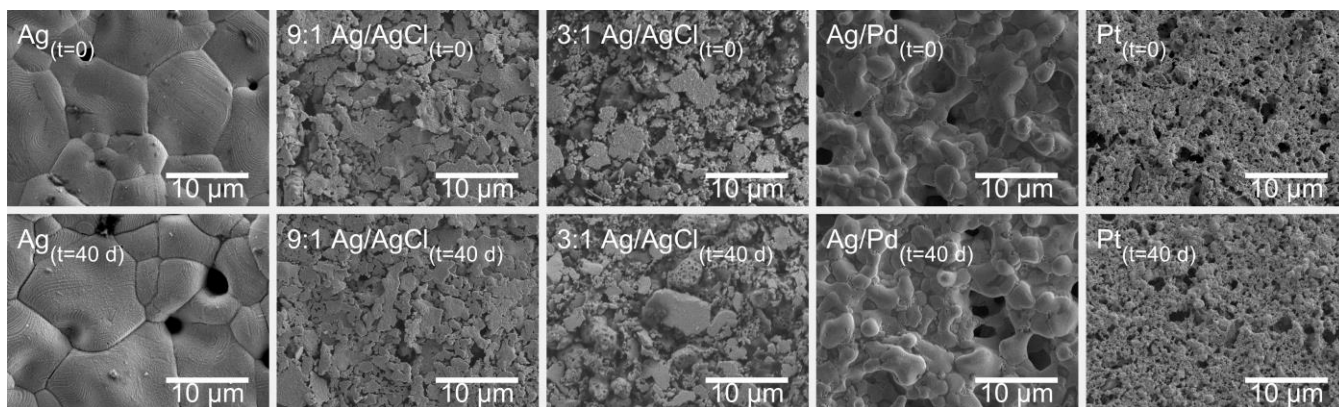
Figure 6. SEM images of SPREs (t=0) before and after 40 days in PBS solution. All images are taken at 4000× magnification with 5 keV acceleration voltage.

## 3.5. Material composition and surface morphology

All electrode surfaces appeared to be structurally unchanged at the micrometer resolution range after a measurement period of 40 days. This can be seen from the electron micrographs presented in Figure 6. Based on these images, large-scale remodeling of the surface is excluded as a major contributor to any net potential change during the 40 day period.

The relative elemental surface composition of the SPRE materials measured before (t<0) and after (t>40d) the long-term stability test, is shown in Figure 7. Considering the pure Ag electrode, the most notable change was a decrease in oxygen content after 40 days. The electrodes are all exposed to air for a significant period of time prior to the testing procedure as well as dissolved oxygen in the PBS during the measurement. A sudden change in the potential determining reaction is likely the cause of the observed jumps in the OCP. This could be related to a change from a silver oxide or silver hydroxide equilibrium to an Ag/AgCl(s) equilibrium. The latter is supported by the observed potential on the Ag electrodes, which is similar to the potential of electrodes pre-loaded with AgCl(s). Thus it is likely to be a similar potential controlled reaction occurring on both types of electrodes. In fact spontaneous precipitation of AgCl(s) on Ag surfaces in solutions with high KCl concentration has been reported previously.[35] In addition, Ag will form more complex anions with Cl⁻, mainly $AgCl_x^{(x-1)^-}$ for (x = 2-4), which is likely to pull the potential away from that associated with the Ag/AgCl equilibrium.[34,36] A diagram of other equilibria, and hence contributors to the potential, formed in the presence of Ag⁺(2 µM) [1] and Cl⁻ in aqueous media is provided in the supporting literature (Figure S4). Therefore, the collective conclusion for the Ag electrodes is that they mainly take on the Cl⁻ exchange reaction mechanism in PBS with assumingly only very small quantities of AgCl(s) being formed on the Ag electrodes, albeit too small a quantity to be picked up by the EDX analysis (Figure 7). These electrodes are thus easily affected by any parallel reactions, leading to mixed potential and more pronounced potential variations as compared to the electrodes with a significant initial AgCl(s) loading.
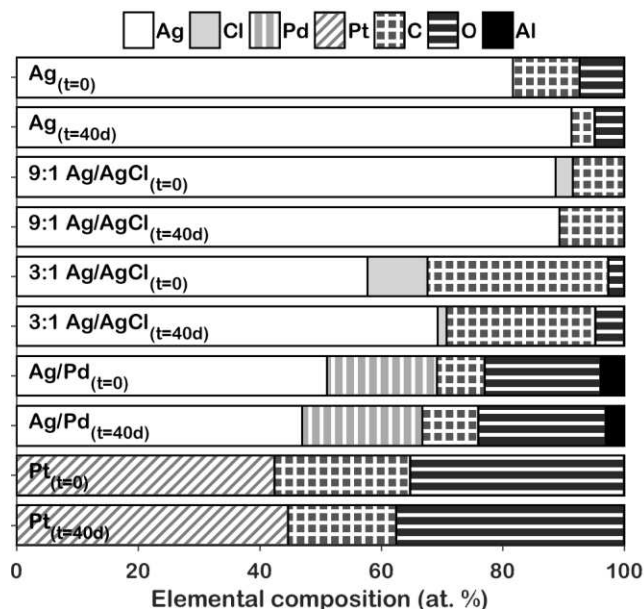
Figure 7. Elemental composition acquired from EDX elemental analysis before and after 40 days of continuous OCP monitoring for five SPRE materials. The estimated interaction depth was 0.1 μm.

The 9:1 Ag/AgCl electrodes generally showed good OCP stability and low spread in the electrode potential over the timeframe of the measurement. The dip in OCP observed from day 30 is a common feature which suggests that the total consumption of AgCl has happened, and which marks the end of the SPRE's lifetime.[1,19,34,37] One might argue that the different in structure due to the electrode materials being from different suppliers might the failure mode observed. There are however three observation discounting this: (i) The SEM images show very similar structure and particle size, meaning the solid particles are not significantly different. (ii) If the organic binders in the ink was the cause of release of the particles into the solution the EDX would have shown a significant increase in organic materials. (iii) The absence of Cl after 40 days for the 9:1 Ag/AgCl electrodes strongly suggests total AgCl depletion to be the root cause. In essence, the electrode potential is no longer dominated by (1), but is influenced also by other equilibria,[37] as is the case for the pure Ag electrodes. A significant loss of Cl was also observed for the 3:1 Ag/AgCl electrodes. The small amount remaining is presumably what ensured stability past 40 days. It is expected that if the experiment was allowed to continue, they would also exhibit the characteristics of a pure Ag SPRE at some point. The total amount of Cl lost was greater than for the 9:1 Ag/AgCl electrodes and demonstrates the larger availability of AgCl for dissolution. Therefore, in selecting the AgCl(s) loading when designing the reference electrode on a sensor there seems to be a tradeoff between its lifetime and drift in potential.

Ag/Pd sees little overall elemental change, which, in conjunction with the unchanged microstructure, excludes compositional changing processes as major potential determining factors. The observed traces of aluminium is presumed to be a consequence of interdiffusion from the Al_2O_3 substrate to the surface during manufacturing (curing at 850 °C). It could also be an effect of where the highly porous structure of the material permits the electron beam to see some exposed parts of the substrate material. Their long-term OCP evolution is quite erratic. Such large variations are characteristic of polarizable electrodes. It seems likely that the small current flowing through the instrument (<400 aA) causes a charge build-up, and then at some point discharges. Indeed, a charge-discharge behavior can be seen repeatedly throughout the Ag/Pd potential evolution (Figure S3). A likely cause for the high polarizability is that weakly solvated cations, like Cl⁻, tend to adsorb on metallic surfaces.[38–42] This process is much slower than the facile Ag/AgCl redox reaction, and will as such be a rate determining step incapable of keeping up with small current surges without severely adjusting the electrode potential. Furthermore, salt formation has been reported to favor Ag/Pd alloys where the Ag content exceeds 61.9 wt% in NaCl solution.[32] While Ag/Pd

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SPREs have been successfully employed in short-term assays,[43] the instability exhibited here makes them unsuited for use over longer periods of time.

The relative material composition of the Pt SPRE appears to be largely unchanged, suggesting that the Pt dissolution must have happened at a rate that is too low to be visible in the overall surface composition. Its net drift along with erratic variations in potential makes it a poor contender for long-term operation in a PBS type environment.

3.6. Cyclic voltammetry

The performance of the SPREs under load was quantified by voltammetry. Voltammograms for a $[Fe(CN)_6]^{3-/4-}$ system in 1 M KCl (pCl 0) employing the different SPRE materials before and after the 40 day period are shown in Figure 8. The translatory shifts along the voltage axis is characteristic of the electrodes operating at different half-cell potentials. The voltammograms obtained with Ag/Pd reference electrode sit at a lower potential range, indicating that its half-cell potential is much higher compared to the other Ag-based electrode materials studied here. The displacements of these voltammograms relative to the reference voltammogram for the KCl(4M)/AgCl/Ag half-cell reveals that the Ag and Ag/AgCl electrodes have potential differences in line to those recorded at the same pCl for the Cl^- sensitivity test (Figure 3). Thus they show resilience towards load effects from the potentiostat and the presence of the electroactive $[Fe(CN)_6]^{3-/4-}$ species. Contrarily, Ag/Pd displays a much higher half-cell potential, showing a difference of about 240 mV, some 180 mV higher than the average OCP value for the same pCl (Figure 3). This means that the Ag/Pd electrodes are either affected by the solution composition to a much greater extent than the other materials, or it is a sign of high polarizability as a response to a small current drawn from the terminal of the potentiostat. Evidence points towards the former, seeing as their half wave potentials $E_{1/2}$ are centered around 0 mV, indicating that electrodes have taken on the $[Fe(CN)_6]^{3-/4-}$ equilibrium reaction. Similar behavior is observed for the Pt electrode, but with a +50 mV shift in half-cell potential. Indeed, pseudo-reference electrodes utilizing this equilibrium have been reported, and have found to be adequate for weakly polarized amperometric applications for up to two months.[44] The +50 mV may be attributed to the electrolyte containing highly concentrated Cl^-, and the positive overpotential associated with Pt dissolution by way of Pt and Cl complexes (Pt^{n+}, $PtCl_4^{2-}$, $PtCl_4^{6-}$).[29]
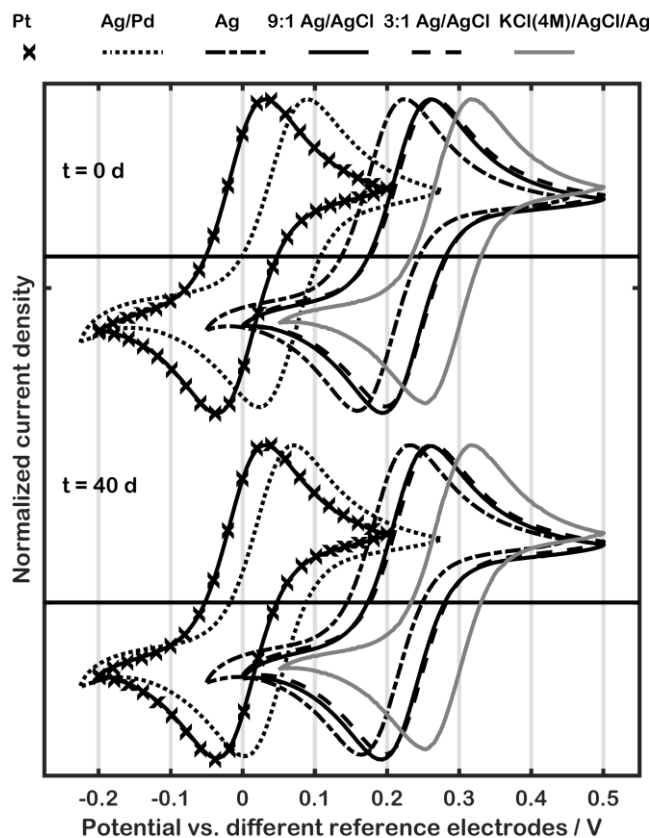
Figure 8. Cyclic voltammograms (10 mV s^{-1}) in a [Fe(CN)$_6$]$^{3-/4-}$ system with 1 M KCl as supporting electrolyte employing the different SPREs before (top) and after 40 days of OCP measurement (bottom). The current is normalized with respect to the oxidation peak value for easier comparison of the peak potentials.

There appears to be no significant change when comparing voltammograms before and after 40 days in PBS. Coupled with the fact that most electrodes did not undergo significant permanent change in either morphology or elemental composition means the chemical processes the electrodes have undergone under open circuit conditions have not caused any permanent impact on their half-cell potentials. Although there were potential variations during the 40 day period, a rinse was sufficient in order to re-use the SPREs.

4. Conclusions

The reference potential of five different screen-printed reference electrode materials: Ag, 9:1 Ag/AgCl, 3:1 Ag/AgCl, Ag/Pd and Pt, were examined in a PBS solution over a period of 40 days. The four Ag-based electrodes displayed similar dependence in OCP towards variation in Cl$^-$ activity, which suggests that Cl$^-$ is active in the potential determining reaction(s) with the same reaction order. In contrast, the Pt SPREs displayed a response to Cl$^-$ sensitivity that was opposite to the Ag-based electrodes. This was attributed to Pt dissolution as opposed to a Ag/AgCl equilibrium giving rise to the potential determining reaction. The micrometer scale surface morphology did not show any visible changes over the measurement period and all the electrodes reached a stable potential in less than 20 sec after immersion.

The ability to generate and maintain a stable potential under long-term operation varied greatly between the materials: Electrodes with a printed Ag ink displayed long-term potential stability of around two days, and exhibited potential steps indicating abrupt changes in surface reactions. Their average potential was similar to that of a Ag/AgCl electrode, suggesting that they mainly take on the Cl$^-$ exchange reaction, provided the involved species are available. However, the electrodes are prone to shift to potentials that are determined by other equilibria. The authors therefore claim that the Ag SPREs are better suited for

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short-term, than for long-term measurements. The Ag/Pd and Pt reference electrodes appeared to be less stable during the long-term potential measurement, owing to its erratic potential variation, charge- discharge characteristics. The lack of potential stability seen during the long-term measurements was reflected in the potential variability seen under Cl^- sensitivity analysis. The Ag/Pd and Pt SPREs cannot be recommended for long-term operation under the given conditions. In contrast, these two electrode materials was able to withstand high temperatures and organic solvents, rendering them suitable for short-term use (as well as applications in harsh environments). Inks containing AgCl exhibited the greatest stability and generally maintained their potential within 30 mV of its initial value for a period of 40 days. EDX analysis revealed a loss of Cl content for both the Ag/AgCl electrodes. The associated consumption of AgCl caused a daily drift in the OCP of -0.1 mV and -0.2 mV for the 9:1 and 3:1 loadings respectively. AgCl depletion additionally caused some of the 9:1 electrodes to take on a behavior that was found to be similar to pure Ag. Given sufficient time, all Ag/AgCl electrodes run a risk of total AgCl depletion. The authors therefore suggest focusing on exhaustive stability tests involving a wide range of Ag/AgCl ratios in future work. For biosensor applications where the conditioning time can be accepted, and where the sample medium is well known, Ag/AgCl SPREs are found to be suitable candidates.

SUPPORTING INFORMATION

Images of the long-term potential measurement circuit and cyclic voltammetry setups, raw data from long-term potential measurement, phase diagram of Ag and Cl aqueous system (PDF).

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