Iridium Oxide Based Potassium Sensitive Microprobe with Anti-fouling Properties

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Abstract—Here, we present a new type of potassium sensor which possesses a combination of potassium sensing and anti-biofouling properties. Two major advancements were required to be developed with respect to the current technology; Firstly, design of surface linkers for this type of coating that would allow deposition of the potassium-selective coating on Iridium (Ir) wire or micro-spike surface for chronic monitoring for the first time. As this has never been done before, even for flat Ir surfaces, the material’s small dimensions and surface area render this challenging. Secondly, the task of transformation of the coated wire into a sensor. Here we develop and bench-test the electrode sensitivity to potassium and determine its specificity to potassium versus sodium interference. For this purpose we also present a novel characterisation platform which enables dynamic characterization of the sensor including step and sinusoidal response to analyte changes. The developed sensor shows good sensitivity (<1 mM concentrations of K\textsuperscript{+} ions) and selectivity (up to approximately 10 times more sensitive to K\textsuperscript{+} than Na\textsuperscript{+} concentration changes, depending on concentrations and ionic environment). In addition, the sensor displays very good mechanical properties for the small diameter involved (sub 150 µm), which in combination with anti-biofouling properties, renders it an excellent potential tool for the chemical monitoring of neural and other physiological activities using implantable devices.

Index Terms— Potassium sensing; anti-fouling; surface linker; iridium oxide; impedance spectroscopy, neurochemical measurements

I. Introduction

ABNORMAL values of potassium (K\textsuperscript{+}) concentration in tissue have been associated with several conditions such as epilepsy, neuromuscular ataxia, cardiac arrhythmia, and Alzheimer’s disease [1]. This observation, in addition to fundamental studies in neuroscience, has resulted in attempts to monitor the in vivo concentration of the cation. Such measurements involving standard glass microelectrodes dates to the 1970s [2,3]. More recent times have seen the introduction of probes based on optical science such as the micro-optrode or fluorescence spectroscopy [4,5]. The aim of the present work is to investigate the possibility of creating a potassium sensor based on an Iridium substrate. This is prompted by the successful testing and demonstration of the closed-loop capabilities for pH sensing achieved using IrOx microelectrodes [6,7]. Such an approach should offer additional benefits such as greater temporal and spatial resolution of neural activity, including the peripheral nervous system. In addition, it also offers the possibility to chronically record cation concentration as an implantable sensor. Until recently, this was unachievable due to the lack of sufficiently hard K\textsuperscript{+} sensitive substrates within such a small form factor. Several techniques, such as ionophore entrapment within polyphenol coatings on Pt microelectrodes, with Ni hexacyanoferrate layers using carbon screen printed electrodes (PEDOT doped with ATP) have been attempted but failed to function [8].

As mentioned above, the standard technique for detecting potassium ions is to use a glass capillary electrode (a pipette) with a gradient cation-sensitive ionophore and a carbon fiber conductive wire [9]. However, these sensors are fragile and bulky, not biocompatible, and cause inflammation when used as potential implants or in in vivo preparations. Another option is to make a ‘solid-state’ electrode, but the issue in this case is that the ionophore layer which is specific to potassium ions becomes saturated for very low potassium concentrations and needs a ‘protective’ layer above it. Furthermore, current technologies can only produce a minimum thickness of about 200µm, which is far too thick for many applications involving the peripheral and central nervous system. Given these issues, the employment of Ir-based electrodes offers attractive potential for the detection of K\textsuperscript{+}. The overall aim is to develop electrodes that are thin (sub-150 µm diameter) such as a microspike, which can be scaled up to a length of about 1cm. The

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devices must be mechanically stable enough to penetrate the epineurium and perineurium of the nerves or brain membranes, with tight coupling to nerve axons, or be placed in the extracellular space in the brain. Iridium is a biocompatible material which has very good mechanical and electrical properties. These properties are crucial for measuring potassium concentration inside tissue. Iridium micro-wires have been used previously to interface with peripheral nervous system by fabricating and demonstrating a pH sensor which reliably detects in vivo neural activity, with much better signal to noise ratio (SNR) and much less interferences than electrical recordings [6,7].

A strategy for the development of a potassium-specific iridium electrode is offered by a recently developed biosensor employed for in vivo measurement in a mouse cranium for measurement of K+ concentration [10]. In this work, a monolayer on a planar gold microelectrode surface for K+ sensitivity was fabricated with capabilities for tandem anti-fouling [11,12] and selective potassium binding via a crown ether [13]. An analogous anti-fouling layer, based on trichlorosilane linking chemistry, has also been developed which is capable of being attached to medical-grade stainless steel [14]. Based on this approach, a further anti-fouling molecule, 3-(3-(trichlorosilyl)propoxy)propanoyl chloride (MEG-Cl), which incorporates an acid chloride end group for linking to other compounds, has also been developed [15]. This chemistry allows the possibility to attach a MEG-Cl monolayer to Iridium, which can then be extended with 18-crown-6 ether to introduce K+-sensitivity to the substrate (Fig. 1).

Successful development of this micro-sensor enables the fabrication of a fully functional bimodal platform that will utilize both chemical and electrical recordings of neural activity. Such sensory capability can be combined with a signal processing and decision algorithm, and stimulation module. These technologies together with the attractive possibility to form a novel closed-loop stimulation system will allow the opportunity to answer two main questions for closed-loop stimulation, that is, when to stimulate as well as the type of stimulation dosage invoked [16,17,18]. The sensor can be readily extended into sensor arrays [19,20].

II. EXPERIMENTAL

A. Materials

3-(3-(Trichlorosilyl)propoxy)propanoyl chloride (MEG-Cl) was synthesized according to previously published methods [10]. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). Epoxy Araldite (ARA400012) was purchased from Farnell (UK). This epoxy is non-biocompatible. A biocompatible epoxy such as EPO-TEK MED-310-2 could also be used in future.

B. Cleaning and surface modification of iridium wire

Iridium plates (1cm x 1cm, for XPS analysis) or a section of iridium wire (125 μM diameter) were sonicated successively in pentane, acetone, and 95% ethanol for 15 minutes each. The iridium was then rinsed copiously with 95% ethanol followed by deionized water. The iridium was then submerged in piranha solution (3:1 sulphuric acid:30% hydrogen peroxide) for 30 minutes at 90°C. It was then rinsed copiously with deionized water followed by methanol, and then placed in an oven at 180°C for 2 hours to dry the surfaces. The iridium was then plasma cleaned under atmosphere for 5 minutes. Finally, it was stored overnight in a humidity chamber at 80% humidity to saturate the surface with water.

The iridium was then submerged in 5 mL of anhydrous toluene in a pre-silanized scintillation vial (presilanization performed by filling the vials with toluene and adding several drops of trichloro-hexyl silane and letting sit overnight before washing with toluene). To this was added 5 μL MEG-Cl. The iridium was then mixed at low speed using a rotoplate for 90 minutes. Then it was rinsed with toluene, and then sonicated in toluene for 5 minutes to remove any excess MEG-Cl.

The iridium was then submerged in 3 mL DCM containing molecular sieves to ensure an anhydrous solution. Added to this was 10 μL 18-Crown-6 Ether. The iridium in solution was
rotated overnight on a rotoplate. Then it was copiously rinsed with deionized water and dried under a stream of air.

C. X-ray photoelectron spectroscopy (XPS)

Angle-resolved XPS analysis was performed with a Theta Probe Angle-Resolved X-ray Photoelectron Spectrometer System (Thermo Fisher Scientific Inc., Waltham, MA, USA) located at Surface Interface Ontario (University of Toronto, Toronto, ON, Canada) for iridium plates set aside after cleaning, and each step of chemical surface modification. The samples were analyzed with monochromated Al Ka X-rays (elliptical spots of 400 µm along the long axis) with take-off angles of 90° relative to the surface. Peak fitting and data analysis were performed using the Avantage software provided with the instrument.

III. Measurement setup

The measurements were performed using three different setups: one for preliminary measurements, one while working with fluidic chamber and another one for measurements in artificial cerebrospinal fluid (aCSF).

A. Preliminary measurement setup

During preliminary experiments CH Instruments 760E electrochemical Workstation was used to perform measurements. The rest of the equipment included a magnetic stirrer, 11x100mL beakers and a laboratory stand.

The sensor consisted of 3 electrodes placed on the polystyrene plate, Fig.2. Three pieces of 0.125mm platinum wire twisted together were used as a counter electrode (CE). A piece of 0.125mm platinum wire served as a pseudo reference electrode (RE). 0.178mm iridium wire coated with K+ sensitive layer served as a working electrode (WE). To make the sensor, a 4-wire (2 twisted pairs) double shielded (with drain wire) cable was used. One twisted pair was used to connect to WE and the RE, one wire from the second pair was used to connect to the CE. Remaining wire was tied to the shield at the device end. The electrodes were glued to the corresponding wires with silver conductive epoxy glue. RE was positioned close to the WE (~0.5mm) and CE in some distance from those two (~5mm). The sensor was covered with thick layer of epoxy glue, except the small opening where electrodes were exposed.

B. Fluidic chamber measurements setup

The fluidic system for testing potassium sensors was built to provide the possibility to characterize sensors in controlled, dynamic conditions, Fig.3. This system was used to obtain constant flow of the base fluid in which the concentration of the specified ion changes according to a predefined profile (i.e. sinusoidal, linear, stepwise). The targeted concentrations are obtained by mixing two fluids with different concentration of ions inside the fluidic chamber. The flow of each fluid was digitally controlled with syringe pumps to follow required profile while keeping the total flow rate through the chamber constant at selected level.

The system consisted of four major parts (Fig.3): (a) fluid delivery system, (b) mixing chamber, (c) impedance analyzer and (d) control software. For impedance measurements the Solartron 1260 Impedance Analyzer was used. Fluid delivery system consisted of 2 syringe pumps (custom design based on the open syringe project [21]), and custom-made driver (built around an inexpensive Arduino® platform with CNC shield and Trinamic® TMC2203 motor drivers).

Software to control the fluidic system and embedded program for the driver were written using widely available software platforms (AtmelStudio® and VisualStudio®). The impedance analyzer was controlled via custom software written in LabView® environment. All data processing and analysis was performed with Matlab®.

A few different designs were evaluated to minimize material required to manufacture the chamber and to provide appropriate fluid mixing. Prior to manufacturing, in-silico studies were performed to ensure the fluids will be mixed in the chamber appropriately (Fig. 4).
Each module after printing was covered with thin layer of PVB varnish to make the structure waterproof. Modules of the chamber were glued with silicone. The mixing chamber consisted of two inlets, premixing chamber, static mixer, sensor chamber and the output (Fig. 5, bottom).

To deliver fluids to the chamber a silicone hose with 4mm outside and 2mm inside diameter was used. The final chamber design is shown at Fig. 6.

The sensor was built in a similar way as during preliminary experiments. However, this time 3D printed support/jig was used. Using the support provides repeatability in electrodes manufacturing. The shape of the support also immobilizes the sensor inside the chamber. The methodology of the electrode manufacturing assumes fabricating a sensor without soldering to avoid unnecessary heating of the electrode wire which could lead to damage of the potassium sensitive coating.

Four ~7mm pieces of 0.125mm platinum wire were prepared. Three of them were twisted together and placed in the bottom wire socket of the sensor support to serve as a CE electrode. One piece was placed in the middle socket to become RE electrode. A ~7mm 0.178mm Ir wire coated with K⁺ sensitive layer was placed in the top socket to serve as a WE. The distance between WE and RE was ~1mm and between RE and CE ~ 4mm. A drop of an epoxy glue was placed at the end of each wire (marked a in Fig. 7) and in place selected as (b) in Fig. 7 to immobilize wires. Next, cable wires were placed into corresponding slots and junctions of wires and electrodes were protected with silver conductive glue. The cable was immobilized by applying epoxy at point c Fig. 7. When silver leaving only electrode wires exposed. Finally, connection wires were bent at the right angle and covered with silicone (Fig. 7, right). When electrode is placed in the chamber, silicone around the connection wires serves as a sealant for electrode cable.

C. Artificial cerebrospinal fluid (ACSF) measurements

Impedance measurements were performed using CH Instruments 760E. A frequency of 1 kHz and an AC amplitude of +/- 10 mV was used to measure impedance. A step change in concentration of potassium ions and sodium ions was introduced by adding potassium chloride and sodium chloride solutions to ACSF in a controlled manner.

IV. METHODS

A. Preliminary measurements

Impedance measurements were performed using standard 3-electrode configuration at 5kHz, 10mV amplitude, 1S/s. The
frequency was chosen based on the impedance spectrum measurements - in the region of phase characteristic transition. Five 100mL beakers were filled with solutions containing 100mM of NaCl and 8mM, 4mM, 2mM, 1mM and 0mM KCl respectively (set 1). Solutions were prepared by serial dilution. Another set of five beakers was filled with solutions containing 108mM, 104mM, 102mM, 101mM and 100mM NaCl respectively (set 2). First, the sensor was placed into the beaker containing DI water for a few minutes. Next, it was placed in the beaker containing 100mM of NaCl and 1mM of KCl for around 60 minutes and during this time the open circuit potential (OCP) was measured. Then, successively beakers were placed on magnetic stirrer in the following order: from one containing lowest concentration of KCl, to one containing highest concentration of KCl and then from highest to lowest (set 1). Every time sensor was immersed in the solution and the recording was taken for around 30s. This sequence was repeated twice. Then the same procedure was repeated for solutions containing only NaCl (set 2).

**B. Measurements with the fluidic chamber**

As in the case of the preliminary experiments impedance measurements were performed using a standard 3-electrode configuration. Parameters used were as follows: frequency 1 kHz, amplitude 10mV, sampling frequency 1S/s. Again, the frequency was chosen based on the impedance spectrum measurements – in the region of phase characteristic transition, this time for a new sensor prepared.

Syringe pumps were used to apply two different patterns of changes in ionic concentration: sinusoidal and pulse. Two solutions were prepared: A, containing 100mM NaCl and B, containing 80mM NaCl and 20mM KCl. The speed of both pumps was controlled differentially. When one pump was speeding up, the second one was slowing down at the same rate, so the total flow through the chamber was held constant at 4ml/min.

In the case of the pulse pattern, for the first 75s only solution A was flowing, then the flow was changed to solution B for 100s and then changed back to solution A for ~130s. In the case of the sinusoidal pattern, for the first 75s the speed of both pumps was equal, then for next 100s, in a sinusoidal way, the pump containing solution A was slowing down to minimum, speeding up to maximum and then returning to its initial value. The pump containing solution B was working in opposite way. For last ~130s the speed of both pumps was equal again. For comparison, the measurements were repeated with both syringe pumps filled with the same solutions containing 100mM NaCl. This was made to ensure that results are not influenced by measurement setup itself (i.e. flow fluctuations).

**C. Artificial cerebrospinal fluid measurements**

ACSF was made as the following: 124 mM NaCl, 5 mM KCl, 26 mM NaHCO$_3$, 1.25 mM NaH$_2$PO$_4$, 10 mM D-glucose, 1.3 mM MgCl$_2$, and 1.5 mM CaCl$_2$ in deionized water. The detailed protocol was extracted from [22,23]. This solution was used as the base fluid to which KCl or NaCl was added in a controlled manner to change the ionic concentration. The sensor was submerged in ACSF for 1 minute for OCP to stabilize, then measurement was made for 5 minutes to measure impedance/phase response of the sensor to the solution. The solutions were subjected to step response changes in concentration of potassium and sodium ions while the phase response was monitored.

**V. RESULTS AND DISCUSSION**

**A. Modification of iridium wire**

XPS analysis was used to verify the surface composition of iridium plates, and thus also the modified iridium wire, at each stage of surface modification. The XPS atomic percentages were calculated from high resolution scans. Detailed values are provided in Table 1. The Iridium samples show clear changes to their atomic compositions at each stage of the modification (Fig. 8). Iridium from the package without cleaning was predominantly found to have carbon, oxygen, and silicon on the surface with virtually no iridium signal present. This suggests a large amount of surface contamination as well as possible oxidation of the surface. The iridium signal drastically increased following cleaning, suggesting successful removal of contaminants from the surface.

**Figure 8.** Elemental composition of iridium surfaces at each stage of modification as measured using XPS.

**TABLE I**

<table>
<thead>
<tr>
<th></th>
<th>Unclean Iridium</th>
<th>Cleaned Iridium</th>
<th>MEG-Cl Addition</th>
<th>Crown Ether Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>55.38</td>
<td>27.64</td>
<td>51.62</td>
<td>47.99</td>
</tr>
<tr>
<td>Iridium</td>
<td>0</td>
<td>33.82</td>
<td>2.3</td>
<td>2.55</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.17</td>
<td>2.95</td>
<td>0.75</td>
<td>0.63</td>
</tr>
<tr>
<td>Oxygen</td>
<td>31.25</td>
<td>26.72</td>
<td>27.39</td>
<td>29.91</td>
</tr>
<tr>
<td>Silicon</td>
<td>10.63</td>
<td>3.13</td>
<td>17.22</td>
<td>18.27</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.54</td>
<td>0.64</td>
<td>0.92</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Following silanization there is a notable increase in carbon and silicon signals, a decrease in iridium signal, as well as a slight increase in chlorine signal. This suggests the successful addition of MEG-Cl to the surface. Finally, addition of the crown ether resulted in a slight decrease in carbon signal, and increase in oxygen signal. This suggests the successful addition
of crown ether to the surface, as the ratio of oxygen to carbon is slightly greater in the crown ether, than in the linker.

B. Sensor use in buffer solutions

The OCP measurement results (Fig. 9) indicates that sensor requires more than 30min to stabilize. Over a period of 30 min, the drift in OCP is 3 mV. That suggests the drift in the following measurements, albeit very small, should be expected.

![Figure 9. OCP measured over 60 min. in the solution containing 100mM NaCl and 1mM KCl.](image)

The changes in phase observed during measurements for both sets of solutions (as described in section 4.1) are presented in Fig. 10. The bias value of phase was removed from both measurements to easily compare changes observed. Existing drift in phase was calculated by linear regression of the last minute measurement and subtracted from the data. Data is also inverted for visualisation purposes. The experiment was repeated in both ascending and descending changes of potassium and sodium concentrations to demonstrate repeatability and stability of the sensor measurements. As can be seen from these measurements, the electrode was moved from solutions with low concentrations of K\(^+\) to those with higher concentrations the phase changed accordingly. When the electrode was moved from high to low concentration the phase returned almost perfectly to the previously observed baseline. This process could be repeated multiple times, with the phase corresponding well to the concentration of K\(^+\) in each solution. Solutions containing differing concentrations of Na\(^+\) also showed phase changes which corresponded to the concentration, however these changes were much smaller than those observed for K\(^+\). This suggests that the measurements are fairly specific to K\(^+\) as well as reproducible.

![Figure 10. The sensor response to various potassium and sodium concentrations. Solutions: five beakers with 100mM NaCl and 0, 1, 2, 4 or 8 mM of KCl and for same concentrations of NaCl.](image)

The chosen range of potassium concentrations corresponds to the relevant physiological range for the extracellular K\(^+\) concentrations. For plasma concentrations, the normal range is 3.5 mM – 5 mM, and the highest reported concentration during strenuous exercise was around 8 mM [24]. The concentration of K\(^+\) in the CSF of healthy mammals is about 2.5 mM – 4 mM [25], and measurements made in unstimulated brain and spinal cord confirm the baseline of up to 4 mM, and in epileptic seizures of around 12 mM [26].

C. Sensor characterized in fluidic chamber

To demonstrate that the sensor is able to measure continuously changing concentration of the ions over time, and not just measure drastically different concentrations as in the previous experiment, we have performed experiments using our fluidic chamber. Results of measurements with the fluidic chamber are presented in Fig. 11 and 12.

In Fig. 11 the response for sinusoidal change in K\(^+\) concentration is shown. In Fig. 12 the response for step change is presented. The green line represents when the concentration changes were applied and the pattern used (aligned manually to compensate the lag resulting from chamber volume). It can be observed that phase follows the changes of potassium concentration. The response for control measurements are not visible. That ensures that results obtained are not influenced by measurement setup itself.

The fluidic chamber allows for frequency characterization of chemical sensors. Here (Fig.11) we demonstrate it only for one frequency which corresponds to the period of 100s.
D. Sensor tested in artificial cerebrospinal fluid

We have also tested the sensor in ACSF in order to determine if it works in biological solutions (Fig. 13). As can be seen from the data the sensor showed a much larger response to changes in K+ concentration in this simulated biological fluid, compared to Na+. This shows that not only is the sensor able to operate in a more complicated matrix, which would cause far more surface fouling than the previously tested buffer solutions, but that it is still very selective to K+ in this solution. This gives great promise for the sensor being used successfully in vivo.

For the measurement instrumentation and custom-made calibration used in our experiments, the sensor can reliably and reproducibly detect at least 1 mM potassium concentration change, with the lower limit of sensitivity of 0.5 mM.

Our previous acute study [6] involving Ir-based microwire electrode indicates no significant difference in and adverse effect to neural responses pre and post implantation. Significant damage is not expected for brain studies, since the electrode dimension is of a similar size of typical electrodes used for brain neural recordings, such as Utah array [27].

VI. CONCLUSION

The present work shows that it is possible to coat iridium oxide metal with trichlorosilane surface coatings. In this case, an anti-fouling linker which was extended with a potassium sensitive probe. This modified iridium wire can then be used as the basis for a potassium sensitive impedance sensing electrode, which provides highly sensitive and reproducible results. The validation platform described enabled successful dynamic characterization of the sensor and helped in determining the dynamic response of the sensor to various temporal types (step/sinusoidal) changes in K+ concentration. The electrode was found to not only operate successfully in buffer solutions, but also in artificial cerebrospinal fluid indicating that it could be adapted for use in living nerve bundles. The next step will be to develop and test an implantable version of this electrode for use in in vivo potassium sensing.
Figure 13. Shows the comparison of the sensor measurements for increasing concentrations of K+ (left) and Na+ (right) in artificial cerebrospinal fluid (aCSF).

REFERENCES


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