NEST 4 – Lifetime Testing Protocol

Revision 1 (03 June 2024)

1 Preliminary Inspection

Materials: *Assembled cuff electrode*

1. Visually inspect the device per the instructions in appendix A

2 Setup

Materials: *Glass jar with sealing lid*
Parafilm
Marine epoxy
Rubber Stoppers
Assembled cuff electrode

1. Cut five holes in the top of the jar lid
2. Thread the lead wires of an assembled cuff electrode through one of the holes such that the cuff is towards the inside surface of the lid and the wires thread to the outside surface of the lid
3. Advance the lead through the hole until the cuff electrode is suspended in the jar when the lid is in place
4. Secure the device in place using marine epoxy
5. Place rubber stoppers in the other three holes in the lid
6. Up to two devices may be fixtured in each jar lid

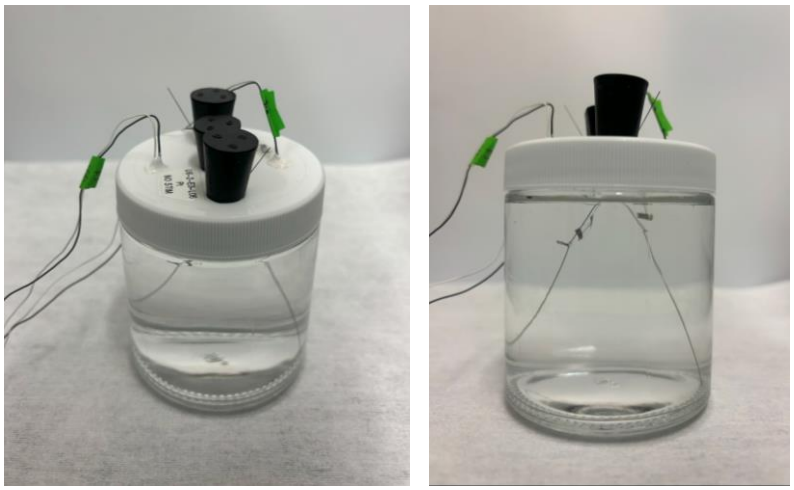


Figure 2-1: Photo of the fixtured devices for lifetime testing. (Left) 5 holes are drilled in the lid of the jar, with devices secured in two holes and the remaining 3 plugged with rubber stoppers. (Right) Devices are suspended in saline and held under the surface using wire threaded through the tab holes.

3 FIXTURING

Materials: *Device/lid assembly and jar*
1x PBS
Parafilm

1. Submerge the electrode end(s) of the cuff(s) in IPA for 1-5 minutes, using a wire or other clamp to hold the cuff underneath the surface if needed
2. Remove the device from the IPA and submerge the electrode end of the cuff in DI water for at least 10 minutes, using a wire or other clamp to hold the cuff underneath the surface if needed
3. Fill the jar with 1x PBS to a depth of 3-4 cm
4. Submerge the electrode end of the cuff(s) in the PBS, using a wire or other clamp to hold the cuff underneath the surface if necessary, and screw the lid of the jar in place
5. Apply parafilm to seal the edges of the jar lid

4 ELECTRICAL TESTING

Materials: *1x PBS*

1. Remove two rubber stoppers from the lid
2. If the solution level is low, top off the solution with fresh 1x PBS
3. In the center hole, insert a platinum wire counter electrode and an Ag/AgCl reference electrode
4. In one of the side holes, insert the N_2 purging tube
    a. Purge for at least 5 minutes before starting CV
5. Perform VT testing and calculate the charge injection capacity per the instructions in appendix B
6. Perform CV per the instructions in appendix C
7. Perform EIS per the instructions in appendix D
8. Disconnect the device from the potentiostat and remove the Ag/AgCl reference electrode, platinum wire counter electrode, and N_2 purging tube
9. Replace the rubber stoppers in the lid

5 ACCELERATED LIFETIME TESTING

Equipment: *Oven or heated water bath*

1. Connect the leads of the cuff electrode to the stimulator
2. Place the fixtured device in the jar in an oven or hot water bath at 50 °C
    a. 50 °C equates to an acceleration factor of 2.46x
3. Begin stimulating the electrode using the following parameters:

Amplitude	0.5 mA
Pulse Width	1500 µs
Interphase Gap	100 µs
Frequency	50 Hz

    a. E1 is the working electrode, E2 is the ground electrode

    b. Note: these parameters were selected based on stimulator limitations; other parameters can be selected so long as they do not exceed the charge injection capacity of the electrodes
4. Record the start time
5. Wait for the designated amount of time
    a. Testing frequency is as follows:
6. Stop the stimulation, disconnect the leads from the stimulator, and remove the fixtured device (in the jar) from the hot water bath or oven
7. Record the stop time
8. Repeat step 4 (Electrical Testing)
9. Replace the jar into the hot water bath or oven
10. Reconnect the leads to the stimulator and resume stimulation
11. Record the restart time
12. Repeat steps 5 through 11 until an accelerated time of one year is reached

APPENDICES

A. VISUAL INSPECTION

Equipment: *Stereoscope with camera*
Microscope with at least 10x magnification

1. Place the device underneath the microscope, taking care not to bend the device harshly with the lid attached
2. Using the stereoscope, inspect the device for any large mechanical failures, such as delamination in the metal, Parylene, or PDMS
3. Using 5x and 10x magnification (or higher), inspect the device for microscopic mechanical failures, such as cracks or delamination in the metal, Parylene, or PDMS
4. Note and photograph any defects

B. VOLTAGE TRANSIENT TESTING

Equipment: *Potentiostat with faraday cage*

Materials: *Ag/AgCl reference electrode*
Platinum counter electrode

1. Verify that the electrodes are fully submerged in PBS and that no bubbles are visible on the active electrode surface
2. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
3. Stimulate one pulse with 1 mA amplitude, 500 µs pulse width, and 100 µs interphase delay and record the resulting voltage
4. Repeat step 3 using the same pulse width and interphase delay for the following amplitudes:
    a. Uncoated electrodes (bare platinum): 1 mA, 2 mA, 3 mA, 4 mA

b. Coated electrodes (platinum/iridium, iridium oxide, or PEDOT): 1 mA, 2 mA, 3 mA, 4 mA, 6 mA, 8 mA
c. Note: if any of the listed pulse amplitudes cause the interphase potential (E_p) to exceed -0.6 V, re-evaluate and use at least three amplitudes with E_p less than or equal to -0.6 V for the following voltage transient tests

5. Move the working electrode connection to the other lead wire
6. Repeat step 3 for the second electrode
7. Save VT data and estimate the charge injection capacity (CIC) by linearly fitting the recorded data (stimulation amplitude vs. E_p, excluding any points where $E_p < -0.7$), finding the amplitude where $E_p = -0.6$ V, and calculating CIC using the equation below:

$$CIC = \frac{\left(current\ @E_p = -0.6\right) \times (500\ \mu s)}{(geometric\ surface\ area)}$$

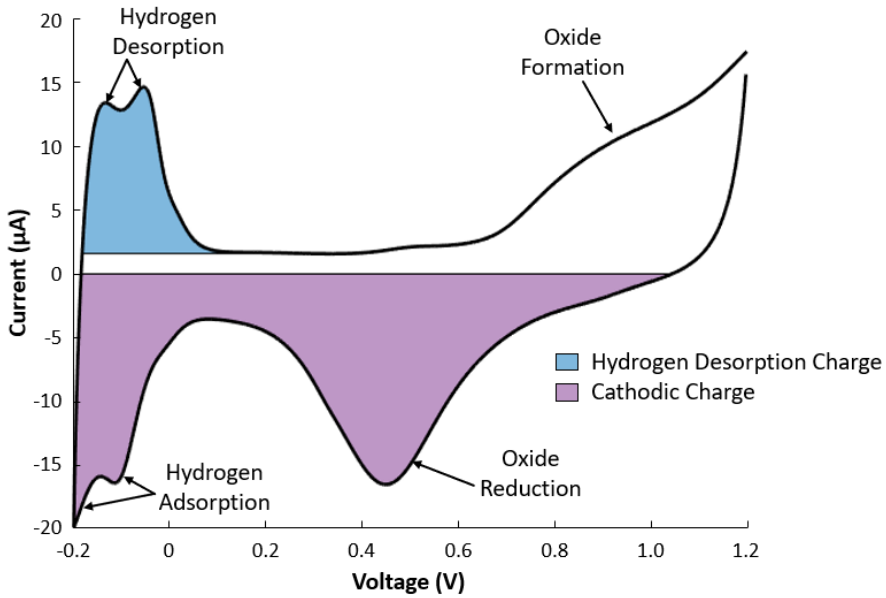
C. Cyclic Voltammetry

Equipment: *Potentiostat with faraday cage*

Materials: *Ag/AgCl reference electrode*
Platinum counter electrode

1. Verify that the electrodes are fully submerged in PBS and that no bubbles are visible on the active electrode surface
2. Purge the PBS with N_2 for at least 5 minutes; continue purging for the duration of CV testing
3. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
4. Perform CV for 3 cycles from -0.6 to 0.6 V with 200 mV/s scan rate
5. Move the working electrode connection to the other lead wire
6. Repeat step 4 for the second electrode
7. Save CV data and calculate the cathodic charge storage capacity (CSC) using the equation below:

$$CSC = \frac{(cathodic\ charge)}{(geometric\ surface\ area)}$$



8. Turn off the N_2 purge

D. Electrochemical Impedance Spectroscopy

Equipment: *Potentiostat with faraday cage*

Materials: *Ag/AgCl reference electrode*
Platinum counter electrode

1. Verify that the electrodes are submerged in PBS and that no bubbles are visible on the active electrode surface
2. Connect the reference electrode, counter electrode, and one lead wire (working electrode) to the potentiostat in a 3-electrode setup
3. Perform EIS using 25 mV_{rms} over the range of 1 to 10^5 Hz with two points per decade
4. Move the working electrode connection to the other lead wire
5. Repeat step 3 for the second electrode
6. Save EIS data and record the 1 kHz impedance