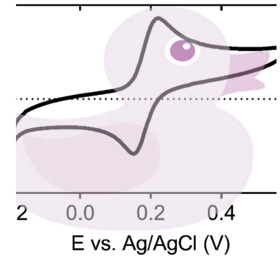
May 09, 2024

Cyclic Voltammetry analysis of laser-inscribed graphene electrodes: Ruthenium(III) chloride trihydrate redox couple



Forked from Electrochemical analysis of laser-inscribed graphene electrodes using cyclic voltammetry (ferri/ferrocyanide redox couple)

This protocol is a draft, published without a DOI.

Nick Cavallaro[1], Carmen Gomes[2,3], Eric S McLamore[2,3]

[1]1Agricultural and Biological Engineering Department, University of Florida;
[2]Department of Agricultural Sciences Department, Clemson University;
[3]Department of Environmental Engineering and Earth Sciences, Clemson University

Eric S McLamore: corresponding author;

SNAPS research group

Eric S McLamore
Clemson University, North Carolina State University

Protocol Citation: Nick Cavallaro, Carmen Gomes, Eric S McLamore 2024. Cyclic Voltammetry analysis of laser-inscribed graphene electrodes: Ruthenium(III) chloride trihydrate redox couple. **protocols.io https://protocols.io/view/cyclic-voltammetry-analysis-of-laser-inscribed-gra-dddq225w**

Protocol status: Working
We use this protocol and it's working

Created: November 28, 2022

Last Modified: May 09, 2024

Protocol Integer ID: 99472

Funders Acknowledgement:
National Institute of Food and Agriculture
Grant ID: 2018-67016-27578

Disclaimer

The authors acknowledge financial support from the NIFA Agriculture and Food Research Initiative Competitive Grant no. 2018-67016-27578 awarded as a Center of Excellence from the USDA National Institute of Food and Agriculture. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Food and Agriculture.

Abstract

This protocol describes use of the cyclic voltammetry (CV) method for electrochemical analysis of laser-inscribed graphene (LIG) electrodes using hexaammine-ruthenium(II) chloride (Hills et al; Oliveira et al). The protocol requires approximately 1 hour (excluding electrode fabrication).

Guidelines

The protocol is based on numerous published manuscripts that use similar methods with the haexamine-ruthenium redox couple, but is not a comprehensive guide. Where appropriate, alternative redox couples or electrolytes are noted.

Materials

MATERIALS

- LIG electrodes (see protocol for LIG fabrication above)
- Hexaammineruthenium(II) chloride (**link to SDS**)
- Potassium nitrate (**link to SDS**)
- Auxiliary electrode. This protocol uses a 7.5 cm long platinum wire or a 3-electrode LIG chip as noted.
- Catalog number for single platinum wire electrode: MW-1032, BASi, West Lafayette, IN, USA (**link here**)
- Reference electrode. This protocol uses a Ag/AgCl with 3M KCl internal solution or a 3-electrode LIG chip as noted.
- Catalog number for single reference electrode: MF-2056, BASi, West Lafayette, IN, USA) (**link here**)
- Optional: QuadHands magnetic workbench (KOTTO, Tokyo, Japan) (**link here**)
- Optional: If 3-electrode chip is used, additional materials include:
- USB type A connector (**link here**)
- Chemical resistant PVC backing (12” x 12” x 1/16“; McMaster-Carr part no. 8747K111) (**link here**)
- Double sided clear tape (**link here**)

HARDWARE

- Magnetic stir plate (**link here**)
- MultiPalmSens4 potentiostat (PalmSens, Houten, Netherlands)
- Optional: ABE-Stat (Diagenetix, Honolulu, USA) and a Tablet or (Android) cellphone

SOFTWARE

- MultiTrace4 software for PalmSens4
- ABE-Stat app if using ABE-Stat potentiostat

Safety warnings

SAFETY

●Lab coat, gloves, and closed-toed shoes are mandatory
●Nitrile gloves (powder-free type as applicable)
●Wrist grounding strap (optional) (**link**)

Chemical safety hazard

●All chemicals should be handled with gloves and a lab coat.
●When using scale to weigh chemicals, ensure the residues are cleaned and discarded in the solid chemical waste container as described by the solid waste disposal procedure and in reference to the SDS for all chemicals.
●After using electrolyte or other solution, for future use, store the solution in a capped glass bottle in 4^{o} C fridge or discard them in a labeled waste solution container.

Eye protection

●Goggles or eye protection is required when handling solutions outside of a chemical hood.

Skin

●If any solutions are spilled and encounter skin, immediately rinse under water and wash with soap for at least 5 min and follow procedures in SDS (links provided in material section).

Disposal

●CV solution should be discarded in the appropriately labeled waste container in the satellite accumulation area and not disposed of in the sink or the trashcan.
●Used LIG electrodes should be discarded in a labeled waste container sorted by appropriate hazard class (e.g., biohazard solid waste if applied for detecting pathogens, heavy metal solid waste, etc.).

ACCESIBILITY

The following guidance is summarized from Perry and Baum [5] for ADA laboratory (building) compliance,where relevant to this protocol.

General building codes for laboratory

- Minimum 2 Exits for labs ≥500 ft^2 net area.
- Minimum 2 Exits for labs using chemical fume hoods or glove box
- Minimum 2 Exits for labs using flammable and combustible: liquids, gases, cryogenics, dusts and solids.
- Minimum 2 Exits for labs using oxidizers, unstable reactives, water reactives, organic peroxides, highly toxics, corrosives.

Wheelchair clearance must be provided as follows:

- Egress for wheelchair 360° Turn is 1.5 m (5 ft) clearance.
- Both sides of Exit and Entry doors to
- Emergency Eyewash & Safety Shower
- In front of wall benches, sinks, equipment

- In front of chemical fume hoods
- At chalk/marker board
- Between benches
- Aisles that lead to Primary Exits, back to front
- Aisles that allow passage side to side in lab

Standard accommodations for use of chemical hood or other exhaust air systems

- knee space obstructions
- adjustable work surface height
- accessible receptacles and alarm control

Common equipment

- Where visual inspection is utilized, alternative technologies should be listed as optional (colorimeters, spectro-radiometers, etc.)

Before start

Always wear proper PPE during experiment (gloves, eyewear, lab coat, proper clothing)

Preparation

1h 20m

1 **Prepare LIG electrodes**

1h

- Use protocol for LIG fabrication to prepare a batch of electrodes (**link here**).

Note

Wear eyewear protection, gloves, and a lab coat during all experiments.

Expected results from step 1:

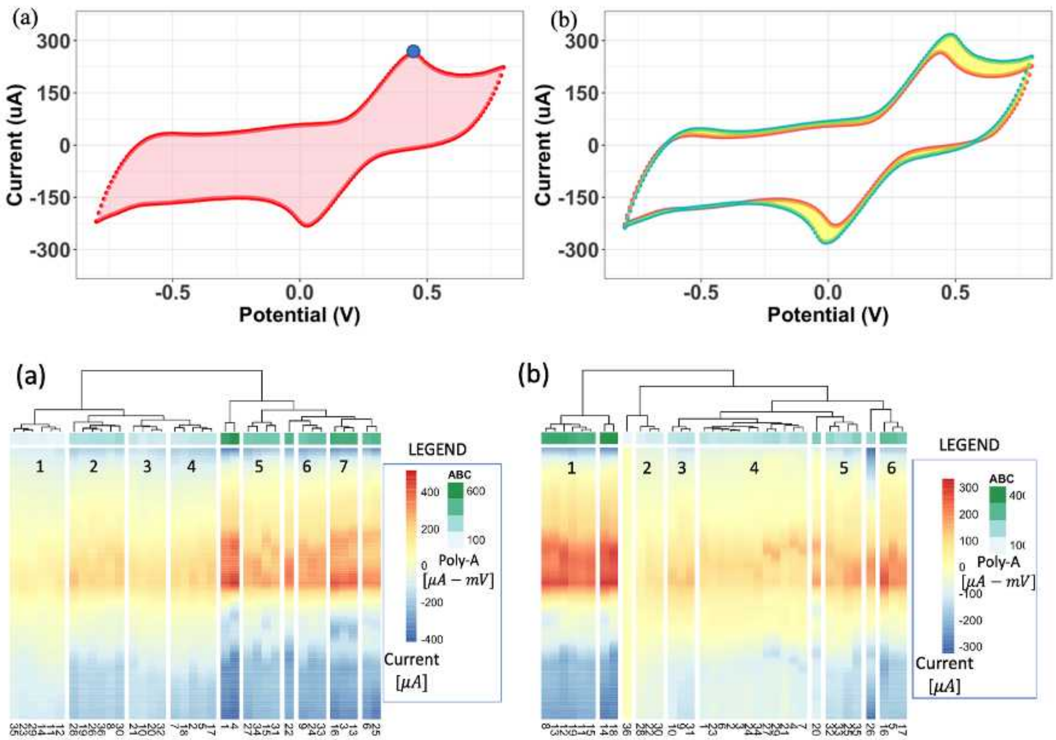
- **Fig 1** shows photographs of the instrument and the expected results from a batch fabrication of six replicate electrodes based on the LIG fabrication protocol here

Figure 1. LIG fabrication protocol used to produce a batch of electrodes.

Note

Store electrodes in a sealed Petri dish or small container until ready for analysis. Be careful not to place heavy or sharp objects on top of the LIG electrodes

- For quality control process to prepare large batches of LIG electrodes, see Qian et al (2024) (**link**). As an example, Fig 2 shows a heat map from the auotmated batch clustering tool.



- **Figure 2**. Example of output from quality control algorithm by Qian et al (2024). (top) Representative CV showing area between curve (ABC). (bottom) Heat map for replicate electrode selection. Dark red/green images are grouped together as replicates by the dendrogram at the top of the image.

2 **Prepare solution** 20m

- Gather a sealed glass storage bottle or Falcon tube with at least 50 mL of capacity
- Label as 4mM $[Ru(NH_3)_6]Cl_2$ + 100mM KNO_3
- In a separate weigh boat/paper, prepare the following:
  1-Weigh 54.6 mg hexamine-ruthenium chloride on a scale.
  2-Weigh 505.5 mg potassium nitrate on a scale.
- Add the potassium nitrate to the labeled glass bottle.
- Fill with 25 mL of DI and stir/agitate well until solution is dissolved
- Add the hexamine-ruthenium chloride.
- Fill with an additional 25 mL of DI (50mL total volume) and stir/agitate well until solution is dissolved
- Transfer 20 mL of the solution to the electrochemical cell (20 mL cell shown in **Tang et al protocol)**

Note

Although this protocol uses KNO3, other electrolytes may be used. In general, the electrolyte concentration should generally be greater than 10 μM to avoid migration effects. The electrolyte concentration has an optimum window that should be studied carefully [8].

Electrochemical testing

30m

3 **Connect electrodes**

5m

Assemble electrochemical cell (Benchtop Palmsens4 system):

Single electrode system

- Connect the single LIG electrode with the potentiostat via the bonding pads using alligator clips
- Ensure the electrodes are not touching, and the distance between the auxiliary and working electrodes is consistent.
- **Tang et al protocol** shows the correct assembly of the electrochemical cell for a single LIG "dip-style" electrode, where a Ag/AgCl reference electrode and Pt wire are used as reference and counter electrode, respectively

Note

The distance between working and reference electrode is an important factor in performance as discussed by Bentley et al. Thus, consistency is critical and care should be taken when using this type of electrochemical cell.

4 **Conduct scan (PalmSens4 benchtop system)**

25m

Note

To ensure that electrodes are positioned correctly (particularly dip-style single electrodes), a QuadHands workbench or similar setup is recommended.

- Turn on Palmsens hardware and the computer.
- Open the PalmSens software (MultiTrace4 software).
- Users may operate 3 channels simultaneously or individually
- Select Cyclic Voltammetry as the Technique.
- After selecting the mode, set up all parameters. For example, see settings below (see MultiTrace manual for details; **link here**)

Note

Cyclic Voltammetry settings
- t equilibration: 3s
- E begin: -0.8 V
- E vertex1: 0.8V
- E vertex2: -0.8 V
- E step: 0.01V
- Scan rate: 0.2 V/s
- Number of scans: 10

- Parameters may be modified based on experiment needs. For example, when conducting experiments to determine the electroactive surface area (ESA), at least four scan rates are suggested. See references for details
- Note that the potential window is reversed from **Tang et al protocol**, since the redox probe here is positively charged.

Expected results from PalmSens4 benchtop potentiostat
- **Fig 3** shows the channels connections to the PalmSens, mode selection panel, and operation panel.
- When the experiment finishes, click export to excel icon to export data.
- When exporting data, make sure all channels' data are exported.

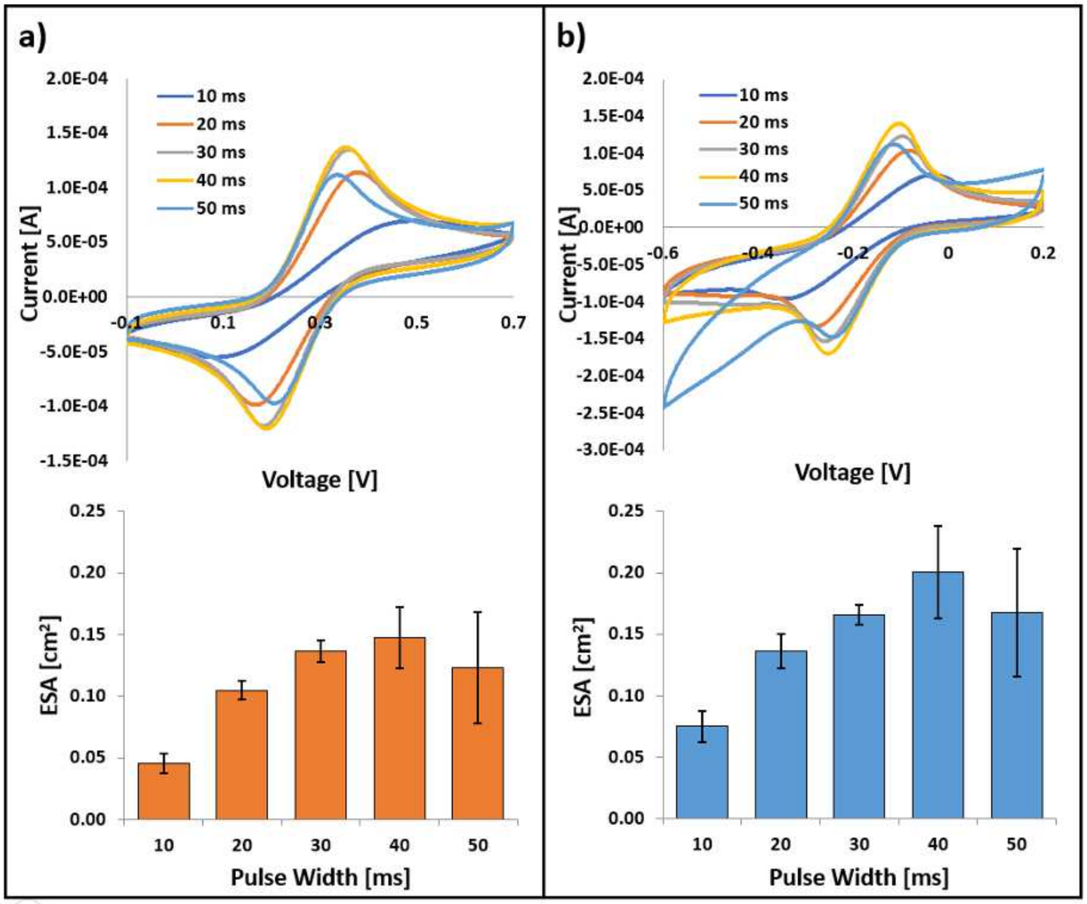


Figure 3. Results of CV pulse width analysis on bare LSG. The columns show the representative cyclic voltammorgrams of bare LSG electrodes carved at varying pulse widths at a 100 mV/s scan rate as well as the average ESA for each pulse width in (a) the negatively charged ferrocyanide probe and (b) the positively charged ruthenium probe.

Clean up

5 **Clean workspace (Timing: 5 min)**

- Ensure chemicals are stored or discarded correctly, and turn off Palmsens and the computer.
- If using external reference and auxiliary electrodes, clean them up with DI water and store them in the appropriate place. Reference electrodes should be stored with a 3M KCl solution.
- Wipe the working desk with 75 % alcohol.
- Store electrodes when not in use.

Protocol references

Qian, H., Moreira, G., Vanegas, D. *et al.* Improving high throughput manufacture of laser-inscribed graphene electrodes via hierarchical clustering. *Sci Rep* 14, 7980 (2024). https://doi.org/10.1038/s41598-024-57932-z

Hills, K.D., D.A. Oliveira, N.D. Cavallaro, C.L. Gomes, E.S. McLamore (2018). Actuation of chitosan-aptamer nanobrush borders for pathogen sensing. Analyst, 2018,143, 1650-1661. DOI: 10.1039/C7AN02039B

Oliveira, D.A., McLamore, E.S. & Gomes, C.L. Rapid and label-free *Listeria monocytogenes* detection based on stimuli-responsive alginate-platinum thiomer nanobrushes. *Sci Rep* 12, 21413 (2022). https://doi.org/10.1038/s41598-022-25753-7