Nov 01, 2022

Microwave Synthesis of Lanthanum-Doped Carbon Dots

Samuel Holberg[1], Addy Cullen[2], Chih-Yu (Jill) Lee[1], Destiny Caulder[3], Geisianny AM Moreira[4,5], Eric S McLamore[4,5,6]

[1]Biosystems Engineering and Earth Sciences, Clemson University, USA;

[2]Biology Department, College of William & Mary, USA;

[3]Department of Biological Sciences, Clemson University, USA;

[4]Department of Environmental Engineering and Earth Sciences, Clemson University, USA;

[5]Global Alliance for Rapid Diagnostics, Michigan State University, USA;

[6]Agricultural Sciences Department, Clemson University, USA

This protocol is published without a DOI.

Clemson University SNAPS research group

Eric S McLamore
Clemson University, North Carolina State University

DISCLAIMER

Any opinions, findings, and conclusions or recommendations expressed in this piece are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the National Institute of Food and Agriculture.

ABSTRACT

This protocol describes the synthesis of lanthanum-doped carbon dots using microwave irradiation. The complete process requires approximately 1h 50 min to complete (including baseline analysis). For this protocol, an Anton-Parr microwave reactor (Monowave 50p) was used throughout. The application example is for water quality sensing, although we have used the particles for cell imaging in other studies. ADA compliant procedures are used throughout the protocol (including both building lab space/design and analysis of synthesized particles).

PROTOCOL CITATION

Samuel Holberg, Addy Cullen, Chih-Yu (Jill) Lee, Destiny Caulder, Geisianny AM Moreira, Eric S McLamore 2022. Microwave Synthesis of Lanthanum-Doped Carbon Dots. **protocols.io** https://protocols.io/view/microwave-synthesis-of-lanthanum-doped-carbon-dots-cih7ub9n

FUNDERS ACKNOWLEDGEMENT

National Science Foundation Science and Technology Center
Grant ID: CBET-2019435

National Institute of Food and Agriculture
Grant ID: NC1194

KEYWORDS

carbon dots, nanoparticle, lanthanum, graphene, microwave, synthesis, graphitization

CREATED

Oct 28, 2022

LAST MODIFIED

Nov 01, 2022

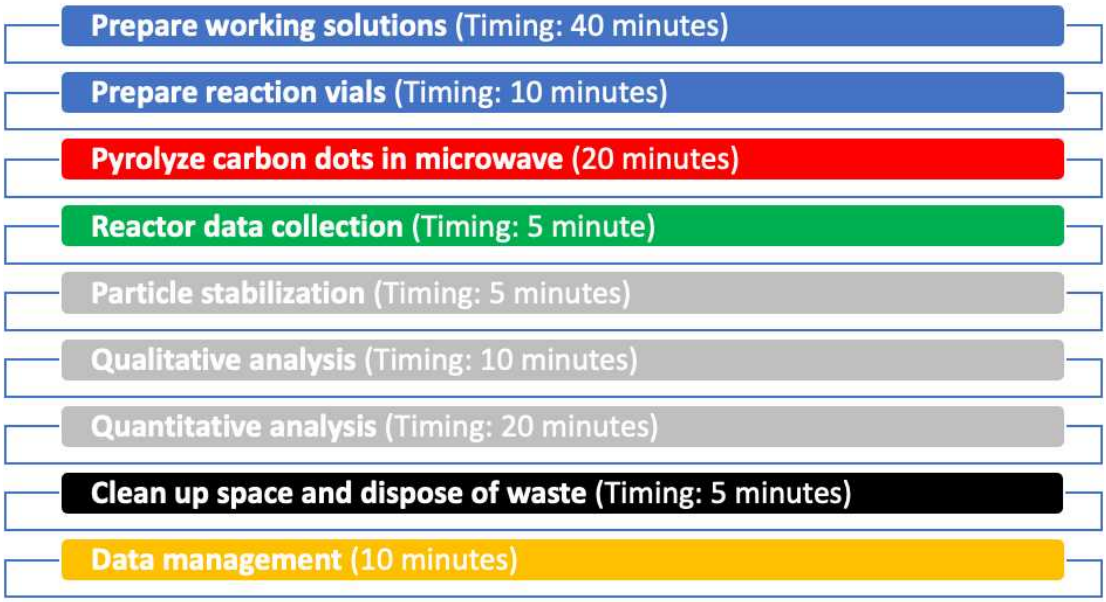
PROTOCOL INTEGER ID

71967

GUIDELINES

This protocol describes the synthesis of lanthanum-doped carbon dots using microwave irradiation. The complete process requires approximately 1h 50m to complete (see diagram for overview of steps).

A link to the Monowave Resource Manual with additional basic information on the instrument is here (link).



Process flow for synthesis of lanthanum-doped carbon dots in microwave reactor. The steps are separated into sections: preparation (blue), particle synthesis (red), stabilization and storage (green), data collection (grey), and cleanup (black). Protocols for data management are also provided.

MATERIALS TEXT

MATERIALS

- D-glucose. Safety Data Sheet for D-glucose (link is here)
- La_2CO_3. Safety Data Sheet for La_2CO_3(link is here)
- Hydrochloric acid (HCl; 6M). Safety Data Sheet for HCl (link is here)
- MES buffer. Safety Data sheet for MES (link is here)
- Potassium chloride. Safety Data sheet for MES (link is here)
- Anton Paar glass reactor vessels (link is here)
- Glass vials with cap (link is here)
- Parafilm (link is here)

HARDWARE

- Chemical hood
- Anton-Parr microwave reactor (Monowave 50p) (link is here)
- UV pen (link is here)
- UV pen (395nm) (link is here)
- Ocean Optics spectrometer (link is here)

SOFTWARE

- Optional: Color Name AR app (link here), colorimeter or (link here), or spectroradiometer (link here)
- Ocean View (link here)

SAFETY WARNINGS

SAFETY

General

- Lab coat, gloves, and closed-toed shoes are mandatory
- Safety issues specific to Monowave 50p
- The maximum pressure for the glass vial is 20 bar
- The maximum volume in the glass vial at any time is 6 mL

Eye protection

- Goggles or eye protection is required when handling acidic solutions outside of a chemical hood (i.e., transport in lab).

Skin

- Immediately rinse under water and wash with soap for at least 5 min

Fumes/aerosols

- Any open containers should be processed/handled under a chemical hood.
- All acidic solutions should be covered with parafilm when outside chemical hood
- The La_2CO_3solution is solubilized in HCl and off-gases CO_2.
- Aerosolization of particles (nano or colloidal) and subsequent health effects are unknown, and samples should be treated as hazardous and capped

Heat and Flammable materials

- The Monowave heats to temperatures as high as 250°C and the undersurface of the instrument may be very hot. Do not touch the undercarriage at any time.
- The instrument panel and handle for the cover are safe to touch when the reactor has cooled to 50°C (default instrument safety standard).

Disposal

- Vials of carbon particles should be discarded in the waste container and not disposed of in the sink.

ADA COMPLIANCE

The following guidance is summarized from Perry and Baum [1] where relevant to this protocol.

1) General building codes for laboratory

- Minimum 2 Exits for labs ≥500 sf (150 sm) 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.

2) Egress for wheelchair 360° Turn is 1.5 m (5 ft) clearance. Wheelchair clearance must be provided for:

- 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

3) Standard accommodations for use of chemical hood or other exhaust air containment 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.)

References

1.Perry, J. & Baum, J. Assessing the Laboratory Environment. in *Accessibility in the Laboratory* vol. 1272 3–25 (American Chemical Society, 2018).

DISCLAIMER:

> Any opinions, findings, and conclusions or recommendations expressed in this piece are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the National Institute of Food and Agriculture.

BEFORE STARTING

- Be sure to wear appropriate safety PPE throughout (lab coat, gloves, eyewear).
- Electronic or physical lab notebook may be used throughout
- See experimental plan guide for tips on planning your work

SECTION 1) Preparation 50m

1 Prepare Solution 1 (200 mM D-glucose) 15m

- Prepare a sealed plastic or glass container that has at least **10 mL** of capacity and label as 200mM D-glucose
- Weigh **0.721 g** of D-glucose and solvate in **200 mL** of DI/nano-pure water
- Mix solution and look carefully to ensure there are no precipitates
- Cover with Parafilm
- Heat in microwave for **00:01:30** to expedite solvation
- Shake/stir well and look carefully to ensure there are no precipitates
- Seal vial immediately (contamination is a common problem)

2 Prepare Solution 2 (2.5 mM KCl) 5m

- Prepare a sealed container with 1L capacity and label as 2.5 mM KCl
- Weigh **0.1864 g** and solvate in **1 L** of DI/nano-pure water
- Mix and inspect carefully to ensure there are no precipitates
- Seal vial

3 Prepare Solution 3: (100mM MES buffer) 5m

- Prepare a sealed plastic or glass container that has at least 1L of capacity and label as 100mM MES buffer
- Prepare a solution of **0.1 Molarity (m)** 2-(N-morpholino)ethanesulfonic acid (MES buffer). This buffer has a pK_a of 6.15 at 25°C; MW=195.2 g/mol (link to Good's buffers)
- For a **1 L** buffer solution, weigh **19.52 g** of MES in a plastic weigh boat
- Transfer the MES powder from the weigh boat to the labeled storage container
- Add **1 L** of DI water or nano-pure water
- Mix solution and look carefully to ensure there are no precipitates
- Check the pH of the solution (should be approximately **6.2**).
- Seal vial

4 Prepare solution 4 (200mM La_2CO_3) 15m

- Prepare a sealed plastic or glass container that has 9.9mL of MES buffer and label as MES+200mM La_2CO_3
- Prepare solution of [M] **6 Molarity (M)** HCl (see guidance here)

> 6M HCl should be handled under the chemical hood at all times. When storing under the chemical hood after use, be sure to place inside a secondary container to avoid spill accidents. The pH of HCl is 1.5 to 2.0

- Weigh **0.916 g** of La_2CO3 in weigh boat capable of holding 1mL of fluid
- Transfer the weigh boat and container to the chemical hood
- Pipette **0.1 mL** of HCl (6M) into the petri dish and carefully agitate the dish until all powder is dissolved
- Carefully transfer the solvated La_2CO_3 solution to the container
- Mix solution and look carefully to ensure there are no precipitates
- Calculate the theoretical pH according to Henderson/Haselbalch equation:

$$pH = 6.15 + log\left(\frac{moles_{MES} - moles_{HCl}}{moles_{MES} + moles_{HCl}}\right)$$

Equation 1. Calculation of pH using Henderson/Haselbach equation for mixture of MES and HCl.

- Check the pH of the solution (should be approximately pH **5.5**), or within 2% of calculation based on pKa
- Seal vial

5 Prepare reaction vials

5m

- Check vial cap and Teflon liner to make sure neither is damaged, and both are clean (**Fig 2A-B**)

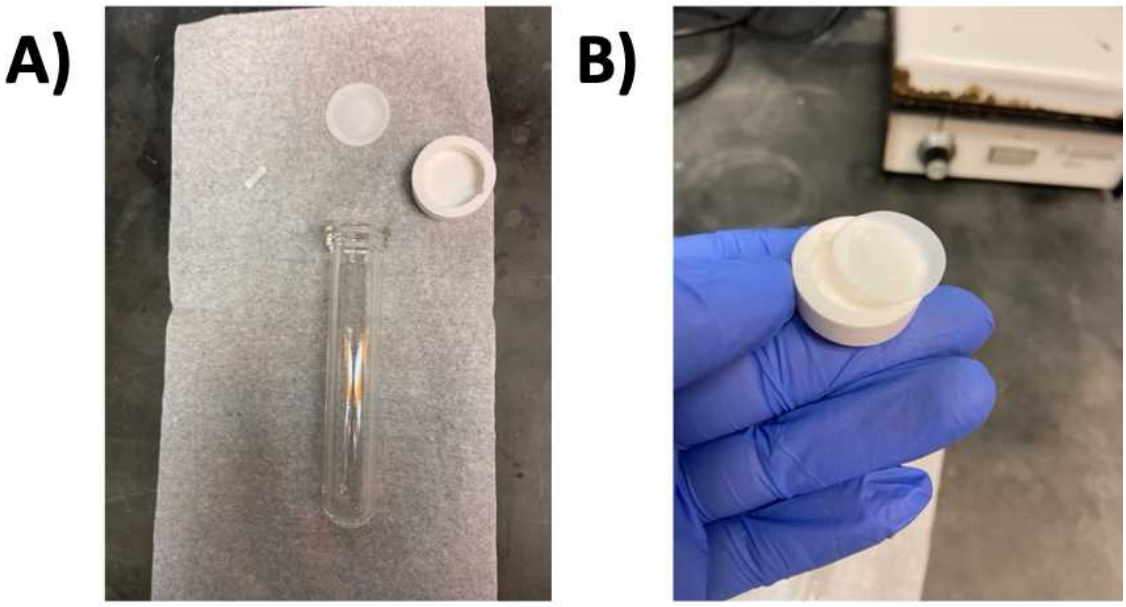


Figure 2. Reaction vials for monowave. **A)** Check vial cap and **B)** Teflon liner. Both the cap and liner should be free of damage and clean.

Critical step: If Teflon liner is damaged, or if reaction vial is capped, dispose and replace with a new cap/liner. If there is damage to the cap that is not observed, solutions will likely be "over cooked", see **Figure 5A**.

6 Add solution to reaction vial

5m

- Pipette **3 mL** of **0.2 Molarity (m)** D-glucose solution to a glass reactor vial
- Add a nano stir bar to the reactor vial
- Add **3 mL** of the La_2Co_3 + buffer solution to the reactor vial
- Place Teflon liner in cap
- Seal reaction vial with cap
- Invert vial three times to mix solutions
- Place in test tube rack

Critical step: Be careful not to lose the nano stir bars, they are the most frequently misplaced item in this protocol.

SECTION 2) Particle synthesis 20m

7 Set up microwave reactor

20m

- Ensure that Anton-Paar reactor is in chemical hood and plugged in to the power outlet (**Fig 3A**)
- If not placed in the chemical hood, the institutional safety committee must approve of the exhaust hose setup (using either a snorkel exhaust and/or valid tube/hose system)
- Open lid and place glass reactor in Anton-Paar reactor chamber (**Fig 3B**)
- Close chamber lid and seal shut with handle

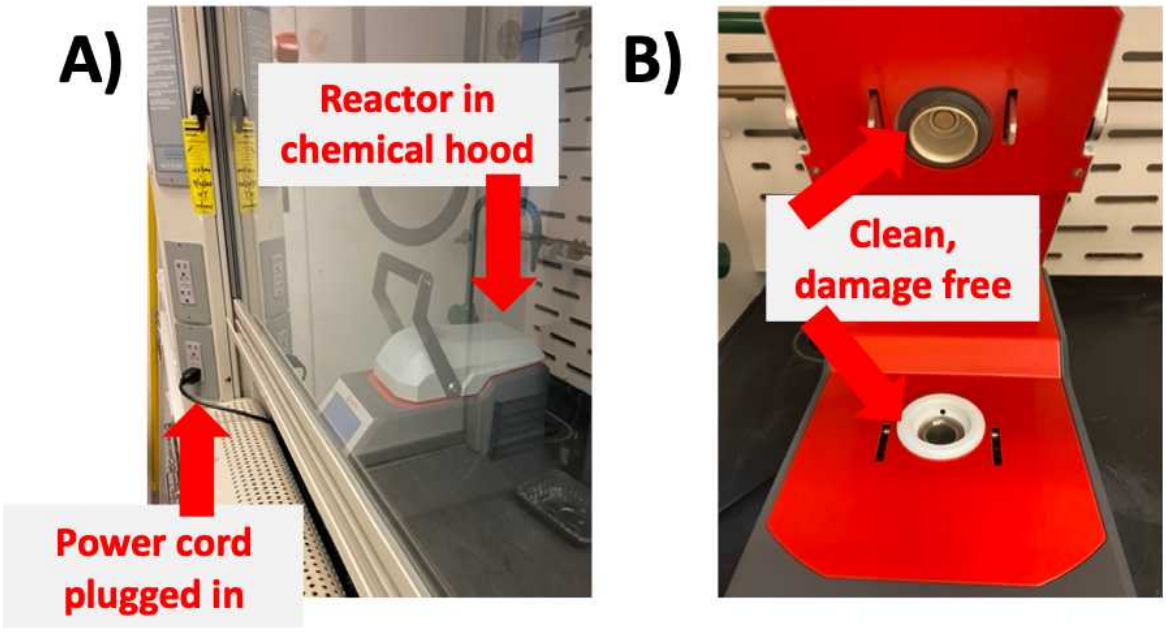


Figure 3. Microwave reactor (Anton-Paar Monowave) for pyrolysis/graphitization of carbon dots. **A)** Ensure reactor is in chemical hood and plugged in to power outlet.**B)** Open lid showing that lining is not damaged or dirty.

- Select LaCD settings in the "Run by Methods" (**Fig 4**) by pressing the Menu button
- Press Menu
- Press Methods button
- Choose LACD1.1
- Click Done
- Click back

Critical step: When running for the first time, LACD1.1 will need to be created. Create a method that has a maximum set temperature of 170 °C, a time to ramp as 5 minutes with a hold time of 1 minute.

- Choose Run Name
- In the text box, name the file as "LACD-today's date" (**Fig 4**)
- Prior to starting the Monowave, make sure that the settings are correct and have not been

changed

- Maximum set temperature= **170 °C**
- Time to ramp setting= **00:05:00**
- Hold time= **00:01:00**

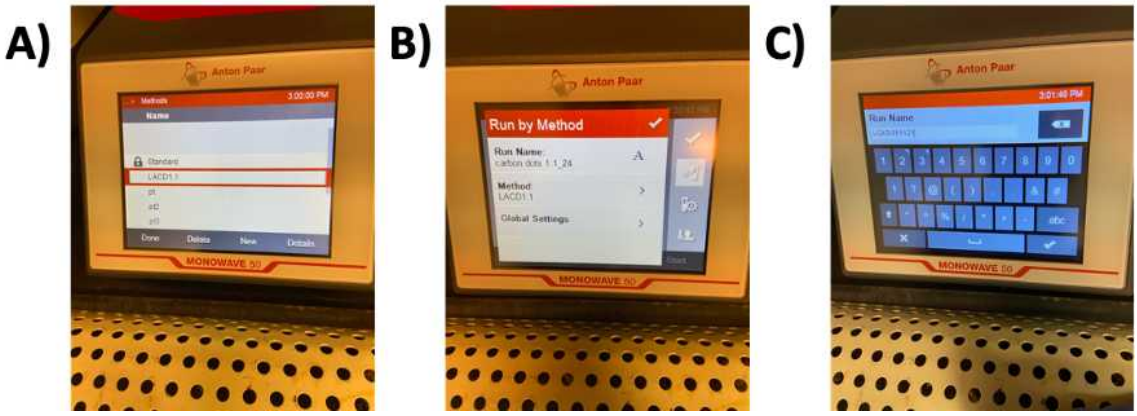


Figure 4. Photos of Monowave 50 reactor touch screen used for setup. **A)** Select LACD1.1 method. **B)** Choose "Run by Method**"**. **C)** Enter File name for saving data.

- Click run
- This protocol is similar to other lanthanum-doped carbon dots in the literature and has a variety of uses from cell labeling to water quality analysis (REF 1-3).

SECTION 3) Reactor data collection 5m

8 Download reactor temp/pressure profile (Timing: 5 minute) 5m

- Insert USB to port on right side of Monowave 50 p
- Press "Details"
- Press "Export"
- Choose the Export option, press "OK" when done
- Remove USB

SECTION 4) Stabilization and analysis (post cook) 35m

9 Stabilize particles in buffer 5m

- Prepare a sealed plastic or glass container that has **10 mL** of capacity and label (Lanthanum-doped carbon dots + KCl).

> **Critical step**: Be careful not to lose the nano stir bars, they are the most frequently misplaced item in this protocol.

- Mix the 6mL of pyrolyzed solution with **4 mL** of **0.0025 Molarity (m)** KCl. This results in a final concentration of 1mM KCl + LACD

Note: If the recipe is altered, the storage buffer must be optimized. For this protocol, we used Zeta potential as an indicator of particle stability (analyzed daily over a 2 week experimental study)

- Inspect solution carefully for any colloids or particulate.
- Seal vial

10 Qualitative analysis (color)

5m

- The first step of qualitative analysis is visual inspection of the solution color
- Analysis of polychromatic visible (VIS) color is an important quality control mechanism in particle synthesis (**Fig 5**)

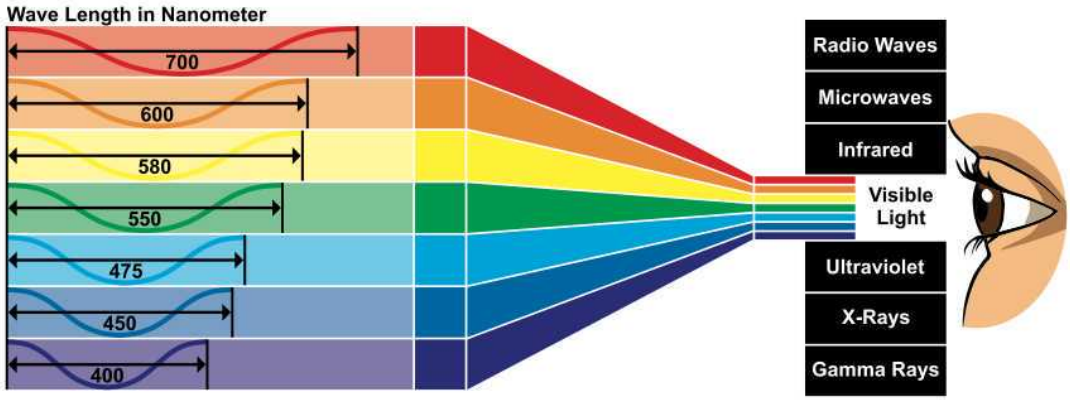


Figure 5. Visual inspection is an important quality control mechanism in particle synthesis. If visual inspection is not possible,, a colorimeter or other device may be used to visually inspect the samples (see note below for example of mobile phone app). Image courtesy of ShutterStock standard license (no. 514067857)

If preferred, a cell phone app may be used to detect the color of the sample. See **Fig 7**.

For example, Color Name AR is a useful tool that is available for both iPhone and Android (as well as tablets) (https://apps.apple.com/us/app/color-name-ar/id906955675)

- Observe the glass vials under white light (common lab lighting)
- Representative photos of "as prepared" particle solutions are shown in **Fig 6**.
- The images depict a “burned sample” (**Fig 6A**), “undercooked sample” (**Fig 6B**) and an optimum sample (**Fig 6C**)
- In addition to visual inspection, UV-VIS may be used to analyze the samples

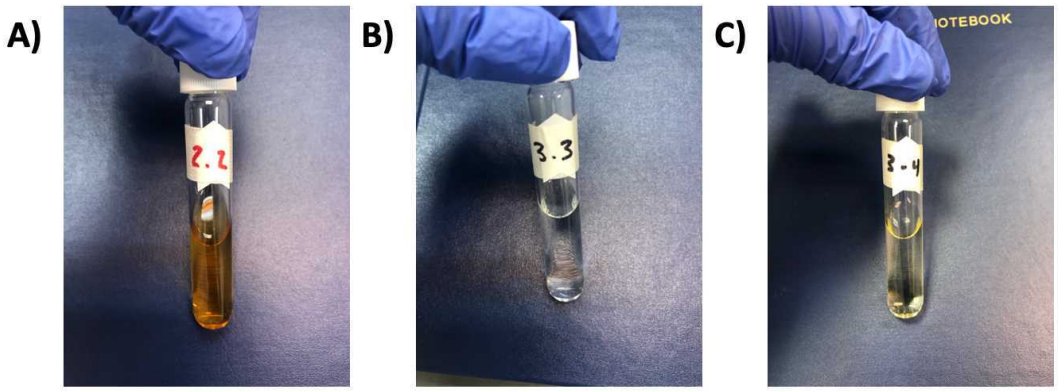


Figure 6. Representative photos of lanthanum-doped carbon dot solutions after microwave reaction. **A)** Sample with dark brown color that has been "burned" by either: reaction settings incorrect, contamination of the sample with a strong acid, or other problems. **B)** Sample that underwent insufficient reaction (clear color). **C)** Sample that is the optimum color for this protocol (based on extensive spectroscopy and material analysis).

- Representative results of using a mobile phone app for identifying color are shown below (**Fig 7**).
- The same photos as shown in **Fig 6** are analyzed using an iPhone app, providing RGB and CMYK color space data for the identified color.

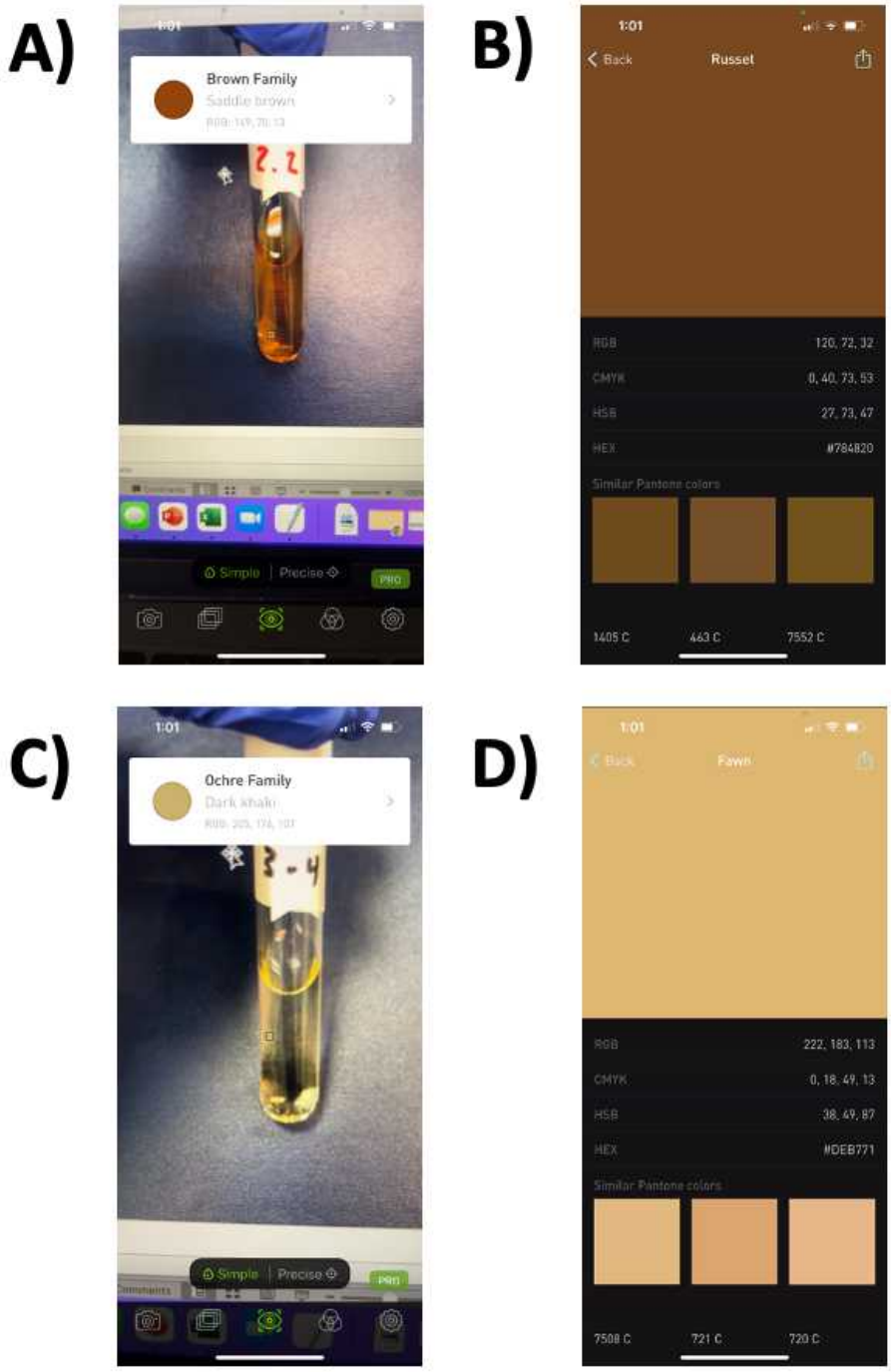


Figure 7. Analysis of solution color using an iPhone app (Color Name AR). **A)** Burned sample with dark brown color and **B)** associated color identification using Color Name AR (identified as Russet; RGB 120, 72, 32). **C)** Optimum sample for this protocol with tea color and **D)** associated color identification using Color Name AR (identified as Fawn; RGB 222, 183, 113).

5m

11 Qualitative analysis (fluorescence emission)

- Using either low light conditions or a prepared light box for analysis (e.g., black styrofoam),

position a 395 nm UV pen perpendicular to glass storage vial,

- Turn on pen and inspect for an emission beam
- **Fig 8A** shows an example of fluorescence for a sample following the protocol described here
- This rapid analysis is useful for quality control immediately after synthesis of particles, but should be supported by subsequent quantitative analysis (shown in step below).

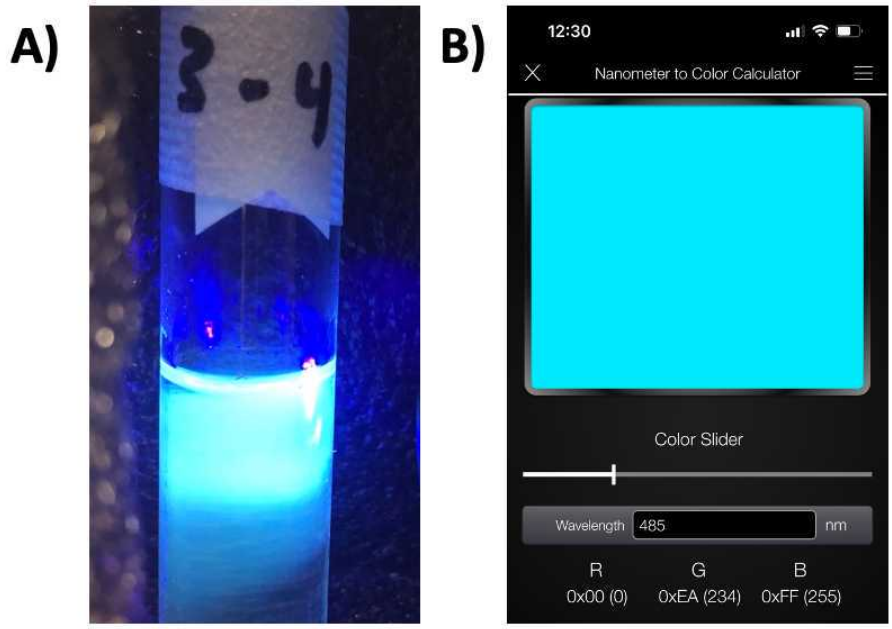


Figure 8. Reference comparison for UV pen fluorescence check of carbon dots. **A)** Photo of carbon dot solution in light box under 395nm excitation using UV pen. **B)** EE Toolkit used for analysis of emission signal. Based on visual observation, an emission peak of approximately 485 nm was detected using EE Toolkit (https://ee-toolkit.com/). This emission is represented by (0,234,255) in the RGB color space.

12 Quantitative analysis (fluorescence emission)

20m

- If available, use a spectroscopy instrument to analyze the emission spectra under UV excitation
- **Fig 9** shows a representative experiment with a 370nm excitation source (fiber optic spectrometer from Ocean Optics; Ocean HR2 UV-VIS)
- The figure shows changing fluorescence emission during addition of a quenching molecule
- Three peaks are identified (denoted as λm_1, λm_2, λm_3), each with a different characteristic response to increasing concentration of the quencher molecule.

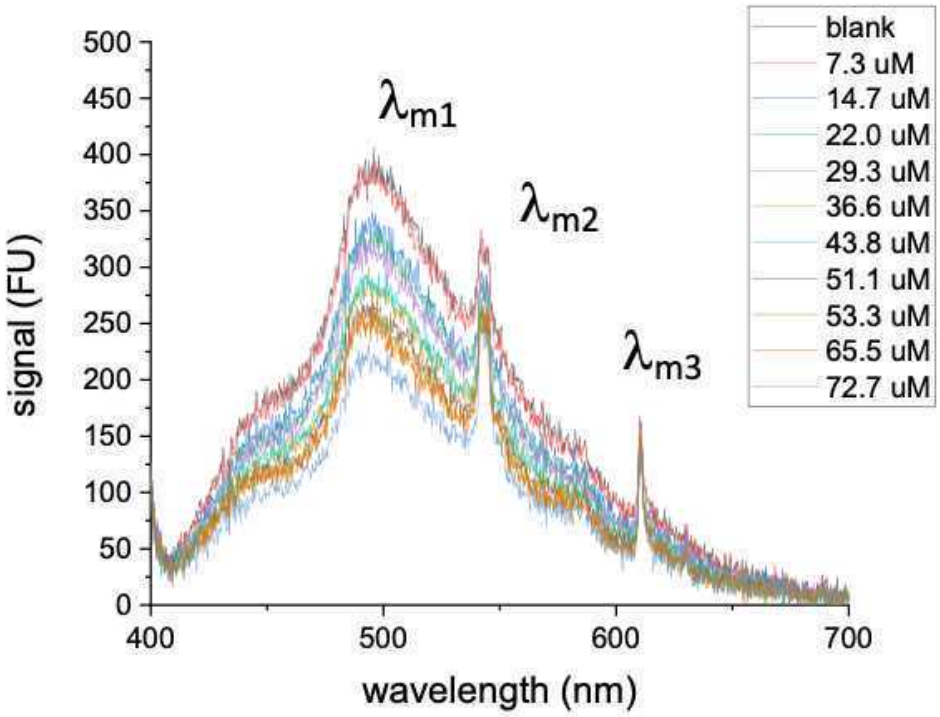


Figure 9. Representative UV-VIS emission under an excitation of 370nm using a fiber optic spectrometer. One broad peak and two distinct peaks are noted (denoted as λ_{m3}, λ_{m2}, λ_{m3}),. The curves represent characteristic response to increasing concentration of the quencher molecule.

- When analysis is complete, place LACD in dark for up to 2 weeks (shelf life currently unknown; 2 weeks is conservative estimate)

SECTION 5) Cleanup 10m

13 **Clean up space and dispose of waste** 10m

- Turn off Monowave and unplug it from power outlet
- Dispose of used chemicals according to the lab safety plan

Note: There is a dedicated chemical disposal container for carbon dots in the waste area.

- Wash all glass reactors with mild detergent and warm water. A bottle brush may be required to remove carbon residue from the inner wall of glass vials
- Carefully remove the Teflon lining from the cap and wash with mild detergent and warm water

oIf the cap or the Teflon lining is damaged, they should be discarded according to the lab safety plan.

Note: Be sure to clean up the chemical hood and process pipette tips or other materials used for handing HCl prior to discarding them according to the safety plan (otherwise they contain acid residue

SECTION 6) Data management 5m

14 5m

- Data management (reactor data only)
- File naming: For saving all versions of this protocol, use the following file structure: LACD1.1_(insert date here).ai
- File storage: Store all methods in the Desktop folder.
- Backup files: At least once per year, ensure that the folder is backed up on the lab external hard drive.
- Note: data management for spectroscopy data is covered in a separate protocol.