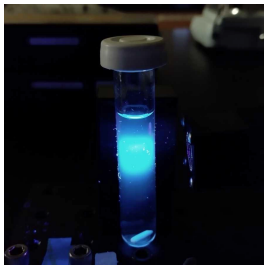
Jun 19, 2024 Version 1

Mycrowave synthesis of low molecular weigth deacylated chitosan V.1



Forked from Microwave Synthesis of Lanthanum-Doped Carbon Dots

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We use this protocol, and it's working

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Abstract

This protocol describes the synthesis of low molecular weight deacylated chitosan using microwave irradiation. The process requires approximately 2.5 hours (including baseline analysis).

An Anton-Parr microwave reactor (Monowave 50p) was used throughout this protocol. ADA-compliant procedures are used throughout the protocol (including building lab space/design and analysis of synthesized particles).

Guidelines

This protocol describes the synthesis of chitosan nanobrushes using microwave irradiation. The complete process requires approximately 2.5 hours (see diagram for overview of steps).

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

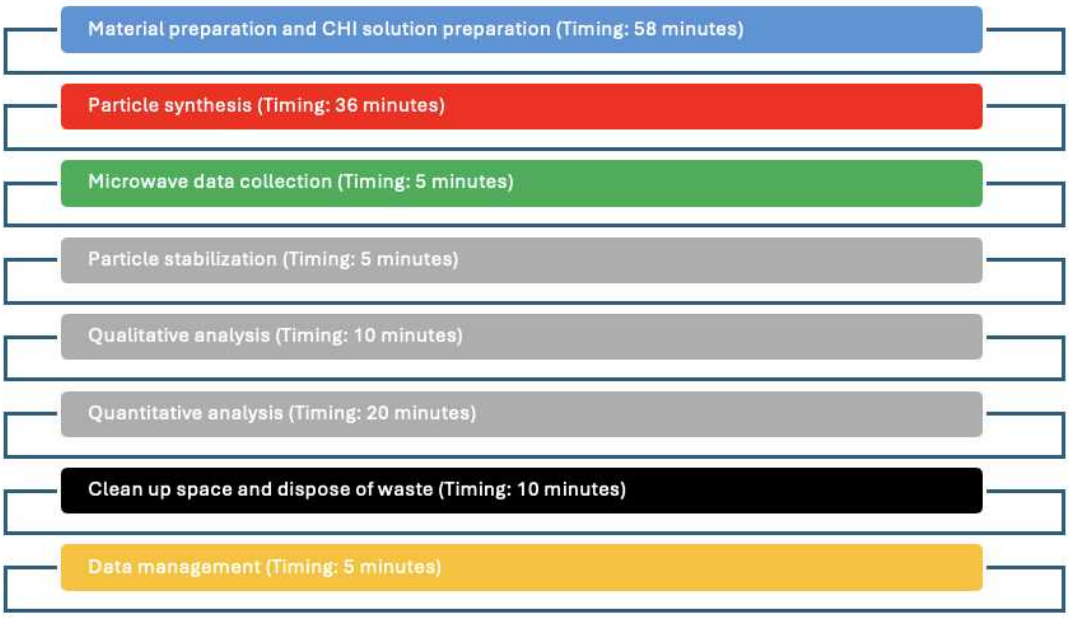

Figure 1. Process flow for the synthesis of nano brushes in a microwave reactor. The steps are separated into sections: preparation procedures (blue), particle synthesis, including the setup of the settings in the equipment (red), stabilization and storage of the solution (green), data collection (grey), and cleanup of the working station and dispose of waste (black).

Materials

MATERIALS

- Anton Paar glass reactor vessels (**link is here**)
- Glass vials with cap (**link is here**)
- Parafilm (**link is here**)
- Magnetic stirrer base (**link is here**)

HARDWARE

- Chemical hood
- Anton-Parr microwave reactor (Monowave 50p) (**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 a chemical hood (i.e., transport in the 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 the chemical hood.
- 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 instrument undersurface 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 by 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, dust 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).

Before start

- Wear appropriate safety PPE throughout (lab coat, gloves, eyewear).
- An electronic or physical lab notebook may be used throughout.
- See the experimental plan guide for tips on planning your work.

SECTION 1) Materials and solution preparation

58m

1 **Prepare Solution** (1% deacylated chitosan -0.05M Acetic Acid)

42m

- In a glass container or sealed plastic weight 2 g of Chitosan (CHI) 85% deacetylated and solvated in 98 mL of 0.1M of acetic acid, mix and look carefully to ensure no precipitates. Use the magnetic stirrer base to provide a mix of the solution.
- Add 49 mL of the previous solution and mix with 49 mL of DI/nano-pure water to obtain 1% deacylated CHI - 0.05M acetic acid solution. Use the magnetic stirrer base to provide a mix of the solution.
- Shake/stir well and look carefully to ensure there are no precipitates. Use the magnetic stirrer base to provide a mix of the solution.
- Seal the vial immediately (contamination is a common problem).

Note

Shelf life solution: The same solution can continue to be used for about one week of storage.

2 **Prepare reaction vials**

10m

- Check the vial cap and Teflon liner to make sure neither is damageddamage to the cap and both are clean (**Fig 1A-B**)

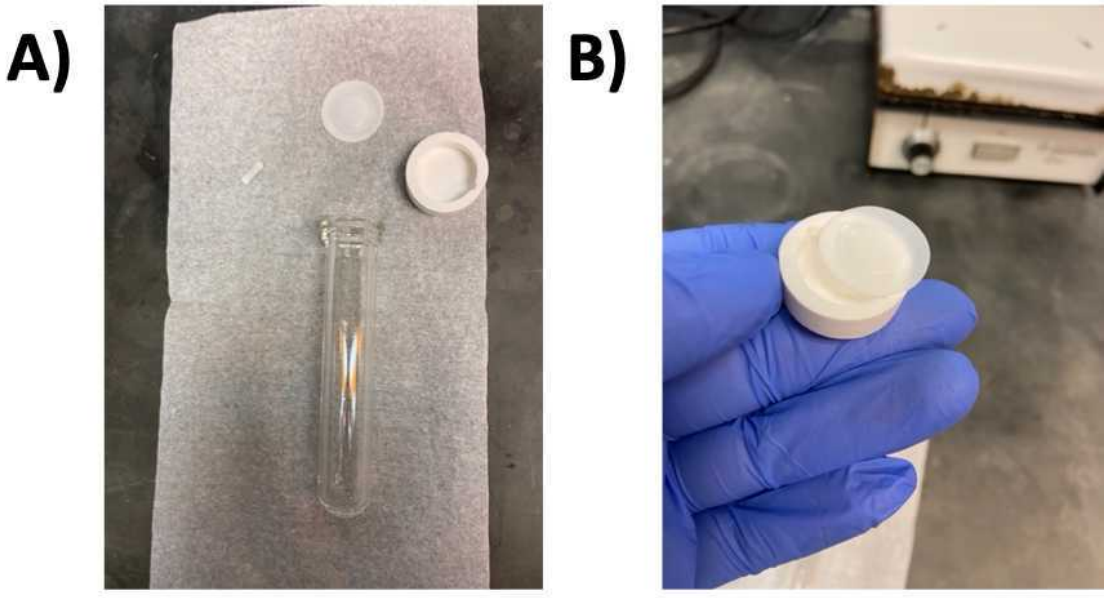

Figure 1. Reaction vials for monowave. **A)** Check vial cap and **B)** Teflon liner. Both the cap and liner should be free of damage and clean.

Note

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

3 **Add solution to the reaction vial**. 6m

- Pipette 8000 µL of 1% deacylated chitosan -0.05M acetic acid solution to a glass reactor vial.
- Add a nano stir bar to the reactor vial.
- Place Teflon liner in cap.
- Seal the reaction vial with cap.
- Invert the vial three times to mix solutions.
- Place in a test tube rack.

Note

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

36m

4 **Set up microwave reactor**.

26m

- Ensure the Anton-Paar reactor is in the chemical hood and plugged into the power outlet (**Fig 2A**).
- If not placed in the chemical hood, the institutional safety committee must approve the exhaust hose. setup (using either a snorkel exhaust and/or a valid tube/hose system).
- Open the lid and place the glass reactor in the Anton-Paar reactor chamber (**Fig 2B**).
- Close the chamber lid and seal it shut with the handle.

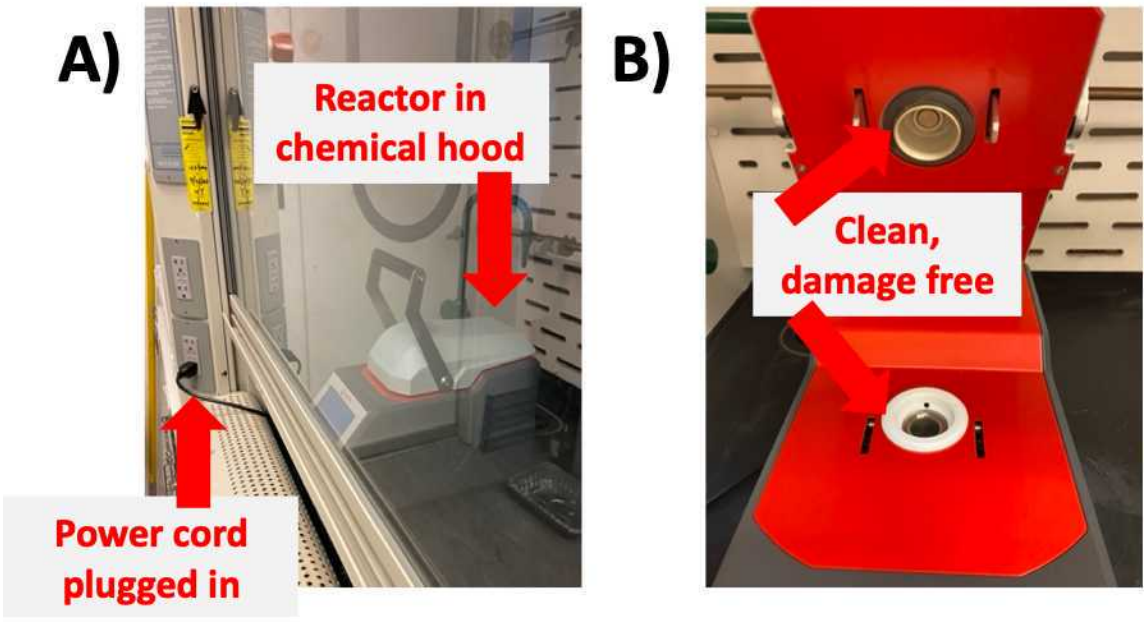

Figure 2. Microwave reactor (Anton-Paar Monowave) for hydrolysis of deacylated chitosan. **A)** Ensure the reactor is in the chemical hood and plugged into the power outlet. **B)** Open the lid to show the lining is not damaged or dirty.

- **Before starting the Monowave,** go to the third bottom option on the screen to ensure the settings are correct and have not been changed. Then, press "step type" and "ramp to temp" and click "✓".

Note

Critical step: When running for the first time, method must be created.

Create a method with the following settings:
- *Target cooling temperature: 50°C*
- *Stir speed: 600 rpm*
- *Maximum set temperature: 170 °C*
- *Time to ramp: 5 minutes*
- *Hold time: 1 minute.*

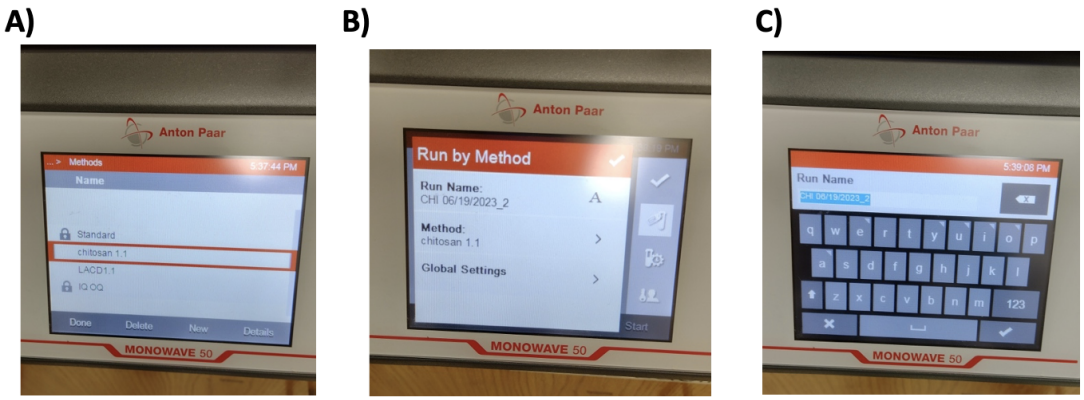

Figure 3. Photos of Monowave 50 reactor touch screen used for setup. **A)** Select the method. **B)** Choose “Run by Method.” **C)** Enter a file name to save data.

- Press “Run by Methods” section (**Fig 3**) by pressing the Menu button.
- Check the settings above (see note) and ensure “Run by Methods” is correct in the instrument (**Fig 3**).
- Press Menu
- Press the "Methods" required to complete button required to complete
- Choose the method, then "Done."
- Click back.
- Choose Run Name and name in the text box as your preference.
- Click "START".

Note

The time required to complete the synthesis of the chitosan nanobrushes is approximately **10 minutes.**

SECTION 3) Reactor data collection

5m

5 Download reactor temp/pressure profile

5m

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

SECTION 4) Stabilization and analysis (post cook)

35m

6 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 4**)

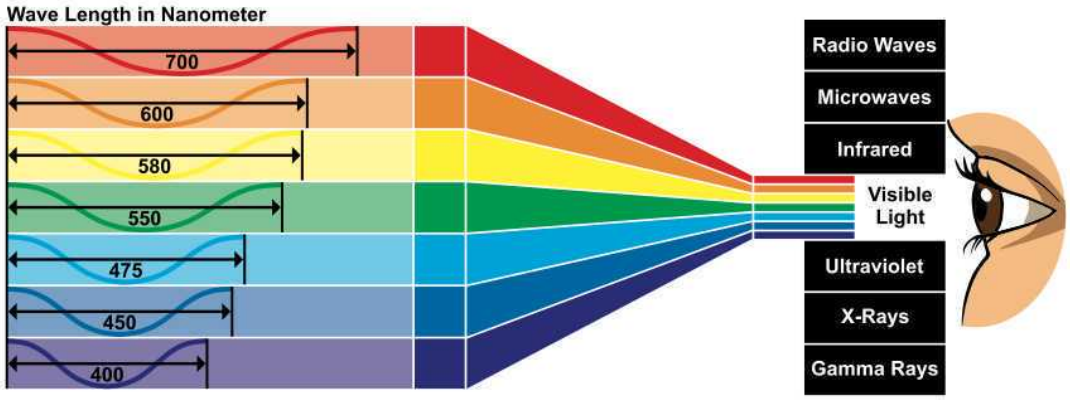

Figure 4. Visual inspection is an important quality control mechanism in particle synthesis. If visual inspection is impossible, a colorimeter or other device may be used to visually inspect the samples (see note below for an example of a mobile phone app). Image courtesy of Shutterstock standard license (no. 514067857)

Note

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

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 (valuable lab lighting)
- Representative photos of "as prepared" particle solutions are shown in **Fig 5**.

- In addition to visual inspection, UV-VIS may be used to analyze the samples.

7 **Qualitative analysis (fluorescence emission)** 10m

- Using either low light conditions or a prepared lightbox for analysis (e.g., black styrofoam), position a 395 nm UV pen perpendicular to the glass storage vial,
- Turn on the pen and inspect for an emission beam.
- **Fig 7A** shows an example of fluorescence for a sample following the protocol described here.
- This rapid analysis is helpful for quality control immediately after synthesizing particles but should be supported by subsequent quantitative analysis (shown in the step below).

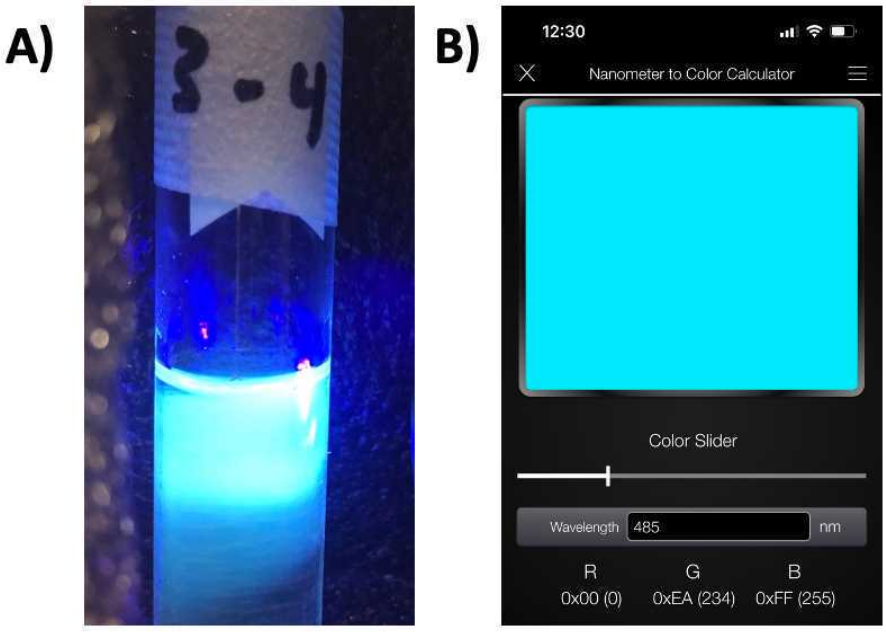

Figure 7. Reference comparison for UV pen fluorescence check of carbon dots. **A)** Photo of low molecular weight deacylated chitosan in a lightbox under 395nm excitation using a UV pen. **B)** EE Toolkit was used to analyze the emission signal. Based on visual observation, an emission peak of approximately 488 nm was detected using EE Toolkit (**https://ee-toolkit.com/**). This emission is represented by (150,222,209) in the RGB color space.

SECTION 5) Cleanup 10m

8 **Clean up space and dispose of waste**. 10m

- Turn off Monowave and unplug it from the waste area. It has a dedicated chemical disposal container for the chitosan outlet.
- Dispose of used chemicals according to the lab safety plan.

Note

Note: The waste area has a dedicated chemical disposal container for chitosan residues.

- Wash all glass reactors with mild detergent and warm water.
- Carefully remove the Teflon lining from the cap and wash with mild detergent and warm water.

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

Note

Note: Clean up the chemical hood and process pipette tips or other materials used for handling HCl before discarding them according to the safety plan (otherwise, they contain acid residue.

SECTION 6) Data management

5m

9 **Data management (reactor data only).**

5m

- File naming: For saving all versions of this protocol, use the following file structure:
  CHI1.1_(insert date here).ai
- File storage: Store all methods in the Desktop folder.
- Backup files: At least once per year, ensure the folder is backed up on the lab's external hard drive.