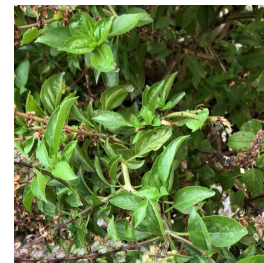


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# 🌱 Green Synthesis of Fluorescent Carbon Dots from Sweet Basil (*Ocimum basilicum*) Leaves via Hydrothermal Method



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Hugo Monreal-Contreras<sup>1</sup>, Manoj-Kumar Arthikala<sup>1</sup>, Ravichandran Manisekaran<sup>2</sup>

<sup>1</sup>Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores Unidad-León, Universidad Nacional Autónoma de México (UNAM), C.P. 37689 León, Guanajuato, México.;

<sup>2</sup>Nanostructures & Biomaterials, Escuela Nacional de Estudios Superiores Unidad-León, Universidad Nacional Autónoma de México (UNAM), C.P. 37689 León, Guanajuato, México.



**Hugo Monreal-Contreras**

Ciencias Agrogenómicas, Escuela Nacional de Estudios Superio...

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**We use this protocol and it's working**

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## Abstract

Carbon-based materials play significant roles in the development of material science. Carbon dots (CDs), a new rising star in the carbon family. Carbon dots (C-dots) have gained more attention among researchers due to their outstanding fluorescent property which can be tuned based on several factors like high photostability, excellent water solubility, tunable fluorescence and optical properties, high quantum yield (QY), low toxicity, good biocompatibility, and environmental friendliness. One among them is based on a carbon source, so scientists have widely investigated several synthetic and natural materials to produce C-dots for diverse applications. Among these approaches, hydrothermal/carbonization treatment is frequently applied for the preparation CDs because of the outstanding advantages, such as high yield, simple manipulation, easy control, uniform products, lower air pollution, low energy consumption and so on. In this protocol we intend to use the sweet basil, scientifically known as *Ocimum basilicum* leaves as a precursor to produce C-dots using water as a solvent by adopting the hydrothermal methodology. The green synthesis methods by means of green precursors of synthesis involves the usage of inexpensive or recycled materials, while the chemical synthesis methods involve toxic chemical reagents or organic solvents as precursors. The synthesized C-dots have been confirmed by their fluorescent image and UV-Visible spectrometer.


## Materials

### List of equipment's required

1. Magnetic stirrer
2. Heating oven
3. Sonicator
4. Centrifuge
5. UV light source
6. UV-Visble spectroscopy
7. Pestle & mortar
8. Sterile syringe filters
9. Quartz cuvette
10. Polytetrafluoroethylene (PTFE) lined stainless steel autoclave reactor





## Troubleshooting

## Step 1: Preparation of basil leaves

- 1 Freshly collected  Sample should be sorted first to ensure only healthy leaves are chosen for the C-dots synthesis.



**Figure 1.** *Ocimum basilicum* (Sweet Basil), depicting the plant at the developmental stage appropriate for the collection of fresh leaves.

- 2 Then it is washed thoroughly and rinsed with distilled water to remove the debris, dust or any other unwanted substance attached on to the leaf surface.
- 3 Excess water is decanted.
- 4 The leaves are allowed to dry at  60 °C ± 5 °C for a duration of  24:00:00 ± 2:00:00 in a heating oven with a temperature increment of  10 °C ± 2 °C /  00:01:00 .

1d 0h 1m



**Figure 2.** Dried *Ocimum basilicum* (Sweet Basil) Leaves Post Oven-Heating.

#### Note

To ensure homogeneous drying process it is recommended to separate the leaves and placed on a tissue paper.

- 5 Dried leaves are crushed to fine powder using pestle and mortar.

#### Note

This process plays an important role in the optical property of C-dots.

- 6 The resulting powder can be stored at  $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for less than a week.

#### Note

More than week time is NOT recommended for the synthesis.





## Step 2: Preparation of precursor solution

7 To prepare the precursor solution, deionized water can be used as a solvent.

8 1 g of powder is mixed with 10 mL of water and dispersed under magnetic stirring or sonication process.

### Note

The solution is better to prepare before initiating the synthesis instead of storing the mixed solution for longer duration.

### Note

Step 1 & 2 should be planned for at least 1 day in advance as the step 3 process is carried out for 10:00:00 .

## Step 3: Synthesis process


9 As this methodology uses hydrothermal process, a non-corrosive Polytetrafluoroethylene (PTFE) lined stainless steel autoclave reactor must be used.



**Figure 3.** Detailed Structure of a Non-corrosive Polytetrafluoroethylene (PTFE) Lined Stainless Steel Autoclave Reactor.


**Note**

PTFE chamber must be cleaned before the synthesis process.

- 10 The characteristic of the reactor – it should withstand a temperature of  $250\text{ }^{\circ}\text{C} \pm 30\text{ }^{\circ}\text{C}$  and an applicable pressure is  $3.0 \pm 1.0\text{ MPa}$  . 
- 11 Depends on the synthesis volume the reactor should be filled only 60–70 % capacity.
- 12 Then the precursor solution is added to the reactor completely.
- 13 Finally, the reactor should be tightened to its maximum limit.

**Safety information**

To avoid any accidents or breakage of the reactor, it is necessary the whole process is carried out under the supervision of experienced personal.

- 14 The whole setup is placed inside the oven which is pre-heated to  $180\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  for a period of  $10:00:00 \pm 00:30:00$  without any external disturbances. 10h 





**Figure 4.** Oven with the complete setup, pre-heated to  $180\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ , shown here during a strictly controlled experiment conducted over a period of  $10\text{ hours} \pm 30\text{ minutes}$ .

- 15 After the process, the reactor is allowed to be cooled down naturally inside the oven.




#### **Step 4: C-dots purification**

- 16 After completing the step 3, the resulting black turbid solution is transferred to the centrifuge tubes and C-dots are collected.

- 17 The solution is purified by centrifugation with  4000 rpm for  00:30:00  $\pm$  00:05:00 .

30m

- 18 Discard the pellet which is burnt residue of leaf powder, and the brownish solution is collected without disturbing the pellet at the bottom.

- 19 Later, to obtain pure C-dots the solution is centrifuged at  13000 rpm for  00:10:00  $\pm$  00:02:00 at   $8\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  .

10m





- 20 The solution collected from (step 4-19) is passed through 0.22  $\mu\text{m}$  pore size nylon sterile syringe filters.
- 21 The final solution is preserved at  $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for further use.

**Note**

Solution is stable for 8-10 months without contamination.

**Step 5: C-dots formation confirmation**

- 22 Qualitative testing 1 - The formation can be checked primarily by exposing the solution under the UV light to visualize the blue fluorescence.



**Note**

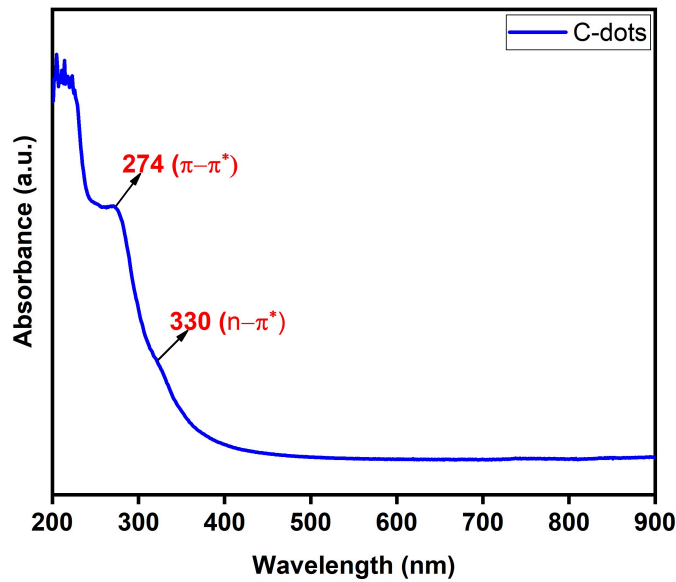
Dilution factor is proportional to the fluorescence intensity but not applicable for all C-dots.

- 23 The fluorescence of C-dots is shown in Figure 5 at 1:3 dilution.



**Figure 5.** Fluorescence under UV light: the left cuvette shows a non-fluorescent water control, while the right cuvette displays bright fluorescence of carbon dots (C-dots) at a 1:3 dilution.

- 24 Qualitative testing 2 - The C-dot solution can be diluted for example  500  $\mu\text{L}$  with  3 mL of water in a quartz cuvette and measured in UV-Visible spectroscopy without any background noise.
- 25 The UV-visible spectroscopy data is shown in Figure 6.



**Figure 6.** UV-visible spectra of synthesized C-dots.

## Application of C-dots:

26 The synthesized can be used for wide range of applications and are broadly classified into:

### 1. **Biomedical Applications**

- Bioimaging
- Drug delivery
- Photodynamic therapy

### 2. **Optoelectronic Applications**

- Light-emitting diodes (LEDs)
- Photodetectors
- Photovoltaic devices

### 3. **Sensing Applications**

- Biosensors
- Chemosensors
- Environmental monitoring



#### 4. **Security and Anti-Counterfeiting**

- a. Security inks
- b. Security labels
- c. Anti-counterfeiting coatings

#### 5. **Agricultural and Environmental Applications**

- a. Plant growth promotion
- b. Crop yield enhancement
- c. Soil remediation

## Protocol references

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