



# Synthesis of Carbon Dot Nanoparticles (C-Dot) from Seeds and Seedpods of Kesumba Keling (*Bixa orellana*) using Hydrothermal and Solvothermal Methods

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## Abstract

C-dot is a 0-dimensional nanoparticle with photoluminescence properties and can be synthesized from plants, such as the Kesumba Keling plant. Kesumba Keling contains a red pigment sourced from the bixin and norbixin dyes containing functional groups like  $\text{-COOH}$  and  $\text{-COO-}$ . These functional groups are anticipated to enhance the luminescence intensity produced by C-dot. This research focuses on synthesizing C-dots from Kesumba Keling seeds and seedpods using hydrothermal and solvothermal methods. It also involves an analysis of how different solvents and passivation agents affect the luminescence of C-dots, along with a comparison of the resulting fluorescent colors. The highest yield, at 73.26%, was achieved when using Kesumba Keling seedpods and ethanol as the solvent without adding urea. Furthermore, C-dots synthesized using ethanol as the solvent display a stronger luminescent glow compared to those produced using double-distilled water as the solvent. Additionally, all C-dots synthesized in this study emit a blue luminescence. Characterizing C-dots using a UV-Vis spectrophotometer reveals absorption peaks at two different wavelengths: 260–280 nm and 320–340 nm. These absorption peak results align with C-dot characteristics, as confirmed by Fourier transform infrared spectrophotometry. When comparing the intensity of C-dots, those derived from Kesumba Keling peel using the double-distilled water solvent with the addition of urea exhibit a higher intensity (measuring at 0.99) than C-dots obtained from Kesumba Keling peel using ethanol as a solvent with added urea. The solvothermal method is deemed the most effective for C-dot synthesis, as it yields the highest luminescence intensity, accompanied by an emission wavelength shift to 491.65 nm.

## 1. Introduction

C-dot is a 0-dimensional nanoparticle known for its photoluminescent properties [1]. It has several advantages, including fluorescence, excellent water solubility, low toxicity, and high conductivity [2]. C-dot can be applied as biolabeling, biomedicine, sensors, photocatalysts, photoelectronic devices [3], luminous devices like LEDs, macrostructure materials, biosensors, bioimaging, and drug delivery [4]. The C-dot surface contains many  $\text{-COOH}$ ,  $\text{-OH}$ , and  $\text{-NH}_2$  functional groups [5]. Notably, a higher concentration of functional groups

on the C-dot surface is reported to increase its luminescence intensity [6]. Additionally, C-dot emission is influenced by passivation agents. These agents, consisting of functional groups containing sulfur and nitrogen atoms, serve to enhance both luminescence and solubility [7].

C-dot synthesis initially used precursors derived from fine coal and soot. As research advanced, the range of materials for C-dot synthesis expanded. The primary requirement for C-dot synthesis is a carbon source, and one potential source is plant materials. Most parts of

plants can serve as precursors for C-dot synthesis, for instance, onion peels [8], lychee seeds [9], durian [10], sago dregs [11], cassava peels [12], and mango leaves [13].

The Kesumba Keling plant is a promising candidate as a precursor for C-dot synthesis. This wild plant bears red fruits and seeds, traditionally used for coloring food and cosmetics. However, the cosmetics industry has gradually shifted towards synthetic dyes, leaving Kesumba Keling seeds largely underutilized and contributing to waste. These seeds contain red pigments, namely bixin and norbixin [14]. Interestingly, Kesumba Keling peel also contains these pigments. Bixin possesses –COOH and –COO– functional groups, while norbixin has the –COOH functional group. These functional groups can significantly augment the range of functional groups available on the surface of C-dots. Furthermore, C-dot synthesis can utilize various dyes, including those sourced from henna [15], lemons [16], and pandan leaves [17].

C-dot synthesis using plant parts as a carbon source precursor typically employs the bottom-up approach. This includes methods like hydrothermal and solvothermal processes. In the hydrothermal method, a chemical reaction occurs in water within a closed, high-temperature, and high-pressure vessel. On the other hand, the solvothermal method is an evolution of the hydrothermal technique; however, it employs organic solvents instead of water [18]. The choice of organic solvents in the solvothermal method can influence the resulting emissions during C-dot synthesis. Notably, solvothermal is the most cost-effective and suitable approach for synthesizing C-dots from dye-containing materials [16].

This research was based on previously reported C-dot synthesis from natural dyes [15, 16, 17]. The dyes used as precursors in this research were the seeds and seedpods of Kesumba Keling. This research focuses on the effect of the solvent used in the solvothermal method and passivation agents on the intensity of the carbon dots produced. The solvents chosen were aquabides and ethanol. This was done to determine the effect of organic solvents on emissions produced by C-dot. Urea is chosen as a passivation agent. After that, the C-dot was characterized by looking at the luminescence intensity under an ultraviolet (UV) lamp, an ultraviolet-visible spectrophotometer (UV-Vis) instrument, and a Fourier transform infrared (FTIR) spectrophotometer.

This research was conducted based on previous studies on C-dot synthesis using natural dyes, as documented in prior research [15, 16, 17]. In this study, the precursors employed were derived from the seeds and seedpods of the Kesumba Keling plant. This research focuses on the effect of the solvent used in the solvothermal method and passivation agents on the intensity of the carbon dots produced. Two solvents, double-distilled water and ethanol, were selected for the investigation to assess their influence on the emissions generated by C-dots. Additionally, the passivation agent chosen for this study was urea. Subsequently, the C-dots were characterized by evaluating their luminescence

intensity under an ultraviolet (UV) lamp, utilizing an ultraviolet-visible spectrophotometer (UV-Vis), and employing a Fourier transform infrared (FTIR) spectrophotometer.

## 2. Experimental

### 2.1. Materials and Tools

The materials were Kesumba Keling seeds and seedpods, distilled water, urea (Merck), H<sub>2</sub>SO<sub>4</sub>, ethanol, and KBr. All chemicals used in this research were not further purified. The tools used in this research were glassware, filter paper, magnetic stirrer, oven, hot plate stirrer (IKA CMAGH), porcelain cup, desiccator, litmus paper, centrifuge (Hermle), 0.45 μm PTFE filter syringe, 100 mesh sieve, mortar, Teflon-lined autoclave, analytical balance (OHAUS), UV lamp (UV Transmitter CA MAG UV Cabinet), UV-Vis spectrophotometer (Hitachi U-2800), Fourier Transformation Infrared (FTIR) spectrophotometer (Prestige 21 Shimadzu), and a fluorescence spectrophotometer (Cary Eclipse Fluorescence Spectrophotometer).

### 2.2. Preparation of Kesumba Keling samples

The seedpods and seeds of the Kesumba Keling were separated and then left to sun-dry for 1 to 2 days. A total of 2 g of the seeds was weighed and placed into 17.5 mL of 6.5 M H<sub>2</sub>SO<sub>4</sub>. The mixture was stirred for 30 minutes at 45°C using a magnetic stirrer. Subsequently, the stirred mixture was washed with distilled water until the pH reached neutral. The Kesumba Keling seeds were then heated in an oven at 200°C for 2 hours. Following this, the seeds were allowed to cool to room temperature and subsequently crushed and sieved until a smooth consistency was achieved. The same procedure was applied to the Kesumba Keling seedpods [9, 19].

### 2.3. Analysis of water content of Kesumba Keling in accordance with AOAC 2012

The porcelain cup was dried in an oven at 104°C for 30 minutes. Subsequently, it was cooled in a desiccator and then weighed. A total of 5 g of fresh samples of seeds were put into a cup and dried at a temperature of 104°C for 5 hours. After that, the dried seed was cooled in a desiccator and weighed. This procedure was repeated three times until a consistent weight was achieved. The water content of the sample was calculated using Equation (1). The same procedure was repeated for the Kesumba Keling seedpods.

$$\text{Water content} = \frac{A-B}{A} \times 100\% \quad (1)$$

where, A is the initial sample weight (g), and B is the dry sample weight (g).

### 2.4. Synthesis of C-dots using the hydrothermal and solvothermal method without passivation agent

A 3 g Kesumba Keling seed powder was weighed and dissolved in 60 mL of distilled water (or ethanol). The mixture was homogenized until fully dissolved. Subsequently, the homogenized mixture was transferred into a Teflon-lined autoclave. The mixture was then heated in an oven at 200°C for 5 hours. Following this, the

mixture was allowed to cool to room temperature. The same procedure was applied to the Kesumba Keling seedpod powder [3].

### 2.5. Synthesis of C-dots using the hydrothermal and solvothermal method with passivation agent

A 3 g Kesumba Keling seed powder was weighed and dissolved in 60 mL of distilled water (or ethanol). The mixture was added with 1.5 g of urea as a passivation agent. The homogenized mixture was then transferred into a Teflon-lined autoclave. The mixture was then heated in an oven at 200°C for 5 hours. Subsequently, the mixture was allowed to cool to room temperature. The same procedure was applied to the Kesumba Keling seedpod powder [3].

### 2.6. Purification of C-dot

After completing the synthesis process, the C-dot filtrate was separated using a centrifuge at 5000 rpm for 15 minutes to separate the precipitate and the supernatant. The result was subsequently poured and filtered using a 0.45 µm PTFE filter syringe. The resulting materials were then dried on a hot plate at a temperature of 100°C for C-dots with double-distilled water solvent and at 80°C for C-dots with ethanol solvent until they solidified. Following this, the obtained materials underwent a characterization process.

### 2.7. C-dot characterization

#### 2.7.1. Luminescence measurement

The luminescence intensity of the synthesized C-dots was measured using a UV lamp (UV Transmitter CA MAG UV Cabinet). A C-dot solution with a concentration of 500 ppm was made and then irradiated under a 366 nm UV lamp.

#### 2.7.2. Absorbance measurement

The objective of measuring C-dot absorbance was to assess C-dot absorption at a specific wavelength. This measurement was conducted using a UV-Vis spectrophotometer (Hitachi U-2800) within the wavelength range of 200 to 800 nm, with intervals of 5 nm. The C-dot absorbance assessments were performed on C-dot samples obtained from Kesumba Keling.

#### 2.7.3. Measurement of functional groups using FTIR

C-dot samples derived from Kesumba Keling seeds were analyzed utilizing an FTIR spectrophotometer (Prestige 21 Shimadzu). The FTIR spectra were acquired through a process involving heating 0.1 g of KBr, subsequent grinding, and adding 0.02 g of the C-dot sample. The resulting pellets were then heated in an oven for 12 hours, followed by identifying functional groups using an FTIR spectrophotometer.

#### 2.7.4. Emission measurement using a fluorescence spectrophotometer

C-dot emission was measured using a fluorescence spectrophotometer (Cary Eclipse Fluorescence Spectrophotometer) at wavelengths between 350–600 nm with a range of 5 nm.

## 3. Results and Discussion

The water content in seeds and seedpods is 41.41% and 46.01%, respectively, as shown in Table 1. The results differ from the values reported in the previous literature. According to Souhoka et al. [14], the seeds were reported to have a water content of 78.74%. Several factors may contribute to these variations in water content values for Kesumba Kelings, including humidity, environmental conditions, storage methods, and the absorption process by materials and the surrounding environment. This factor is supported by Dinarto [20], highlighting the influence of environmental conditions and storage methods on water content in plant samples.

**Table 1.** The water content of Kesumba Keling

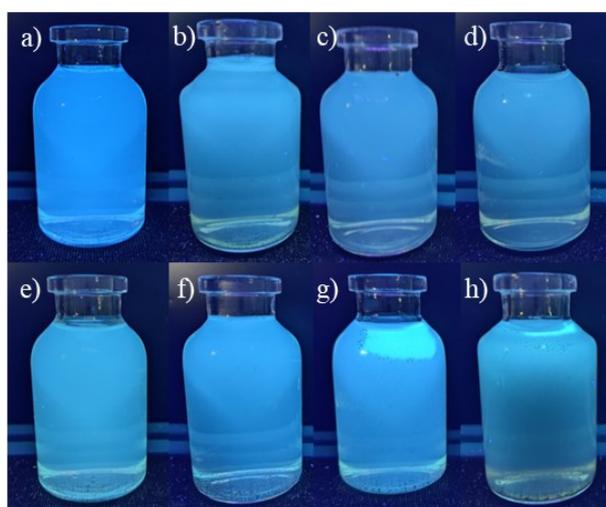
Precursor	Repetition	Water content (%)
Seeds	1	44.50
	2	38.87
	3	40.87
Average		41.41
Seedpods	1	48.16
	2	48.77
	3	41.10
Average		46.01

The yields achieved in synthesizing C-dots using this type of solvent and adding a passivation agent ranged from 8.13% to 23.08% for double-distilled water solvent and 16.07% to 25.50% for ethanol solvent. These yields nearly align with results reported in prior studies. According to the literature, C-dot yields using double-distilled water solvents typically fall between 8.5% and 13.7%, while ethanol solvent yields range from 17.6% to 26.1% [20, 21]. The synthesized C-dot has the form of a black paste after the purification process, as shown in Figure 1. This observation is consistent with Sjahriza et al. [22], who also reported that the synthesized C-dot product was in the form of a black paste following the purification process.



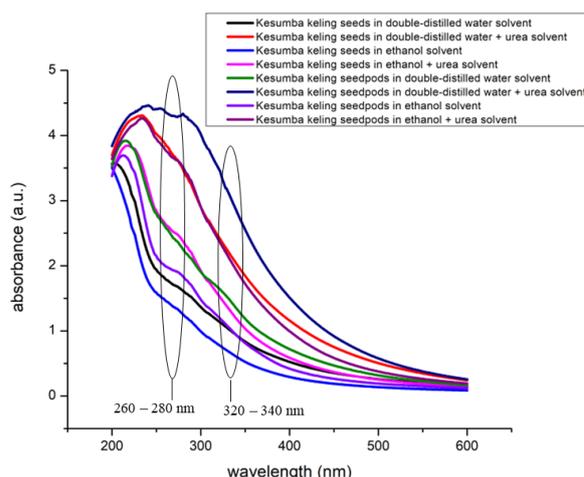
**Figure 1.** C-dots synthesized using double-distilled water solvent: a) seeds, b) seedpods. Double-distilled water+urea solvent: c) seeds, d) seedpods. Ethanol solvent: e) seeds, f) seedpods. Ethanol+urea solvent: g) seeds, h) seedpods

The C-dots were then examined for their luminescence under UV light with a wavelength of 366 nm. The luminescence color emitted by the C-dots derived from both the seeds and seedpods of Kesumba Keling was entirely blue. Notably, there was a noticeable difference in luminescence between C-dots synthesized using double-distilled water and ethanol solvents. The C-dots produced with ethanol solvent exhibited a brighter luminescence due to the ethanol, an organic solvent that can adhere to the core of the precursor, thus slowing down crystal growth and stabilizing smaller crystals [23]. Furthermore, introducing urea as a passivation agent in the C-dot formulation resulted in enhanced luminescence under a 366 nm UV lamp. These findings align with existing literature, which underscores how the inclusion of passivation agents can boost luminescence intensity due to the presence of additional functional groups on the C-dot's surface [24]. The luminescence of C-dots is depicted in Figure 2.



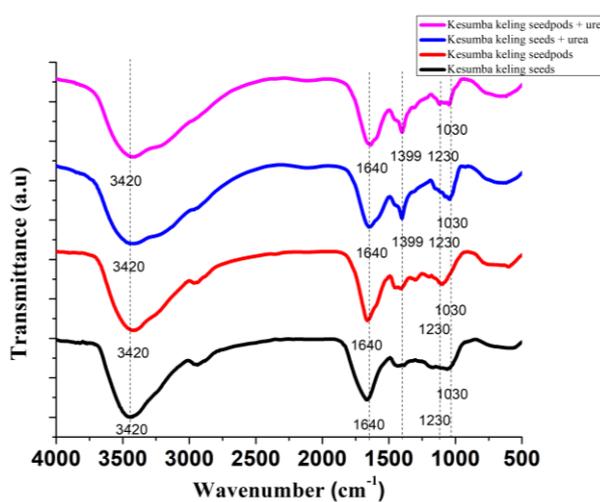
**Figure 2.** C-dots (under 366 nm UV lamp) synthesized using double-distilled water solvent: a) seeds, b) seedpods. Double-distilled water+urea solvent: c) seeds, d) seedpods. Ethanol solvent: e) seeds, f) seedpods. Ethanol+urea solvent: g) seeds, h) seedpods

The success of C-dot synthesis can be confirmed by analyzing the sample's absorption pattern using a UV-Vis spectrophotometer. The C-dots were measured using a UV-Vis spectrophotometer at the wavelength range of 200 to 800 nm using the UV-Vis spectrophotometer. As depicted in Figure 3, the UV-Vis absorption spectra of C-dots display eight samples with absorption peaks at 260–280 nm wavelengths and 320–340 nm. These findings align with previous research results by Newman Monday *et al.* [20], which attribute the absorption peak at 260–280 nm to the  $\pi$ - $\pi^*$  transition of the aromatic C=C bond within the  $sp^2$  core hybridized carbon fragment. Additionally, another absorption peak at 320–340 nm indicates the  $n$ - $\pi^*$  transition of non-bonding electrons in C=C and C=O. Notably, variations in spectral patterns are observed in the UV-Vis spectrum, likely due to the use of different solvents leading to distinct functional groups on the C-dot surface. The broadening of peaks among the synthesized C-dots can occur because they possess different sizes, resulting in variations in the wavelength of the emitted fluorescence [25].



**Figure 1.** UV-Vis spectra of C-dots

In addition to utilizing a UV-Vis spectrophotometer, the success of C-dot synthesis can also be verified through measurements conducted with a Fourier transform infrared (FTIR) spectrophotometer. Figures 4 and 5 display the FTIR spectra of C-dot Kesumba Kelings. Across the eight samples, observable shifts occurred in OH and NH (amine) peaks at  $3420\text{ cm}^{-1}$ , C=O (carbonyl) at  $1640\text{ cm}^{-1}$ , as well as  $sp^3\text{ CH}$  and  $sp^2\text{ CH}$  shifts at  $1230\text{ cm}^{-1}$  and  $1030\text{ cm}^{-1}$ , respectively. The C-dot sample incorporating urea as a passivation agent exhibited a CN shift peak at  $1399\text{ cm}^{-1}$ , while the C-dot synthesized using ethanol solvent displayed a CO (alcohol) shift peak at  $1000\text{ cm}^{-1}$ . Additionally, the C-dot sample employing ethanol solvent showed a shift peak at wavenumber  $2923\text{ cm}^{-1}$ , attributed to the  $sp^3\text{ CH}$  group of ethanol, likely due to incomplete drying of the C-dot sample. The distinction between the FTIR spectra of C-dots with and without urea addition lies in the presence of a CN shift in the C-dot with urea, confirming successful nitrogen modification on the surface structure. Importantly, the FTIR spectrum of C-dot synthesized from Kesumba Keling seeds and seedpods nearly resembles the research results from Sugiarti and Darmawan [1] and Newman Monday *et al.* [20].



**Figure 2.** FTIR spectrum of C-dots synthesized using double-distilled water solvent

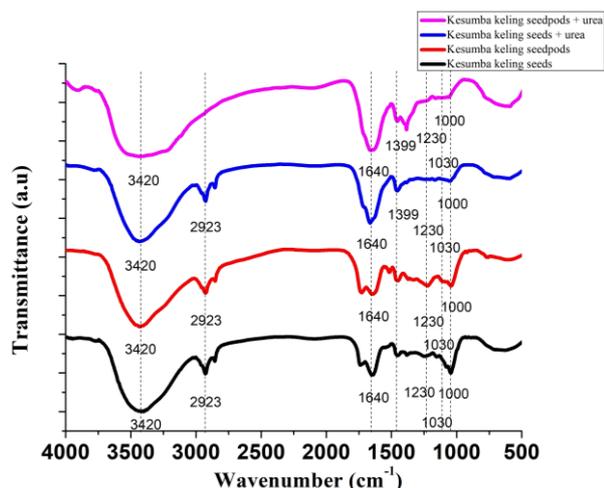


Figure 3. FTIR spectra of C-dots synthesized using ethanol solvent

The success of C-dot synthesis can also be validated through measurements performed with a fluorescence spectrophotometer. Figure 6 shows the C-dot fluorescence spectrum of Kesumba Keling seedpods synthesized using double distilled water+urea and ethanol+urea as solvents. The C-dot emission peak of seedpods with double distilled water+urea solvent is at 496.52 nm with an excitation wavelength of 438.80 nm. Meanwhile, for C-dot derived from seedpods with ethanol+urea solvent, its emission peak is 491.65 nm, and its excitation wavelength is 433.58 nm. The excitation and emission wavelengths in the two solvents are quite similar. Based on Li *et al.* [26], the results of the C-dot fluorescence spectrum reveal an emission wavelength shift towards the red part of the spectrum, a phenomenon known as redshift. The emission wavelength in the redshift range typically falls between 455 nm and 595 nm.

The C-dot intensity of Kesumba Keling peel was higher when double-distilled water was used as the solvent with the addition of urea, compared to using ethanol+urea. This difference in intensity is influenced by the presence of dyes bixin and norbixin in the Kesumba Keling seedpods. Bixin is insoluble in water, while norbixin is water-soluble [27]. During the C-dot synthesis process employing double-distilled water as the solvent, norbixin dissolves more readily in the water. This leads to a higher concentration of norbixin functional groups adhering to the C-dot surface, consequently affecting the intensity of the C-dot. Quantum yield is essential, as it represents the ratio between the number of emitted and absorbed photons (UV). A higher quantum yield value signifies increased application sensitivity [28]. The quantum yields of C-dot with double-distilled water+urea solvent and C-dot with ethanol+urea solvent were calculated using Equation (2).

$$QY = QY_r \times \frac{I}{I_r} \times \frac{A_r}{A} \times \frac{n_r^2}{n^2} \quad (2)$$

where, r is the reference (quinine sulfate), I is the emission intensity, A is the absorbance, and n is the refractive index of the solvent.

C-dot prepared using double-distilled water+urea solvent, and C-dot synthesized with ethanol+urea

solvent exhibited quantum yields of 50.60% and 23.77%, respectively. These findings align with previous research [19, 29], which reported that C-dots synthesized with water solvent tend to have higher quantum yield values than those with ethanol solvent. Quantum yield in C-dots is influenced by various factors, including reaction time, temperature, the molar ratio between carbon and nitrogen sources, and reaction pH. Quantum yield values tend to increase with higher reaction temperatures, reaching their peak around 200°C. Elevated temperatures lead to increased particle activity within the reactant materials, while extended reaction times provide more opportunities for interactions among the reactant materials. However, excessive heating can result in the carbonization of amides, leading to a decrease in the quantum yield value, especially if the reaction time is extended to six hours [30].

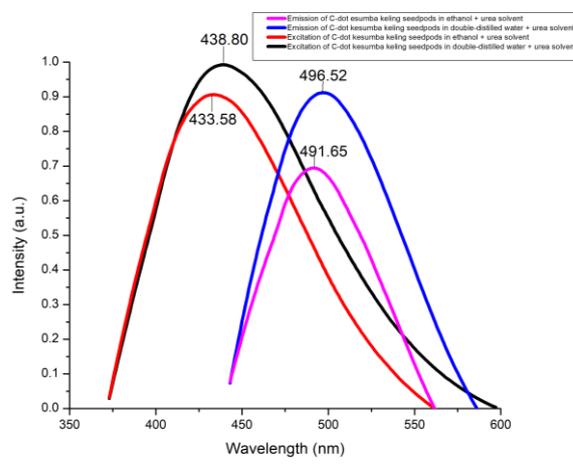


Figure 4. The fluorescence spectrum of C-dots

#### 4. Conclusion

C-dots were successfully synthesized from the seeds and seedpods of Kesumba Keling using hydrothermal and solvothermal methods. The results revealed that C-dots produced with ethanol solvent exhibit a brighter luminescence than those produced with distilled water solvents. The light emitted from the C-dots derived from Kesumba Keling seeds and seedpods is predominantly blue. Furthermore, the addition of urea as a passivation agent has a discernible impact on C-dot luminescence intensity, as evidenced by the brighter glow observed when urea is added compared to when it is not. The fluorescence spectrum of C-dots also demonstrates a redshift in their emission wavelength. Lastly, the intensity of C-dots derived from seedpods synthesized using an aquabides+urea solvent exhibits a greater value, specifically 0.99, in contrast to C-dots synthesized using an ethanol+urea solvent.

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