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Laboratory Experiment

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Carbon Dots: A Modular Activity To Teach Fluorescence and Nanotechnology at Multiple Levels

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Supporting Information

ABSTRACT: In recent years, nanomaterials have entered our daily lives via consumer products; thus, it has become increasingly important to implement activities to introduce these novel materials into chemistry curricula. Here we introduce a newly developed fluorescent nanomaterial, carbon dots, as a more environmentally friendly alternative to heavy-metal semiconductor quantum dots to be used as a model nanomaterial for experiments at multiple educational levels ranging from high school to upper-division college laboratories. These dots, which are polymeric in nature, can be made from a variety of carbon precursors and a cross-linker such as ethylenediamine. The synthesis, which involves heating in a conventional microwave, is quick and straightforward and can be carried out in typical high school chemistry laboratories. The resulting solution is fluorescent without further purification. To increase the complexity for entry-level college students, absorption and emission spectra of the carbon dot solution can be collected as an introduction to spectroscopy. In more advanced undergraduate lab courses, the quantum yield can be determined with a standard reference fluorescent material such as quinine sulfate. Atomic force microscopy or transmission electron microscopy images can also be collected to illustrate the morphology of these particles where such specialty instruments are accessible.

KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General, Upper-Division Undergraduate, Environmental Chemistry, Interdisciplinary/Multidisciplinary, Hands-On Learning/Manipulatives, Fluorescence Spectroscopy, Nanotechnology*

INTRODUCTION

The use of nanomaterials in a wide range of applications has increased in recent years, from optical and storage electronics to energy-harvesting devices to medicine, and education about nanotechnology is important at all levels.[1] By definition, nanomaterials have at least one dimension that ranges in size from 1 to 100 nm; the small size of these materials results in unique chemical and physical properties that benefit their broad applications.[2] The particular phenomenon of interest in the activities presented here is fluorescence: the emission of light by a material upon excitation with higher-energy photons.

Fluorescent nanoscale materials such as semiconductor quantum dots (QDs) have been studied for decades.[3] The ability to label and detect different substances using fluorescence-based nanotechnologies highlights their utility for a variety of applications, including biomedical imaging, forensic reagents, solar cells, light-emitting displays, and consumer electronic products including cellphones and tablets.[4−7] Increased understanding of fluorescent nanomaterials will lead to new and exciting applications.

One of the primary concerns for conventional semiconductor QDs, such as CdSe, is the toxicity of the materials.[8] Such materials may pose potential health hazards for individuals working with them[9] as well as to the environment in the event of improper disposal after use.[10] One approach to address this problem is to engineer fluorescent nanoparticles from less toxic substances. Carbon dots are carbon-based nanoparticles, smaller than 10 nm in diameter, with various chemical

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modifications to enhance their photophysical properties such as fluorescence.[11,12] After their discovery as a byproduct in the synthesis of single-walled carbon nanotubes, a laser-based synthesis technique was developed in 2004 to produce these carbon dots as a product.[12] More recently, a variety of methods have been developed to simplify the synthesis procedures and facilitate the preparation of carbon dots,[13,14] including hydrothermal carbonization,[15] combustion,[16] microwave,[14] and ultrasonic treatment.[17] Some benefits of being carbon-derived include chemical stability and low toxicity.[18−20] Carbon dots are likely to be less harmful to human health and the environment than their inorganic alternatives, which often contain heavy metals like cadmium. Carbon dots have potential applications in biomedicine because of their lower toxicity and in optoelectronics and sensor technologies because of their optical properties.[12]

Per the ACS 2015 Undergraduate Curriculum Guidelines, there is a need to modernize chemistry curricula by introducing mesoscale and nanoscale materials.[21] There have been a few developed activities aimed at introducing fluorescent nanomaterials to students at an entry level. For example, Boatman and co-workers reported the synthesis and absorption characteristics of different sizes of CdSe quantum dots in 2005.[22] In 2007, Hutchins et al.[23] developed a procedure for secondary and postsecondary students to compare the emission properties of different-sized quantum dots to those of organic dyes. More recently, five lab procedures on the synthesis of potentially fluorescent nanomaterials were published,[24−28] with only one of these exploring the fluorescence properties in the lab activity.[26] Despite these efforts, new experiments focused on exploring both the synthesis and the properties of nanomaterials that are suitable at multiple educational levels are needed.

The objective of this experiment is to allow students from high school to upper-division college level to explore the synthesis and characterization of nanoscale carbon dots. The synthesis protocol using a microwave oven is straightforward and quick to carry out; fluorescence quantum yield calculations relate closely to optical spectroscopic techniques commonly taught in upper-division collegiate chemistry courses. The modular nature of the activity allows it to be adopted at various levels, from high school to college. Specifically, at the high school level, students can synthesize the particles and observe their fluorescent glow in ultraviolet (UV) light. At the entry level in college, students can characterize the synthesized carbon dots using spectrometers. For upper-division college level laboratories in analytical and/or physical chemistry, students can also further characterize the carbon dots through absorption, fluorescence, quantum yield, and physical size measurements. Carbon dots prepared from two different precursors, citric acid and malic acid, have both been successfully synthesized and characterized to demonstrate the flexibility and potential variations of this lab activity.

■ EXPERIMENTAL PROCEDURES

Detailed protocols and safety concerns for the synthesis of both citric acid- and malic acid-based carbon dots are provided in the Supporting Information. Synthesis, spectral characterization, and quantum yield determination using the single-point method (module 1, module 2, and part of module 3) can be carried out in one four-hour lab period. The calibration method for quantum yield and atomic force microscopy (AFM) imaging can fit in another four-hour lab period, with transmission electron microscopy (TEM) in an additional four-hour lab period if time and facilities allow. If AFM/TEM instruments are unavailable, sample images provided here can be used to complement the carbon dot synthesis.

Module 1: Synthesis (High School)

A 2 mL aliquot of a 4.0 M citric acid (or malic acid) solution was added to 0.5 mL of ethylenediamine, and the mixture was stirred with a glass rod or a magnetic stir bar for 20 min. Once the stir bar was removed, the solution was heated in a conventional microwave at ∼360 W for 5 min. To dissolve the solid mass of carbon dots formed after heating, 10 mL of water was added, and the solution was swirled until the carbon dots were dispersed. Long-wavelength UV light (365 nm) was used to observe the fluorescence of the carbon dot solution in the dark.

Module 2: Spectroscopic Characterizations (Lower-Division Undergraduate)

Freshly synthesized carbon dot solutions were diluted for spectroscopic characterization with a UV-vis absorption spectrophotometer and a fluorescence spectrophotometer in glass cuvettes. We have found a dilution factor between 1000 and 5000 to be suitable for the citric acid carbon dots (CACDs) and between 100 and 500 for the malic acid carbon dots (MACDs). Absorption spectra were acquired from 300 to 600 nm, and fluorescence emission spectra were taken from 350 to 700 nm with excitation at 340 nm.

Module 3: Quantum Yield (Upper-Division Undergraduate)

The quantum yield of the fluorescent carbon dots can be calculated using one of two possible methods, both utilizing a quantum yield reference, quinine sulfate. In the single-point (single-concentration) method, the absorbance of both diluted carbon dots and a 5 μM quinine sulfate solution (in 0.1 M H_2SO_4) were recorded at 340 nm. The fluorescence emission of these sample solutions from 350 to 650 nm was then collected with λ_{ex} = 340 nm. The quantum yield of the carbon dot solution (ϕ_s) was then determined using the integrated fluorescence emission (F) with the following equation:

$$\frac{\phi_S}{\phi_R} = \frac{F_S}{F_R} \times \frac{(1 - 10^{-A_R})}{(1 - 10^{-A_S})} \times \frac{{n_S}^2}{{n_R}^2}$$

where subscripts “R” and “S” denote the reference material and sample, respectively, A is the absorbance at 340 nm, and n is refractive index (which can be assumed to be 1.33 for both the sample and reference in diluted aqueous solutions).

The second possible method, known as the calibration method, used a series of four to five diluted solutions of carbon dots at various concentrations as well as reference solutions of quinine sulfate (with concentration ranging from 0.5 to 5.0 μM in 0.1 M H_2SO_4) to construct a calibration curve by plotting fluorescence intensity versus absorbance. The absorbance of each solution was recorded at 340 nm, and the corresponding fluorescence intensity was measured at 450 nm. The slopes of the fluorescence intensity versus absorbance plots for the carbon dots and quinine sulfate were used to determine the quantum yield (ϕ_s) using the following equation:

$$\frac{\phi_S}{\phi_R} = \frac{\text{slope}_S}{\text{slope}_R} \times \frac{{n_S}^2}{{n_R}^2}$$

Module 4: Imaging (Upper-Division Undergraduate)

A NaioAFM atomic force microscope (Nanosurf, Woburn, MA) was used to image the carbon dots. Samples with volumes

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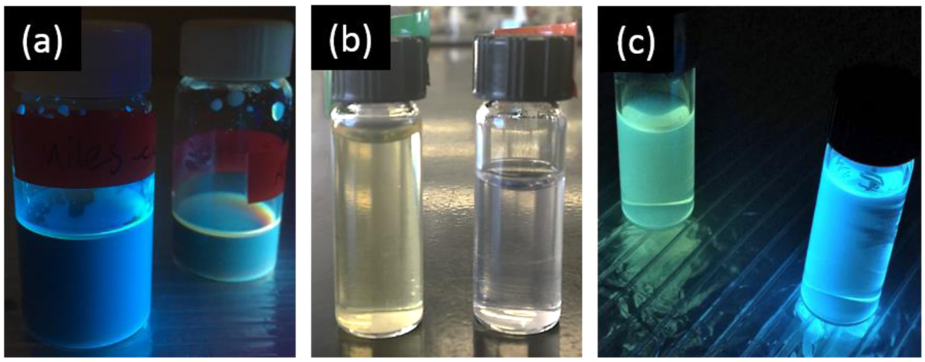

Figure 1. (a) Undiluted, freshly synthesized citric acid carbon dots (CACDs) (left) and malic acid carbon dots (MACDs) (right) under irradiation with 365 nm UV light. (b, c) Diluted MACDs (left) and CACDs (right) under (b) ambient light and (c) 365 nm UV light.

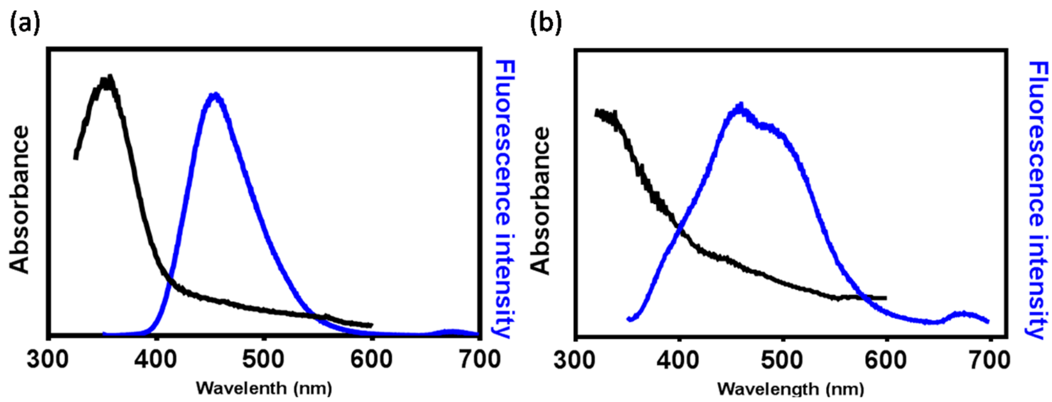

Figure 2. Fluorescence emission (blue) and absorption (black) spectra of (a) citric acid carbon dot and (b) malic acid carbon dot solutions after dilution.

of 2−5 μL were placed on a freshly cleaved sheet of mica and dried in an oven at 40 °C. Images were acquired in tapping (dynamic) mode for 5 μm × 5 μm areas with a Tap190Al-G tip (BudgetSensor). The size of the particles was determined using a cross-section analysis tool in the software to obtain the *z* axis (height) of each particle.

TEM images of both CACDs and MACDs were obtained on an FEI F30 transmission electron microscrope. Sample mounting was achieved by dropping a carbon dot aqueous solution onto a 300-mesh gold grid coated with an ultrathin lacey carbon film.

■ HAZARDS AND DISPOSAL

Ethylenediamine can produce irritant fumes when added to water. Therefore, we recommend that students wear gloves and handle the compound inside a fume hood. Ethylenediamine should be disposed in the nonhalogenated waste. The carbon dots are not as hazardous as ethylenediamine, but are also organic molecules with amino chains and therefore should be disposed in the same waste container. UV light is harmful to skin and eyes. It is strongly recommended that the microwave be placed in a hood during heating as large amounts of steam and smoke are usually generated during the synthesis.

■ RESULTS AND DISCUSSION

Module 1: Synthesis

The synthesis steps are straightforward and can be completed in ∼30−40 minutes. Right after heating in the microwave, the reaction mixture forms a dark-amber-colored solid on the bottom of the container, but it is soluble in water upon agitation. Figure 1 shows that undiluted carbon dot solutions (Figure 1a) are more opaque and with less fluorescence intensity than the diluted ones (Figure 1c).

Module 2: Spectroscopic Characterization

Figure 2 illustrates the absorbance and emission spectra of the citric acid and malic acid carbon dots. Because citric acid has three carboxylic acid groups while malic acid only has two, we expect a more intricately cross-linked network for citric acid-based carbon dots compared with malic acid-based carbon dots. This difference in molecular-level connectivity is likely to have a significant impact on the carbon dots' ability to absorb and emit photons. The citric acid carbon dot sample has a well-defined emission peak centered around 450 nm that corresponds to the blue fluorescence seen in Figure 1c. The malic acid carbon dot spectrum depicts a broader emission band with a maximum containing a shoulder around 480−520 nm, which corresponds to the more green/yellow fluorescent color shown in Figure 1c. Both carbon dot solutions absorbed strongly at 340 nm, so 340 nm was chosen as the excitation wavelength for fluorescence emission experiments.

Module 3: Determination of the Quantum Yield

The quantum yield is defined as the ratio of the number of photons emitted to the number absorbed:

$$\phi_f = \frac{N_{em}}{N_{abs}}$$

Some fluorescence spectrophotometers are equipped with an accessory known as an integrating sphere that allows the

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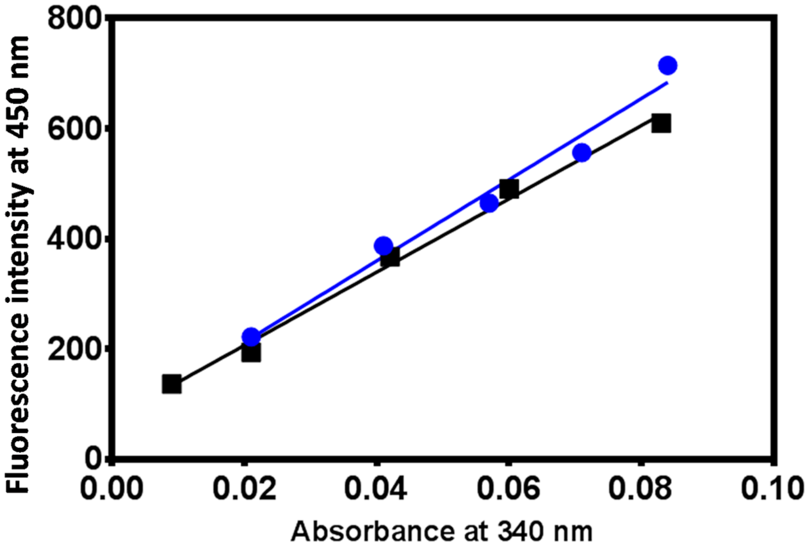

Figure 3. Sample calibration curves for determining the quantum yield of carbon dots. A series of diluted solutions of quinine sulfate (blue circles) and citric acid carbon dots (black squares) were measured to obtain the absorbance at 340 nm and fluorescence at 450 nm (λ_{ex} = 340 nm).

Table 1. Summary of Class Data on Quantum Yields for Citric Acid and Malic Acid Carbon Dots Using Different Methods

	Emission Quantum Yield (%)					
	Citric Acid Carbon Dots			Malic Acid Carbon Dots		
Parameter (N = 10)	Single-Point	Calibration	Integrating Sphere[a]	Single-Point	Calibration	Integrating Sphere[a]
Average	44	48	56	15	13	23
SD	7	6	3	11	8	2

[a]See ref 29.

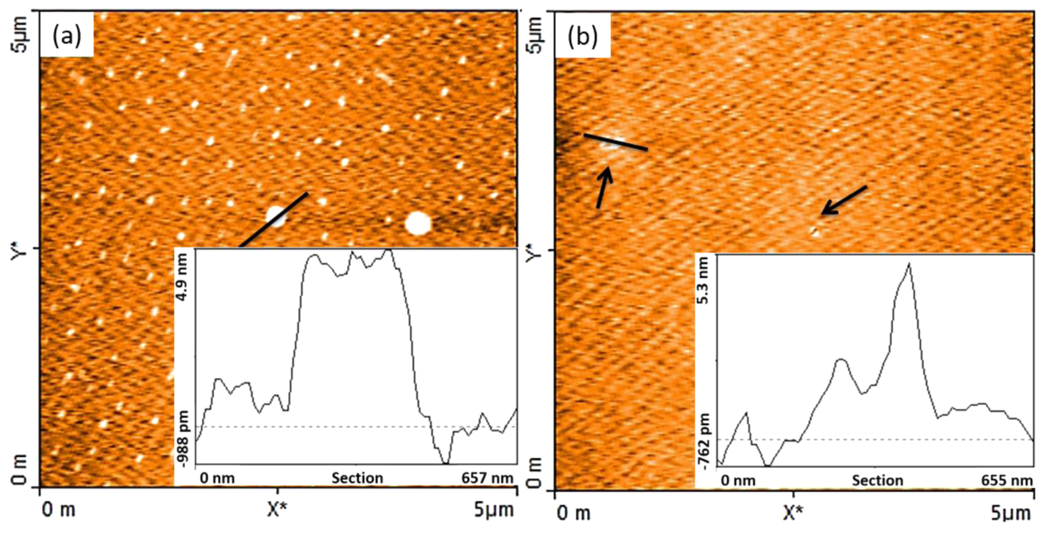

Figure 4. Tapping-mode AFM images of (a) citric acid carbon dots and (b) malic acid carbon dots on mica. The black arrows in the MACD micrograph identify carbon dots. The insets show cross-sections of the dots along the black lines for height measurements.

quantum yield to be measured directly and sensitively. However, when such a component is not available, in order to determine the quantum yield of a new fluorescent material, a reference material with a known quantum yield, such as quinine sulfate, can be used. As mentioned in Experimental Procedures, this can be done either with a single-point method or with a full calibration curve. We note that in order for either method to work properly, it is desirable to have the absorbance of the diluted solutions at less than 0.1 to reduce self-absorption, which may lead to errors in quantum yield measurements. In order to improve the accuracy, it is also recommended to select a reference material that has a similar quantum yield than the unknown sample, as indicated by the similar slopes shown in Figure 3.

Table 1 summarizes the quantum yield values determined in an undergraduate teaching lab where this activity was beta tested, comparing the two methods for quantum yield calculations to those found using a commercial integrating sphere for both CACDs and MACDs. The results show that the two methods yield comparable average quantum yields that are

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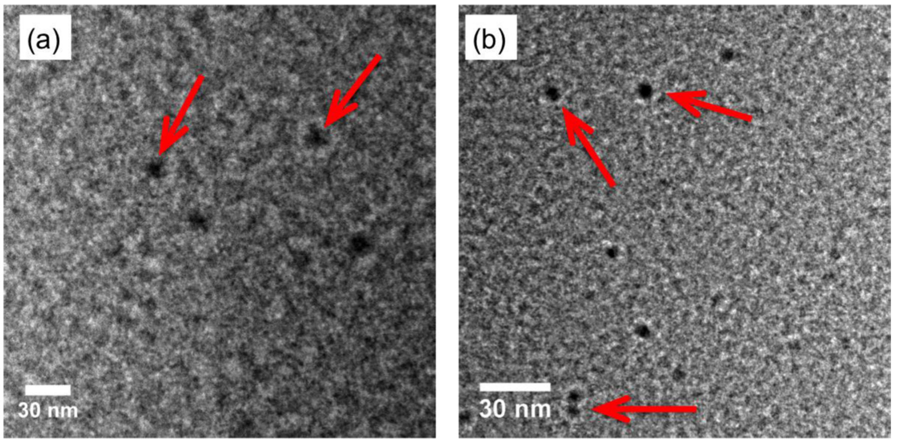

Figure 5. Example TEM images of (a) citric acid carbon dots and (b) malic acid carbon dots. The red arrows in the micrographs identify carbon dots.[29]

close to that obtained from the integrating sphere measurements. This demonstrates that either method for determining the quantum yield could be applied, depending on the class size and instruments available. We note that although the calibration method in both cases resulted in smaller standard deviations among class data collected, in practice the single-point method took significantly less time to carry out, which allowed us to fit the synthesis, characterization, and quantum yield determination into one four-hour lab period.

Module 4: Carbon Dot Imaging

Figure 4 shows sample images of both CACDs and MACDs using tapping (dynamic) mode for AFM measurement. The average particle sizes based on height measurements from samples prepared in the class were 3.1 ± 0.4 nm ($n > 40$) for CACDs and 10 ± 5 nm ($n > 20$) for MACDs. The carbon dots made from citric acid were significantly more evenly distributed (smaller white spots in Figure 4a) on most of the substrates, and the sizes of the particles were more uniform. The carbon dots made from malic acid agglomerated laterally, and their distribution on the substrates was random, which made it more difficult to observe and led to a larger distribution in size. We believe the larger patches in these images are likely due to the lateral aggregation of the carbon dots on the AFM substrates in the drying process. Contact (static) mode was initially attempted, but a strong tip/sample interaction led to a deterioration of the images.

Transmission electron microscopy reveals the two-dimensional morphology of the nanoparticles and serves as a common complementary method to the *z*-profile height determined by AFM. Representative TEM images of both CACD and MACD samples are presented in Figure 5. Determination of the size distribution was conducted on the basis of counting more than 500 particles (CACDs, $n = 500$; MACDs, $n = 545$). The average sizes of the particles were found to be 8.2 ± 3.6 and 6.3 ± 1.8 nm for CACDs and MACDs, respectively.

The advanced microscopic methods described here, if available, can enhance students' understanding of nanomaterials and provide a basic introduction to techniques frequently used in nanotechnology. In case such instruments are not available, sample microscopy images from this article and the Supporting Information can be used for visualization. AFM sample preparation may result in more aggregation and some artifacts, while acquiring TEM images of these samples may be challenging and time-consuming because of the low contrast of these particles compared to the TEM supporting film.

■ PEDAGOGICAL DISCUSSION

In this laboratory exercise, students explored a fluorescent carbon-based nanomaterial. In our experience, the easily observed fluorescent nature of the products generated genuine excitement during the lab, even among upper-division students. The determination and comparison of quantum yields among different carbon dots, in comparison with a commonly used fluorescent reference material (quinine sulfate), solidified their understanding of the concept beyond just a numerical value. The different colors observed for the CACDs (blue) and MACDs (yellowish green) under UV light also helped the students understand the shift in fluorescence emission spectra. The synthesis method was very straightforward and nearly fail-proof, which made carbon dots a great alternative to citrate-capped gold nanoparticles[30] as a model to introduce students to nanomaterials.

Through detailed laboratory reports, students honed their ability to communicate scientific information by describing their results, applying a mathematical equation, and representing data in graphical form. Through this straightforward exercise, students gained knowledge of photoluminescence, enhanced their experience in spectral interpretations, and refined their data analysis skills using a spreadsheet.

The modular nature of the lab activity will allow instructors for various levels, from high school to upper-division college, to adopt and implement this activity in a way that fits their curricular needs. For example, relevant Next Generation Science Standards for high school include HS-PS4-4 ("Evaluate the validity and reliability of claims in published materials of the effects that different frequencies of electromagnetic radiation have when absorbed by matter") and HS-PS4-5 ("Communicate technical information about how some technological devices use the principles of wave behavior and wave interactions with matter to transmit and capture information and energy"),[31] and International Baccalaureate SL and HL courses would find this lab ideal as an Optional-Materials Lab.[32]

The laboratory discussion questions (included in the Supporting Information) engaged students in the developing research field of nanotechnology and the specific challenges researchers face, including considerations of environmental impact, toxicity, instrumentation design, and varied synthesis

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techniques. By referencing primary literature, students were further challenged to compare and contrast the synthesis techniques.

Lastly, carbon dots have been proposed as an environmentally friendly alternative to semiconductor quantum dots. One of the questions for students to consider in this lab was the environmental impacts of a new material, which provided an excellent platform to introduce the concept of environmental sustainability and green chemistry. Among the 12 Principles of Green Chemistry,[33] this synthesis method achieves at least three: less hazardous chemical syntheses (principle 3), safer solvents and auxiliaries (principle 5), and use of renewable feedstocks (principle 7). It was also interesting for students to compare and contrast chemical sources to balance the use of renewable resources with the practicality of scale-up. Source materials and synthesis energy use may be greener in the carbon dot microwave synthesis, yet CdSe/ZnS quantum dots have a longer shelf life[34] and have a protective shell to potentially lower their cytotoxicity.[35]

CONCLUSION

Overall, this activity brings cutting-edge scientific research on carbon dot nanomaterials into high school or undergraduate chemistry laboratories to teach fundamental concepts (absorption, emission, and quantum yield) with modern tools (nanomaterials). Using relatively affordable chemicals and instrumentation, students can synthesize carbon dots and then, depending on educational goals, visually observe their properties (high school), measure absorption/emission spectra (lower-division undergraduate), or calculate emission quantum yields and use advanced microscopies (advanced undergraduate). The activity fulfills several educational standards at the high school level and, more importantly, engages students with visually exciting products and the timeliness of nanoscience topics.

ASSOCIATED CONTENT

Supporting Information

This material can be found at The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00995.

> Additional information for high school teachers to adopt this activity (lab supplies shopping list, procedure handout, and suggested prelab and postlab readings and questions for students) and instructor's notes and lab handout for college students (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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