Preparation and Characterization of Carbon Quantum Dots And Their Application to Fingerprint Imaging

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Overview:

In this laboratory exercise, students will prepare carbon quantum dots (CQDs), purify them, and use them to collect and visualize a latent fingerprint.

Introduction:

Nanotechnology is a rapidly growing area of science. The basic concepts of nanotechnology are reported to have originated in a 1959 talk given by Richard Feynman, a renown physicist.[1] However, it wasn't until the 1980's that nanotechnology began to emerge as a field. The invention of the scanning tunneling microscope in 1981, which allowed the visualization of atoms and bonds, and the 1985 discovery of fullerenes (C_{60}), sparked the growth of nanotechnology.[1] In the early 2000's, commercial products based on research advancements in nanotechnology began to appear. This prompted the National Nanotechnology Initiative, a United States federal government program for the science, engineering, and technology research and development of nanoscale projects, to define nanotechnology.[2] The initiative chose to define it as the manipulation of matter with at least one dimension sized from 1 nm to 100 nm. As one might imagine, nanotechnology is very broad, encompassing fields of science as widespread as organic chemistry, inorganic chemistry, surface science, molecular biology, semiconductor physics, energy storage, and molecular engineering.

The last two decades have seen the development of a host of new nanomaterials including gold nanoparticles, silver nanoparticles, palladium nanoparticles, hollow spheres made of silica, carbon nanotubes, carbon nanorods, graphene, and quantum dots, to name a few. These materials have also been used in the development of a number of applications in a variety of areas including catalysis, drug delivery, biosensors, optoelectronics, and solar cells.[3]

One material class of particular interest is quantum dots (QDs). Quantum dots are namometer scale semiconductors that have unique optical and electronic properties.[4] When quantum dots are exposed to UV light, they absorb the energy, causing an electron to be excited to a higher energy state. This excited electron can then relax to a lower energy state, releasing energy as light in a process called fluorescence. The color of the emitted light depends on the difference in the two energy states, which is tunable in quantum dots based on the size of the crystals. There are a host of potential applications for QDs including solar cells, LEDs, medical imaging, biosensing, single electron

transistors, and a host of other electronic products.[5, 6] Quantum dots have been studied for decades with the most common type being cadmium selenide (CdSe) QDs. While quantum dots have the potential to be useful in a number of areas, the main disadvantage of conventional semiconductor QDs is their toxicity due to the use of heavy metals in their preparation.[7] These materials pose a hazard not only to those individuals working with them, but also to the environment. Ideally, it would be better if QDs could be made of nontoxic materials, and carbon quantum dots (CQDs) provide one possible solution.

Carbon quantum dots, or just carbon dots (CDs) as they are often called, were discovered in 2004 during the purification of single-walled carbon nanotubes prepared through wet-chemical synthesis.[7] Carbon dots are small nanoparticles (generally < 10 nm) that are water soluble, highly photoluminescent, inexpensive to make, have good biocompatibility, and are believed to be nontoxic.[8] This sets them apart from traditional quantum dots and makes them a promising candidate for numerous exciting applications.

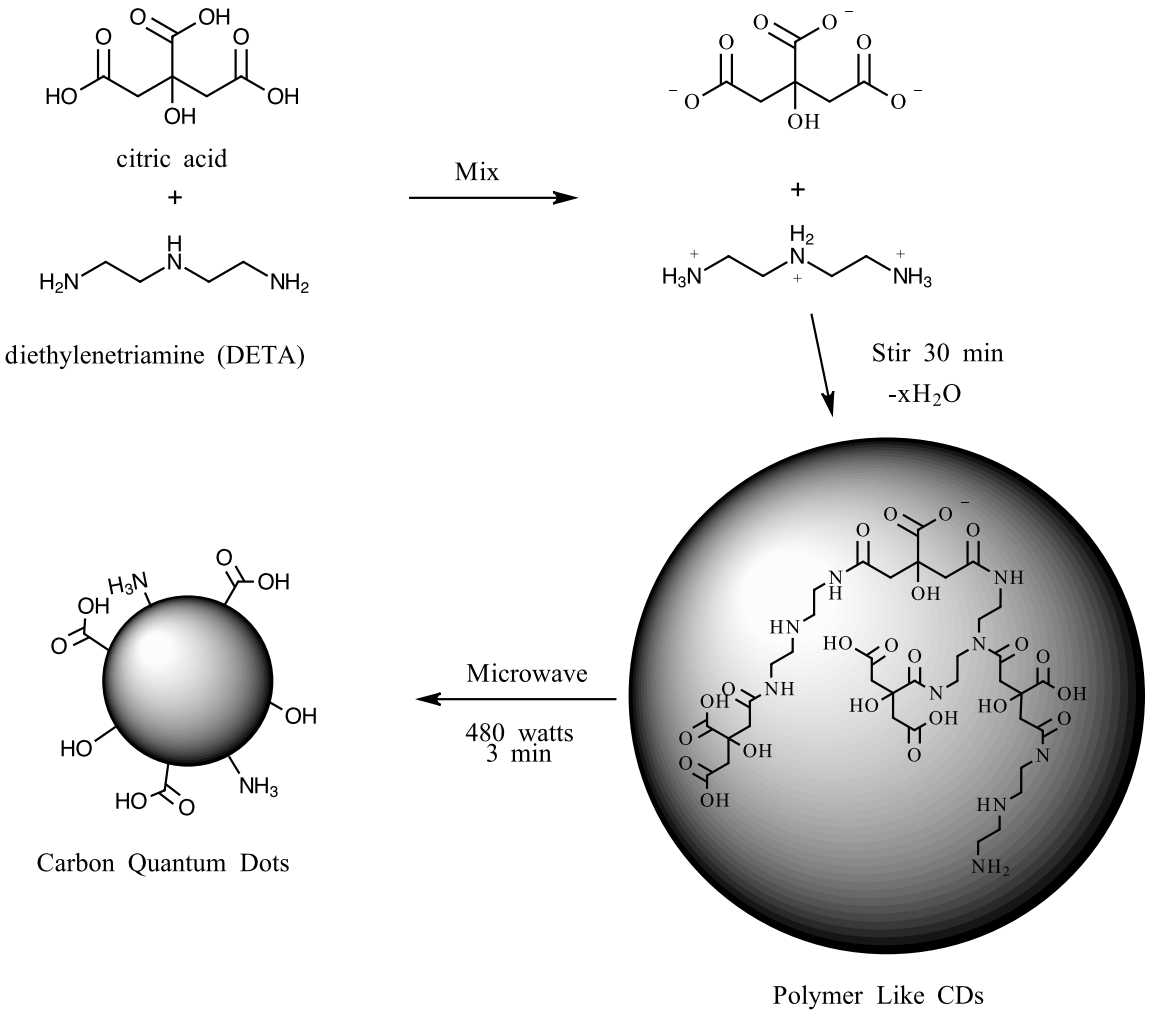

Figure 1. Microwave Synthesis of Carbon Quantum Dots.

Background:

Synthesis

Carbon dots can be synthesized from a host of different materials including lemon juice,[8] apple juice,[9] aspirin,[10] coffee grounds,[11] egg whites,[12] and even chocolate.[13] There are two basic approaches to the synthesis of carbon dots which are generally referred to as the top-down or bottom-up approaches.[8] The top-down approach involves the break-down of larger carbon structures such as graphite or carbon nanotubes to form the CDs. In contrast, the bottom up approach is the formation of CDs from small organic molecules by carbonization of these materials, citric acid being one of the most common precursors. These are often synthesized using hydrothermal or microwave-assisted methods. It has been suggested that since the heating efficiency of the microwave method is not related to the thermal conductivity of the precursors, it conserves both time and energy compared to other methods.[14] It may also help avoid undesirable side reactions and thus, it is the method of choice and will be used in this experiment.

The fluorescence of carbon dots can be enhanced if citric acid is mixed with an amino group-containing small molecule.[15] This is typically referred to as N-doping. A number of different amines have been used, but ethylenediamine (EDA) is probably the most common. However, in this laboratory exercise diethylenetriamine (DETA) will be used as it creates highly fluorescent CDs that can easily be purified.

The basic approach will be to mix citric acid and diethylenetriamine in water, stir the solution at room temperature for an appropriate time, then microwave it. While the complete structure of the carbon dots is not known, the basic process as it is typically envisioned[16] is outlined in Figure 1.

Purification

After microwaving the citric acid/diethylenetriamine solution, a crude mixture of carbon dots is obtained. However, this mixture contains not only the carbon dots, but also some unreacted starting materials and possibly some side products. Consequently, the carbon dots need to be purified. The traditional method in the literature is dialysis. Dialysis uses a semipermiable membrane that allows small molecules (starting materials) to pass through but stops larger structures such as carbon dots. Unfortunately, this technique is difficult and time-consuming (taking several days to a week), so it will not be used here.

A simpler and quicker method is column chromatography.[8] In column chromatography a mobile phase (or moving phase) is passed over a stationary phase (or nonmoving phase). For the purposes of this laboratory exercise, silica gel will be the stationary phase and water will be the mobile phase. Figure 2a shows how to make a mini chromatography column. From a theoretical standpoint, when a sample to be purified is dissolved in a small amount of mobile phase and introduced to the column, the various components separate as they move down the column as additional mobile phase is added. The larger particles (i.e. the carbon dots), which cannot enter the pores in the silica gel, move more quickly and elute earlier while the smaller particles (i.e. the starting materials), which do enter the pores, move much more slowly, thus separating the

materials. A chromatographic separation based on size, like this, is referred to as size exclusion chromatography. While this technique is not perfect, it provides a quick, reliable method to give reasonably pure carbon dots. The basic concept for the separation process is shown in Figure 2b.

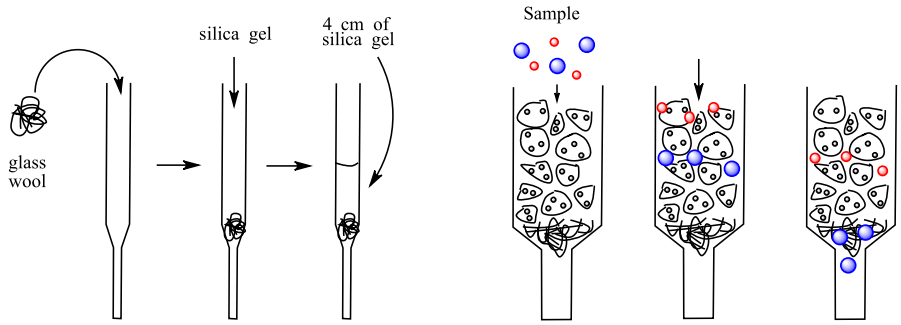

Figure 2. a) Building a silica gel column. b) The size-exclusion separation process.

Characterization

Any time a chemist makes a new material they characterize it, which means they collect basic data to make sure they actually have what they think they do. This can be accomplished by a variety of techniques including infrared spectroscopy (IR), ultraviolet/visible spectroscopy (UV-vis), nuclear magnetic resonance spectroscopy (NMR), and transmission electron microscopy (TEM). In the case of carbon quantum dots, their key properties are their absorption (generally in the UV region) and emission of light (generally in the visible region). Consequently, the primary method of characterization is obtaining a UV spectrum to observe the region of absorption and a visible spectrum to determine the region of light emission, or fluorescence.

Although the UV region of electromagnetic radiation includes much shorter wavelengths, for spectroscopic purposes the ultraviolet (UV) region of light is considered to be from 200 nm to 400nm. This can be broken down further into UV-A (200-280 nm), UV-B (280-315 nm), and UV-C (315-400 nm). Visible light is considered to be from 400 nm to 750 nm, since the typical human eye can see from about 380 nm to 750 nm. The Vernier SpectroVis® Plus or the Go Direct® SpectroVis® Plus spectrophotometers can be used to collect the desired spectra. The Vernier spectrometers are set up to have a range of 380 nm to 900 nm. Although the region of maximum absorption of carbon dots is typically below 380 nm, and thus, cannot be viewed using these instruments, you can still observe part of the absorption spectrum. You might also remember that according to Beer's Law ($A = \varepsilon \, l \, c$) that the absorbance (A) is directly proportional to the concentration (c). Although we will not be using this to make any direct measurements in this laboratory exercise, this can be used to give you an idea of reaction yield or the amount of sample you have in a given set of conditions.

The Vernier equipment can also be used to acquire an emission (or fluorescence) spectrum. Research-grade fluorescence spectrometers allow a person to choose any wavelength for excitation of the carbon dots, and this is typically the wavelength of

maximum absorbance from the UV spectrum. However, the equipment you will be using will only allow choices for excitation of 405 nm or 500 nm. You will need to decide which one to use. A typical absorption/emission spectrum is shown in Figure 3.

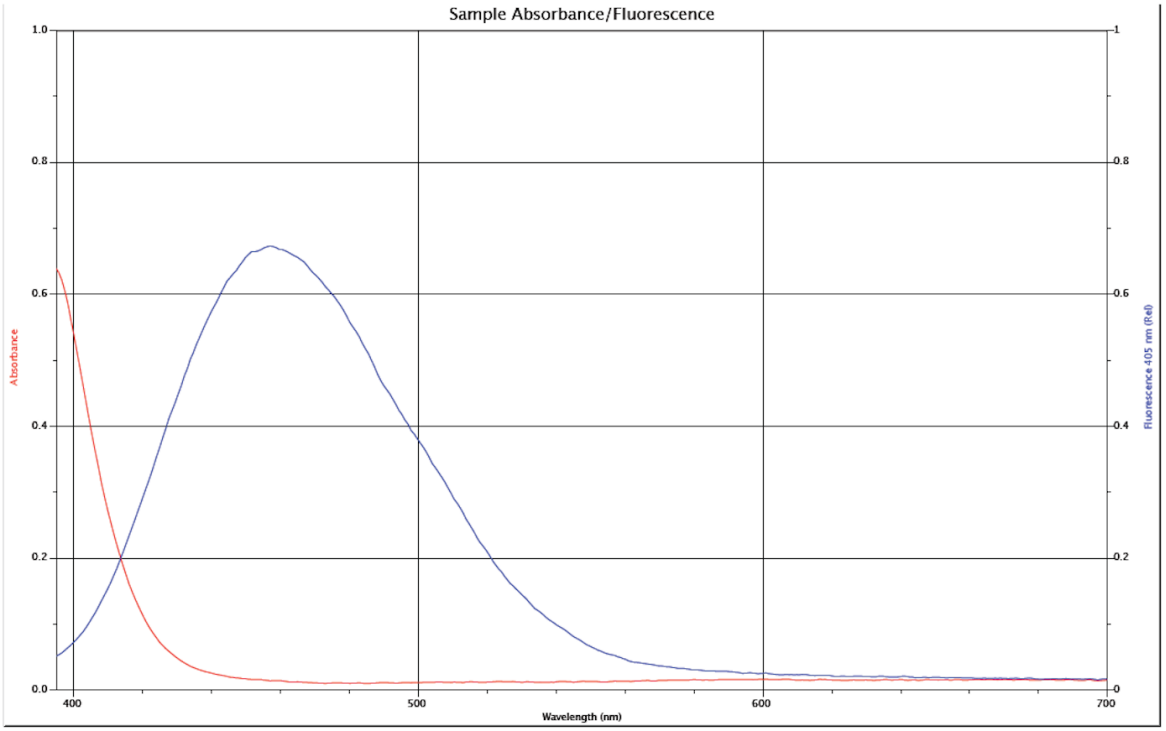

Figure 3. Sample Logger Pro® Absorption/Emission Spectra.

Application for the Collection of Latent Fingerprints

Although synthesis and characterization of new material, like carbon dots, is essential, the goal is the use of the material in some new application. Wang and coworkers reported that carbon dots made from pig intestines could be used to collect latent fingerprints.[17] Latent fingerprints, or those hidden from the eye, have been widely used in forensic investigations, since the friction skin ridge pattern is unique to an individual. Latent fingerprints, in a typical investigation, need to be recovered and preserved. Wang and coworkers showed that carbon dots imbedded in a film of polyvinyl alcohol (PVA) could be used to get a highly detailed image of the fingerprint and that the films had long term stability. Recent research has shown that carbon dots made of citric acid and diethylenetriamine are equally effective. The basic procedure involves mixing the carbon dots in the liquid PVA, drop casting them onto the fingerprint, and letting the film dry. Once dry, the fingerprint can easily be visualized by

holding the PVA film under a UV pocket flashlight that emits light around 395nm to 400 nm. An example of a fingerprint is shown below in Figure 4.

Figure 4. Sample of PVA/CD Latent Fingerprint under 395 nm UV Light.

Materials:

Citric acid (CA)

Diethylenetriamine (DETA)

Deionized Water

20 mL Scintillation vials

UV Lamp w/ 365 nm wavelength

Silica Gel 60 Å (230-400 mesh)

Disposable Pasteur pipets (6 or 9 inch)

Glass wool

Vernier Go Direct® SpectroVis® Plus or SpectroVis® Plus spectrometers w/cuvettes

Glass microscope slides

Polyvinyl alcohol (PVA)

LED UV Flashlight 395 nm wavelength

You should where safety goggles at all times. Handle all chemicals with care. Diethylenetriamine is hazardous to breathe and is an irritant to the skin. The synthesis should be performed in the fume hood and gloves should be worn. In case of skin contact wash the affected area immediately with soap and water. In case of eye contact, rinse with water for at least 15 minutes.

Experimental Procedure:

Synthesis of the Carbon Quantum Dots

1. Obtain a 20 mL scintillation vial and weigh out 0.770 g of citric acid (CA) in the vial.

2. Add 2.0 mL of deionized water to the vial and a small magnetic stir bar. Stir the solution until the citric acid is all dissolved.

3. *The next three steps (steps 3, 4, and 5) should be done in a fume hood.* Once all the citric acid is dissolved, add 0.370 mL (0.353 g) of diethylenetriamine (DETA) to the solution drop-by-drop with stirring. Once all the DETA is added, allow this mixture to stir for at least 30 minutes.

4. After 30 minutes remove the stir bar and stuff a Kimwipe® in the top of the vial. Microwave the solution on a power setting of 4 for 3.00 minutes. (*Note: The power setting depends on the microwave used. These directions are for a 1200 watt microwave.)* Be very careful when you handle this, as the vial is very hot at this point. Use a hot mitt to remove it from the microwave oven and let it cool for about 10 minutes.

5. At this point you should have a very viscous orange-red material in the scintillation vial. This contains your carbon dots (CDs). Add 5.0 mL of water to the vial and stir the solution until the carbon dots completely dissolve.

6. Use a 395 nm LED UV flashlight to view the solution. The solution should "glow" a bright blue color at this point.

Caution should be used when using the 395 nm LED UV light. The light emitted is in the UV region and is potentially harmful to the eyes. Do not look directly at the light.

Purification of the Quantum Dots

1. To purify the quantum dots, this lab will use a mini silica gel chromatography column. To prepare the column, obtain a 6- or 9-inch disposable Pasteur pipette and put a small piece of glass wool in the pipette to plug the outlet (see Figure 2).

2. Add about 4 cm of silica gel (60Å 230-400 mesh) to the pipette. This can easily be accomplished by putting some dry silica gel in a beaker and repeatedly putting the top of the Pasteur pipette into the silica gel until you get the right amount.

3. Clamp the pipette on a ring stand and then use a second Pasteur pipette to add 1 mL of deionized water to the column. Put a beaker underneath the column to catch any waste liquid. You can let the water drain through by gravity or you can apply pressure using a Pasteur pipette bulb. Repeat this a second time using another 1 mL of water. Drain the column until the water level is just at the surface of the silica gel. This wets the silica and rinses the column.

4. Transfer about 1 mL of your carbon quantum dot solution to the column. As before, you can let the solution drain through by gravity or you can apply pressure using a Pasteur pipette bulb. Drain the solution into the waste beaker until the carbon quantum dot solution reaches the top of the column. You can watch the progression of the carbon dots through the column using a 395 nm LED UV light. It will glow blue where the carbon dots are located.

5. At this point replace the waste beaker with a clean 20 mL scintillation vial. Add 1 mL of deionized water to the top of the column and drain it through by applying pressure using a Pasteur pipette bulb until the water reaches the top of the column. Repeat this three (3) more times using 1 mL of water each time. At the end you should have a total of about 4 mL of water in your vial and it should be a pale-yellow color. These are the purified carbon dots (CDs).

Collection and Visualization of the Latent Fingerprint

1. Obtain a clean 20 mL scintillation vial. Using a disposable syringe, transfer about 4 mL of polyvinyl alcohol (PVA) to the vial. This is actually a solution of PVA dissolved in water. It will harden over time.

2. Add to this solution 50 µL of the purified carbon dots and 300 µL of deionized water. Using a small spatula, stir the mixture thoroughly. It will be full of bubbles at this point. Put the vial in the microwave and microwave on power level 3 for 15 seconds. Let the vial cool for 20 to 30 minutes. Most of the bubbles should dissipate during this time. *You can go on and complete the spectroscopic characterization (absorption and fluorescence spectrum) while you are waiting for this to cool.*

3. While the PVA is cooling, obtain a microscope slide. Make sure it is clean. On the center of the slide, make a fingerprint. This is best done by rubbing your finger on the side of your nose or near your hairline to collect oils that are necessary for a clear fingerprint. Carefully touch your finger to the glass slide, but do not press hard as this will lead to a smudged print. If you look at the slide at different angles in the light, you should be able to see the fingerprint. If you do not see a clear fingerprint, clean the slide and repeat the process.

4. After you get a good fingerprint use a 1 mL disposable syringe (without a needle) to draw up the PVA/CQD solution. Do this slowly so that you do not create bubbles. Fill the syringe.

5. Slowly squeeze the PVA/QCD solution onto the fingerprint. Move the syringe around so as to cover the entire fingerprint. Keep the syringe near the slide so that bubbles are not introduced, but not so near that you damage the fingerprint.

6. Move the slide to a fume hood to dry. *It will need at least 24 hours to dry, but it can be left until the next laboratory period.* After it is dry, he PVA film can be removed from the glass slide using a spatula and the fingerprint viewed using the 395 nm UV LED flashlight.

Spectroscopic Characterization of the Carbon Quantum Dots

1. Obtain a laboratory laptop computer and a Vernier Go Direct® SpectroVis® Plus or SpectroVis® Plus spectrophotometer and set them up.

2. Open Logger Pro® using the short cut on the desktop. The spectrophotometer should automatically connect.

3. In the menu bar at the top, select "Experiment", then "Set Up Sensors", and finally "Spectrometer". This should open a spectrometer set-up window. Change the wavelength range from 380 to 950 nm (which it is currently set on) to 395 to 700 nm.

4. Using the "Experiment" menu, calibrate the spectrometer. Use deionized water as a blank.

5. After calibration, remove the cuvette containing the deionized water and add *1 drop* of your purified carbon quantum dot solution. Put the lid on, then gently shake the cuvette. Reinsert it in the spectrometer and click "Go" to obtain a UV spectrum. Let the spectrum collect for about 10 to 15 seconds and then click "Stop". This gives you the "Absorbance Spectrum", which is all the wavelengths at which the carbon dots absorb.

6. Since the key property of carbon quantum dots is their fluorescence, the next step is to obtain a fluorescence spectrum. To do this, you will need to change the Vernier Go Direct® SpectroVis® Plus spectrophotometer to the fluorescence mode. Go to the "Experiment" menu, select "Change Units" and then select "Fluorescence 405 nm" or "Fluorescence 500 nm" (whichever you have decided is best). *You should note that the Vernier spectrometer only has two choices for excitation, 405 nm and 500 nm.*

7. You do not need to calibrate the spectrometer. Once the units are changed, click "Start Collection". After 10 -15 seconds, click "Stop". This is the fluorescence spectrum, which is all the wavelengths at which the carbon dots emit light when they are excited using 405 nm light.

8. You should use Logger Pro® to plot both spectrums on the same graph. Make sure you give the graph a title and that the axes are labeled correctly. Print the graph.

Waste Disposal

Any excess, unused citric acid or diethylenetriamine should be put in the appropriately labeled waste container. The carbon dot solutions can also be placed in the same container.

Post-Lab Questions

1. The carbon dots are very water soluble. Considering what is known about their structure, why do you think they are?

2. What color is the chromatography column after you purified the carbon dots? Why do you think it is that color?

3. What was the wavelength of maximum fluorescence in the spectrum you collected? What color does this represent?

4. What do you think would happen if you used 500 nm to excite the carbon dots when you collected the fluorescence spectrum?

5. Describe the fingerprint you collected in terms of quality and ability to view the print. Why do you think the fingerprints can be viewed when using a 395 nm LED flashlight, but can't be seen under normal daylight?

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Pre-Lab Questions

1. Explain what a nanomaterial is.

2. Explain what fluorescence is in relation to semiconductor nanomaterials.

3. Briefly explain what quantum dots are and what properties make them useful. Also explain what makes carbon quantum dots, in particular, unique.

4. Briefly explain what column chromatography is and why it is done.

5. Explain what an absorbance spectrum is and what a fluorescence spectrum is and how they differ.