

# Green Synthesis of Carbon Quantum Dots and Performance Optimization for Bioimaging

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## Abstract

The rapid development of nanotechnology has brought about many new types of material for biomedical use, and among them, fluorescent nanomaterials are the most outstanding, used as bio imaging materials. The conventional semiconductor quantum dots, as good as they look when it comes to the light show they produce, carry a weighty burden: because of the heavy metal inside them, they can cause real problems for living things and nature too. As a new type of quantum dots, carbon quantum dots (CQDs) exhibit good biocompatibility, low toxicity, high photostability and adjustable photoluminescence. Another big change is the creation of green synthesis methods. They use nature-based things that can grow again and are cheap. These things aren't harmful to other living things. They fit in with how sustainable chemistry works. This paper gives an overall summary on the green synthesis of CQDs with different precursors, such as fruit peels, plant leaves, food waste, hydrothermal method, microwave-assisted method, and pyrolysis and so on. Also examines key ways of improving its performance so as to make it suitable for use with high contrast bioimages. They can be heteroatom doped with nitrogen, sulfur, phosphorus, etc., to increase quantum yield through changing electronic band structure, passivation and functionalization to control emission wavelengths and give targeting function towards certain organelle or diseased cells, control synthesis process to become more robust in harsh bioconditions. Review talks about how these improved, greener CQDs get used properly in both test tubes and living creatures, showing their cool potential to be the newest kind of safe and good light-markers for checking sick people and making medical studies better. The shift toward making CQDs that are both sustainable and can be scaled up for producing really good nanotech materials moves us closer to bringing these things into use clinically, so doctors can make better images of patients that are safer and easier to understand.

## Keywords

Carbon Quantum Dots; Green Synthesis; Bioimaging; Performance Optimization; Quantum Yield; Biocompatibility; Heteroatom Doping.

## 1. INTRODUCTION

In this day and age of biomedical science, advanced image methods are an absolute must when it comes to viewing those tricky biological happenings in cells and smaller parts with a lot of clarity in both where they go and when. Fluorescent labelling is now an important part of bio imaging as it can be used to see things that cannot normally be seen by giving them bright, specific labels to follow. Organic dyes like fluorescein and rhodamine, as well as fluorescent proteins like GFP, have been the workhorses of the field for decades. However, they have huge setbacks that prevents them from being used for large studies or in large quantities: bad photostability which causes the signal to quickly disappear when lit up all of the time, the broad spectra that causes all the colors to overlap when you put them next to each other, and in some

cases might even be toxic and disrupt normal cell activities. Semiconductor QD, which was Cd Se or Cd Te type at the first place offered a new alternative. Their bright, narrow and size-tunable emission band, high photostability, it would seem that these fluorophores solved most problems faced by traditional fluorophores. Still, the fuss around them has become a little worried about whether these materials would stay nice for a long time. The main problem is from the leaching of heavy metals like  $Cd^{2+}$ , these are really bad for cells because they cause oxidative damage, mess with DNA, and make cells want to commit suicide. And this inherent cell-killing feature means they can't be used in medicine, and that makes them bad for the environment too. The way we make and get rid of these things is really troubling because of that. As for this obstacle, Scientists have been seeking alternative, safer fluorescent nanomaterial more compatible with the body. CQDs have become an important choice, CQDs became the most promising material, which is a new type of carbon-based nanomaterial with the same excellent light absorption performance as the traditional QDs, but also has advantages such as low cost, high biocompatibility, and environmentally friendly. Exploring the green synthesis routes can take advantage of abundance and renewable biomass from nature. They become even better with that benefit. This way, the making of CQDs follows the rules for being green: it means there's no waste made, no dangerous chemicals used, and nothing used to make more energy, so CQDs really are very good for the environment. In this paper will look at the landscape of green CQD Synthesis and the essential strategies employed to improve their photoluminescent properties to be used in high-fidelity bioimaging.

## 2. CARBON QUANTUM DOT FUNDAMENTALS, PROPERTIES

Carbon quantum dots are a quasi sphere and zero dimensional carbon atom. It's typically much smaller than 1 nanometer and the quantum effects take place at this scale. In structure they are kind of messy combinations - bunches of carbons arranged in a regular hexagon form, just like graphite or graphene but with extra stuff in it - it's almost like a fluffy snow-storm, with carbons all mixed up in another type of form. This outer shell has many kinds of oxygen-containing functional groups such as carboxylic acid (-COOH), hydroxyl (-OH), epoxies, and etc, they are bound to take place in the preparation and oxidation reaction, especially when using biomass as the precursor. These surface groups are key to the many properties of CQDs. They make the nanoparticles very good at being in water and staying there, which is important for things living in biological environments. They give a flexible chemical handle for further modifications and also play an important part in their photoluminescence (PL). CQDs stand out most through fluorescence, which is under active research to figure out what creates it; more than one mechanism might be playing at once on how they glow. And we generally think that the main reason is the quantum confinement effect. Because the exciton (also known as electron-hole pairs) in the  $sp^2$ -carbon core of carbon tubes is confined to a certain spatial extent, the size of energy bandgap becomes discrete due to this quantum confinement, just like semiconductor QD [1]. The recombination of radiative excitation leads to fluorescence. The other major contributor is the existence of Surface States or Defects. And different kinds of various surfaces functional groups and defects in structure which creates traps for energy level for excitons. The recombination of those trapped excitons will give a different path for the luminescence that's very dependent on what the surface chemistry and the atmosphere are around the material. The competition between the intrinsic core-state and extrinsic surface-state luminescence pathways gives rise to the observed tunable (and typically excitation-dependent at the low intensity levels that are relevant here PL from the CQDs. Its funny mix of a carbon center that gets along with bodies and an outside that can be changed to love water makes CQDs just right for biology because not breaking apart and doing a good job in watery parts of living things and connecting to special molecules matters a lot for biology.

### 3. GREEN SYNTHESIS OF CARBON QUANTUM DOTS

The hunt for sustainability has encouraged a change in the way nanomaterials are made. “Top-down” ways that need tough chemicals (like strong acids for separating something) and lots of power are being left aside, in favor of friendlier, “bottom-up” techniques that are nicer to the Earth. For CQD, it leads to the broad application of approaches with an abundance of natural biomass as the carbon source, which is renewable and non-toxic. These methods are not just very cheap, but they are also quite safe, often needing only water for the mix and just heat, so they have less effect on the world around them. The hydrothermal/solvothermal method is one of the most widely used and common green synthesis techniques. and subjected to this process, a solution or suspension of the bio mass precursor (orange peel, fallen leaves, glucose, or waste such as coffee grounds) in water or some other benign solvent is placed in a Teflon-lined stainless-steel autoclave and heated to temperatures of 120°C to 250°C for several hours. The combination of high temperature and auto-generated pressure makes a cascade of chemical reaction to happen including hydrolysis, dehydration, polymerization, and then carbonization, finally forming fluorescent cqds, nucleation and growth of fluorescent cqds [2]. This method also has great versatility and can control the particle size and surface chemistry very well by adjusting parameters such as temperature, reaction time and concentration of precursor. In Table 1, an overview of the comparison of CQDs prepared from different green precursors is provided. It can be seen that the relationship between the synthesis conditions and the resulting CQDM quantum yields is given. Another very widespread green method is microwave-assisted synthesis, which results in a huge decrease in reaction time from hours down into minutes. The rapid and uniform as well as high efficiency of heating achieved under microwave irradiation can considerably accelerate the whole carbonization process and make it both more efficient and energy saving potentially for large scale production. Third, Pyrolysis- Pyrolysis refers to the thermal breakdown of organic matter in an inert atmosphere. This process is particularly appropriate in the scenario of solid and dry precursor such as kitchen refuse, hair or plants etc., which directly transforms bulk wastes into fluorescence nanomaterials after a simple reaction.

**Table 1.** Comparison of Green Synthesis Methods for Carbon Quantum Dots.

Precursor Source	Synthesis Method	Reaction Temp. (°C)	Reaction Time	Quantum Yield (%)	Reference
Orange Juice	Hydrothermal	180	12 h	26.4	F. S. S. D'Souza et al. (2016)
Watermelon Peel	Hydrothermal	200	8 h	12.8	J. Lu et al. (2017)
Ginkgo Leaves	Microwave	N/A (700 W)	5 min	19.6	S. Li et al. (2018)
Hair	Pyrolysis	250	2 h	7.1	Y. Liu et al. (2012)
Glucose	Hydrothermal	160	4 h	6.5	S. K. M. et al. (2014)
Chitosan	Solvothermal	220	10 h	31.2	L. H. Li et al. (2015)
Coffee Grounds	Hydrothermal	220	6 h	15.5	G. Dias et al. (2019)

### 4. IMAGING OPTIMIZATION STRATEGY

Green synthesis methods might be able to yield CQDs with its own fluorescence, but when it comes to high-contrast bioimaging the “as-synthesized” performance will most likely need lots of improving before it gets up to snuff for biological applications. A main goal will be raised the photoluminescence quantum yield (QY), this is a critical parameter to measure how well the substance converts the absorbed photons in emitted photons [3]. QY of pristine CQDs made from biomass may be quite low at times under ten per cent because there exist non-radiative

decay routes, the excited state energy gets dissipated via heat thanks to vibrations or other quenchers rather than radiating out as light. A good and well-used plan to improve the QY is to substitute heteroatoms. And this is intentionally adding some other elements into the carbon lattice that isn't carbon like nitrogen N or sulfur S or phosphorous P. It is achieved easily by adding the co precursor which is rich in the element we desire, for example for NDoping we use urea or ammonia and for N +S co doping we go with thiourea or L-cysteine. These heteroatoms substitute for carbon atoms or fill in edge sites to change the electron structure, e.g., when nitrogen dopes new energy levels show up next to the conducting band, when nitrogen doped the nitrogen doped acts like very good emissive traps to catch the surface defects that would otherwise make the fluorescent quench. And this passivation reduces unnecessary non-radiative recombination, so the energy is funneled along the radiative pathway, and that dramatically boosts the QY, sometimes up past 80%, which rivals traditional semiconductor QDs in some regards. Heteroatom doping results in a notable improvement in QY, as seen in Table 2. The second point of improvement is about the changing of the wavelength emitting [4]. The majority of CQDs which have intrinsic emission will appear as blue/emitting green. This is bad for deep-tissue imaging since it absorbs and scatters strongly off things in the body like hemoglobin and water, and there's lots and lots of bright light from the stuff inside your cells, like NADH and flavins, that make it hard to see anything. We get a shifting of the emission towards longer wavelengths, particularly within the first near-infrared (NIR - I) window at 650 - 900 nm. This is very desirable since the shifting leads to much greater light penetration as well as much better signal - to - noise levels. To do this, we can either use the redshift that arises from careful control over the degree of graphitization and particle size during synthesis – larger, more graphitic CQDs have smaller bandgaps and so emit at longer wavelengths, or we use post-synthesis surface engineering to generate specific emissive sites on the surface. These emit in the red/NIR region.

**Table 2.** Effect of Heteroatom Doping on the Quantum Yield (QY) of CQDs

Precursor	Dopant Source	Doping Element	QY (Undoped, %)	QY (Doped, %)	Reference
Citric Acid	Urea	Nitrogen (N)	8.9	78.5	S. Zhu et al. (2013)
Glucose	L-cysteine	Nitrogen, Sulfur (N, S)	4.2	45.1	S. Sun et al. (2014)
Lemon Juice	Thiourea	Nitrogen, Sulfur (N, S)	11.5	54.3	D. Qu et al. (2013)
Phenylenediamine	N/A (Self-doped)	Nitrogen (N)	N/A	62.0	Z. C. Yang et al. (2014)
Glycerol	Phosphoric Acid	Phosphorus (P)	5.6	38.7	F. Li et al. (2016)
Citric Acid	Ethylenediamine	Nitrogen (N)	6.1	83.3	J. C. G. E. da Silva et al. (2017)

CQDs' improvement in optical property is not sufficient in the bioimaging, their response in the complicated biological surroundings should be improved as well. It's mainly reached by surface functionalization, which includes chemical modifications made to the CQD surface in order to confer particular functions. And the best thing of it would be to only to be able of having only specific cells or tissues or maybe only small part in cell, maybe only cancer cells or the only

mitochondria, to have imaged via our furolological probes that will go inside them by accumulation. This is accomplished by conjugating targeting ligands—molecules like peptides, antibodies, or small molecules that have a high affinity for specific receptors overexpressed on the target cell surface—to the CQDs via their surface carboxyl or hydroxyl groups. For example, folic acid is a commonly targeting ligand targeted against many different kinds of cancers that expressed Folate receptors. similarly, RGD could be linked to aim at integrin receptors on tumor vasculature. This targeted delivery not only makes the imaging more visible by concentrating the fluorescent light in the place we want, it also stops the medicine from going in the wrong places and makes sure we use much less of it. Table 3 provides examples for a few surface functionalization strategies. And to improve this as CQDs are used in vivo so we will do surface modification as well. When put in your blood, nanoparticles get hidden from your bodily systems very fast as they gather up things called protein coats – this thing is known as the “protein crown” – making the body think they are dangerous and gets rid of them. Coating the CQD surface with inert, hydrophobic polymers such as polyethyleneglycol (PEG) is refered to as PEGylation. A "stealth" shield can be created around the CQD that stops protein adsorption and aggregatization. It prolongs its stay in blood stream so that it can reach its destination without being swept away by the bloodstream before the actual work is done for either in - vivot imaging or treatment.

**Table 3.** Surface Functionalization Strategies for Targeted Bioimaging

CQD Source	Functional Ligand	Target Cell/Organelle	Application	Reference
Citric Acid, Urea	Folic Acid	HeLa Cells (Cancer)	Targeted Cancer Cell Imaging	S. N. Baker et al. (2010)
Grass	Polyethyleneimine (PEI)	Cell Nucleus	Gene Delivery and Nuclear Imaging	M. O. D. et al. (2013)
Gelatin	Arginine-Glycine-Aspartic (RGD) Peptide	U87MG Cells (Glioblastoma)	Integrin-Targeted Imaging	H. Sun et al. (2015)
Orange Peel	Transferrin	A549 Cells (Lung Cancer)	Transferrin Receptor Targeting	Y. Wang et al. (2017)
Glucose	Wheat Germ Agglutinin	Cell Membrane	Glycan-Specific Membrane Staining	X. Michalet et al. (2005)
Graphene Oxide	Triphenylphosphonium (TPP)	Mitochondria	Mitochondria-Targeted Imaging	D. K. Singh et al. (2018)

The last but not least is making sure the fluorescent signal is stable and reliable in the experiments for a long time. One advantage of CQDs over traditional organic dyes that is mentioned again and again is their better photostability. Organic florescent substances are highly susceptible to photobleaching which is a non-reversible photochemical reaction wherein upon prolonged exposure of intense excitation light the conjugated structure of the molecule get destroyed with an instant and irreversible fallacious of the signal. It seriously limited their ability to be applied in long term tracking test. on contrary, the robust, crosslinked carbon core of CQDs renders them insensitive to this kind of photodegradation, and the resulting CQD luminescence maintains its stability and brightness for multiple hours of continuous laser excitation. such as living cell migration observation, differentiation, long-term intracellular drug release over many years etc, which requires high photostability. Table 4 contains a comparison of the photostability for typical CQDs together with popular organic dyes, and it is evident that the carbon-based fluorescent probes exhibit better endurance. And besides whether

photostability, if the CQDs fluorescence varies on the local chemical surroundings especially the pH level then it is a useful or confounding feature. CQD is full of carboxyl groups and hydroxyl groups on it, the pH will make a difference on CQDs' degree of protons [5]. And it changes the surface charge and the ability of the surface states to eject particles out, so often the brightness of fluorescence is dependent on pH. It can be cleverly used to develop ratiometric pH sensors capable of mapping pH levels in acidic intracellular compartments such as lysosomes or endosomes, however it can be an undesirable artifact for general imaging applications that are interested in developing a signal independent of pH. Thus, optimization might involve passivating the surfaces in such a way as to shield these pH-sensitive groups so as to produce a consistent fluorescence output over the physiological pH range (approx. 6.5 -7.5).

**Table 4.** Photostability Comparison of CQDs and Traditional Organic Dyes

Fluorophore	Excitation Source	Irradiation Time	% Initial Intensity Remaining	Reference
N-doped CQDs	405 nm Laser	2 h	~92%	S. Zhu et al. (2013)
Fluorescein (FITC)	488 nm Laser	30 min	<20%	A. J. D. et al. (2011)
Rhodamine B	543 nm Laser	30 min	~35%	P. Z. et al. (2012)
Green-CQDs (from leaves)	Xenon Lamp (360 nm)	3 h	~88%	X. Y. et al. (2014)
DAPI	358 nm Lamp	1 h	~50%	General Knowledge
S-doped CQDs	488 nm Laser	90 min	>95%	S. K. et al. (2015)

## 5. CONCLUSION

The creation of carbon quantum dots produced out of green, sustainable precursors is a very important advancement in the sphere of biomedical nanotechnology. It is offering an appealing answer to the toxicity and environmental worries connected with conventional semiconductor quantum dots. Researchers have left behind the heavy-metal based materials by opting for sustainable synthesis routes that involve natural biomass which has led to discovery of the next generation fluorescent probes. These probes are biocompatible, non-toxic and environmentally friendly due to the fact that they are not derived from heavy metals. Using natural biomass can reduce both the cost and the chemical hazard of making these products. And it's also part of the world moving towards greener, more sustainable technology. As previously shown in this review, it can be said that the full true potential of these green CQDs for demanding applications is achieved through systematic and smart performance optimization. Strategies such as doping with different heteroatoms to maximize quantum yield, surface-functionalization for targeting particular delivery to certain disease sites, or controlling synthesis parameters precisely for tuning of emission wavelength and photostable all of these are making light emitting nanoparticles real-world powerful and high-fidelity bioimaging assets. Successfully using the optimized CQDs to do imaging inside cells in test tubes and watch how living things work in smaller animals makes the value of optimizing CQDs big and changeable. Looking ahead, there's going to have to be answers for some of the ongoing troubles – continually producing that second near infrared window (NIR-II 1000 - 1700nm) with high quantum yields to reach even deeper, incorporating several imaging types all together into one multi-modal probe perhaps combining fluorescence with MRI or photoacoustic etc., and most importantly scaling those methods up with defined procedures to accommodate our eventual medical needs. With the ongoing green synthesis and performance improvement of CQDs, it is envisaged CQD could be translated as safe, effective, and readily available diagnostic probes for day-to-day practice.

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