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RESEARCH ARTICLE

To Detect Pesticide in Food Beverages by Partial Structural Examination of CDs with the Help of Fluorescence Quenching Study

Gaurav Dutta, Pranav Dave*

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Abstract

In recent world, there are lots of crimes happen by adding some hazardous or poisonous materials in the food and food beverages. So it is very hard and cost effective to detect those materials. By one step synthesising carbon dots, the hydrothermal method is very accurate and precise. Carbon dots were synthesised by using hydrothermal methods with different precursors (ethanolamine, urea, ammonium thiocyanate). Fluorescence quenching study has been done by using spectrofluorophotometer by adding some amount of food beverage in carbon dots solution. The structural property of CDs and the fluorescence quenching study have been examined by UV-vis spectrometer, atomic force microscopy, spectrofluorophotometer and FT-IR. The comparisons of precursors conclude that ethanolamine is the accurate and precise precursor to synthesise carbon dots with limited crystalline size (5 nm-40 nm). It shows better and less time-consuming detection of the pesticide in appy fizz drink. By using ethanolamine batch, the fluorescence graph gradually decreased by adding 20 ppm carbon dot solution after each reading.

Key Words: *Carbon dots; Pesticides; Precursors; Fluorescence quenching study; Detection of pesticide*

Introduction

Carbon dots (CDs) have emerged as most precious gifts in nanotechnology because of their magical properties and applications. CDs are typically carbon nanoparticles, most of them with average diameter less than 10 nm. These materials are derived from organic compounds and are stable in aqueous media which is extremely significant in terms of biological points of view [1,2]. Surface engineering plays a significant role for CDs in diversified applications like explosive detection, chemical sensing, food safety, bio imaging, drug delivery, energy conversion, and photo catalysis. Photo physical and chemical properties of CDs vary dramatically by tuning their shapes and sizes and also by doping heteroatoms such as oxygen, nitrogen, phosphorus, sulphur, and boron [3-5]. Moreover, photo stability, high quantum yield, biocompatibility, low toxicity, water solubility, good conductivity, and environmental friendli-

School of Engineering & Technology, National Forensic Sciences University, Gandhinagar, 382007, India

**Corresponding author: Pranav Dave, Researcher, School of Engineering & Technology, National Forensic Sciences University, Gandhinagar-382007, India, E-mail: pranavdave77@yahoo.com*

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ness of CDs receive additional advantages over other well-recognized quantum dots (QDs) like grapheme quantum dots (GQDs), metal oxides (ZnO, TiO_2), and inorganic QDs (ZnO-PbS, CdSe, CuInS/ZnS, and CuInS/ZnS) [6-8]. In fact, non-carbon QDs are not much graceful in their field of applications compare to CDs, because of their serious health and environmental issues. CDs can be synthesized from both natural and synthetic organic precursors. Synthetic methodologies that are very frequently used in this concern are microwave irradiation, hydrothermal treatments, ultrasonic irradiation, laser ablation, electrochemical, arc discharge, and pyrolysis [9-11].

Nanotechnology is a rapidly evolving research area of science. The fundamental principles of nanotechnology are said to arise in 1959 talks given by physicist Richard Feynman but it wasn't the year 1980s that nanotechnology became a recognized field. The discovery of fullerenes (C60) in 1985 fuelled the development of nanotechnology. The National Nanotechnology Initiative, a federal government programme in the United States for nanoscale science, innovation, and technology research and advancement, to describe nanotechnology where the initiative decided to characterise it as the manipulation of matter with at least one dimension ranging from 1 nm to 100 nm in scale [12,13]. A wide range of nanotechnology disciplines includes organic chemistry, inorganic chemistry, surface science, molecular biology, semiconductor physics, energy storage, and molecular engineering [14]. Carbon dots are tiny nanoparticles (generally 10 nm) that are extremely photo luminescent, excellent biocompatibility, water soluble, and are thought to be nontoxic. This distinguishes them from conventional quantum dots and makes them a promising candidate for a wide range of exciting applications [15,16]. Nanomaterials with size less than 10 nm, are said to be the Carbon quantum dots (CQDs), also called as carbon dots (CDs) CQDs have robust physicochemical properties, as well as strong biocompatibility and water dispersibility, so easy to be functionalized. CQDs with quasi-spherical microstructures have excellent optical properties such as controllable and stable fluorescent characteristics, photo bleaching resistance, and excellent ultraviolet (UV) absorption efficiency, which has piqued the interest of researchers and developed a new research hotspot in the field of materials [11]. They are said to be liable and highly active to be connected with other atoms or chemical groups to establish or to serve different purposes [17,18].

CQDs are having preferable optical properties to reduce the participation of toxic metal elements in the preparation process, thus minimising the negative effects on the environment and realising low-cost and renewable synthesis on compared with conventional semiconductor quantum dots and organic fluorescence dyes [19]. Carbon-based quantum dots are a new class of carbon nanomaterials with sizes less than 110 nm, composed of graphene dots (QGDs) and carbon quantum dots (CQDs, C-dots, or CDs). They have been procured in 2004 during the preparative electrophoresis purification of single-walled carbon nanotubes, and then in the year 2006 during the laser ablation of graphite powder and cement. Because of their innocuous, plentiful, and low cost, carbon-based quantum dots with intriguing properties have rapidly become an emerging star as a new Nano carbon member. Carbon is a natural black substance that was previously thought to have low water solubility and moderate fluorescence. Because of their solubility and endurance factor, carbon-based quantum dots are been attentively focused in environment with the researchers and also for their luminescence, they have the noticing factor of carbon Nano lights [20-22].

Experimental Details

Materials and Method

All the materials and solvents were purchased from commercial sources (Sisco research laboratories Pvt. Ltd., India) and used as received unless stated otherwise. Citric acid (anhydrous, extra pure AR, 99.5%), ammonium thiocyanate (ACS reagent, 97.5%), urea (anhydrous, 95%) and Ethanolamine (extra pure AR, 99%) were obtained from SRL company and used without further purification process. Milli-Q water and spectroscopic grade solvents were used for all measurements. Fourier-transform infrared (FTIR) spectra were measured on a JASCO FT-4600 spectrophotometer by putting directly the sample solution into it. Solution phase optical absorption spectra were recorded on a JASCO V-670 spectrophotometer by using a quartz cell with optical path length of 1 cm after diluting the sample in Milli-Q water. Steady state photoluminescence (PL) spectra were recorded using a JASCO FP-6500 fluorophotometer by using a quartz cell with optical path length of 1 cm. All solution phase PL measurements were carried out at RT (room temperature) and without removing dissolved O_2. Atomic force microscopy (Nanosurf C300) was used to find the particle size after diluting the sample into Milli-Q water and dried it on MICA sheet at room temperature. The gel was characterised by Brookfield viscometer after diluting the sample in 4:6 ratio with Milli-Q water. After synthesising, all the carbon dots batches are been stored in freezer because CDs are hydrophilic by nature; it can easily absorb the atmospheric moisture.

Synthesis process of carbon dots with precursor Ethanolamine

The synthesis was followed by procedure reported by Gregory Ethan Le Croy et al. in 2014 [23]. In brief, the one step hydrothermal process was used to synthesis crystalline carbon dots. 500 mg of Citric acid (CA) was dissolved in 45 mL of Milli-Q water along with 1 mL of ethanolamine. The solution was mixed for few minutes on the stirrer and then it was transferred into the Teflon coated 90 mL of white chamber, which was a part of small autoclave system. After that the autoclave was heated along with the solution in it in muffle furnace at 175°C for 4-5 h. The solution was cooled and characterised by FT-IR and AFM for structural property. The fluorescence property was characterised by UV-Vis-spectrometer and fluorophotospectrometer.

Synthesis process of carbon dots with precursor Ammonium Thiocyanate

400 mg of Citric acid (CA) was dissolved in 30 mL of Milli-Q water along with 400 mg of ammonium thiocyanate. The solution was mixed for few minutes on the stirrer and then it was transferred into the Teflon coated 90 mL of white chamber, which was a part of small autoclave system. After that the autoclave was heated along with the solution in it in muffle furnace at 175°C for 4-5 h similar to the first process. The solution was cooled and characterised by FT-IR and AFM for structural property. The fluorescence property was characterised by UV-Vis-spectrometer and fluorophotospectrometer [24].

Synthesis process of carbon dots with precursor Urea

400 mg of Citric acid (CA) was dissolved in 30 mL of Milli-Q water along with 400 mg of urea. The solution was mixed for few minutes on the stirrer and then it was transferred into the Teflon coated 90 mL of white chamber, which was a part of small autoclave system. After that the autoclave was heated along with the solution in it in muffle furnace at 175°C for 4-5 h similar to

the first process. The solution was cooled and characterised by FT-IR and AFM for structural property. The fluorescence property was characterised by UV-Vis-spectrometer and fluorophotospectrometer [8].

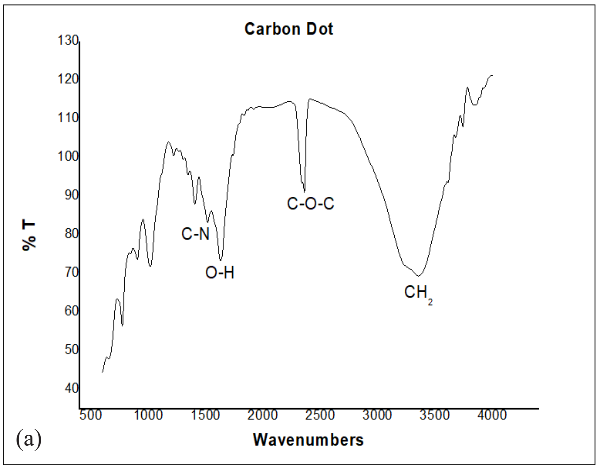
Preparation of chemo sensor solution

Take 1 mL of appy fizz apple-based fruit juice and 500 µL of carbon dot solution with the different ratio of Glyphosate pesticide (20, 40, 60, 80 ppm) and analyse it with spectrofluorometre. By the instrument, we are about to know the quenching or degradation of the intensity of the carbon dots, which concludes the detection of the pesticide by lower the intensity of carbon dots.

Result and Discussion

Characterization study of CDs

For analysing the structural property of carbon dots, the XRD instrument was not useful because of the hydrophilic property of carbon dots. So the structural property was analysed by the FT-IR instrument. FT-IR instrument can detect the functional group of the material, so different materials can be detectable by the FT-IR graph. (Figure 1) shows the FT-IR graph, which concluded the different carbon, oxygen and hydrogen bonds.



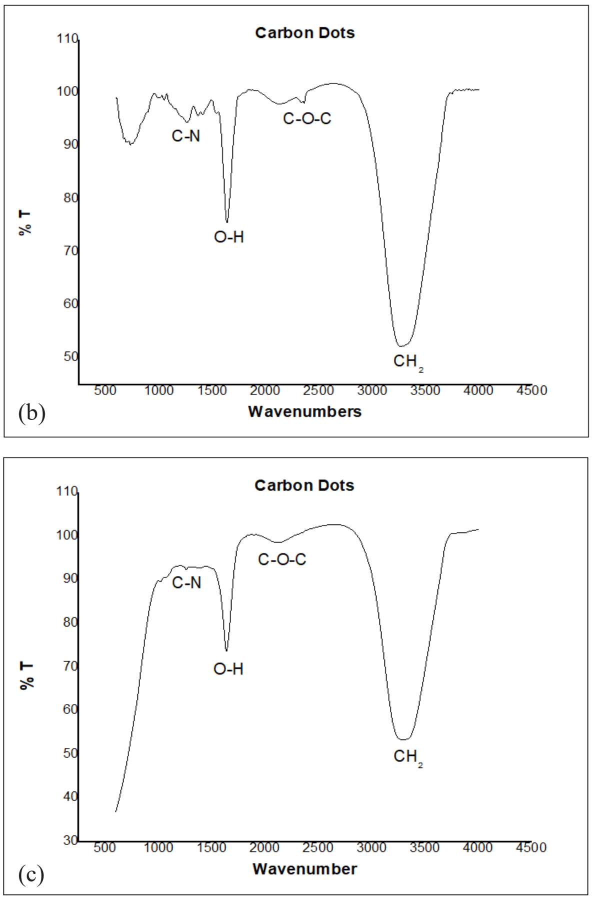


Figure 1) *FT-IR spectra of Carbon Dots by using different precursors (A) Ammonium thiocyanate, (B) Ethanolamine and (C) Urea.*

Here the absorption band 3450 cm^{-1} shows the presence of Methylene group, which correspond to the saturated hydrocarbon from amorphous carbonic framework in the CDs. And 3500-3100 cm^{-1} concludes the O-H and N-H stretching vibrational peaks. Due to little bit vibration of the O-H group, small peak can be detected on 3441 cm^{-1}. The peaks at around bands 1600 cm^{-1} and 2400 cm^{-1} conclude the carboxylic group. Here another stretching vibration peaks found nearest to the 1500 cm^{-1} with O-H and C-N group. A strong absorption peak nearest to 2450 cm^{-1} is assigned to C-O stretching vibration peak, revealing the existence of abundant C-O or C-OH groups. Further, the fluorescence property of CDs was analysed by the UV-Vis-spectrometer and Fluorophotospectrometer. (Figure 2) shows the UV-Vis spectrometer spectra of CDs. The graph shows some oscillator peaks between 300 nm to 500 nm wavelength.

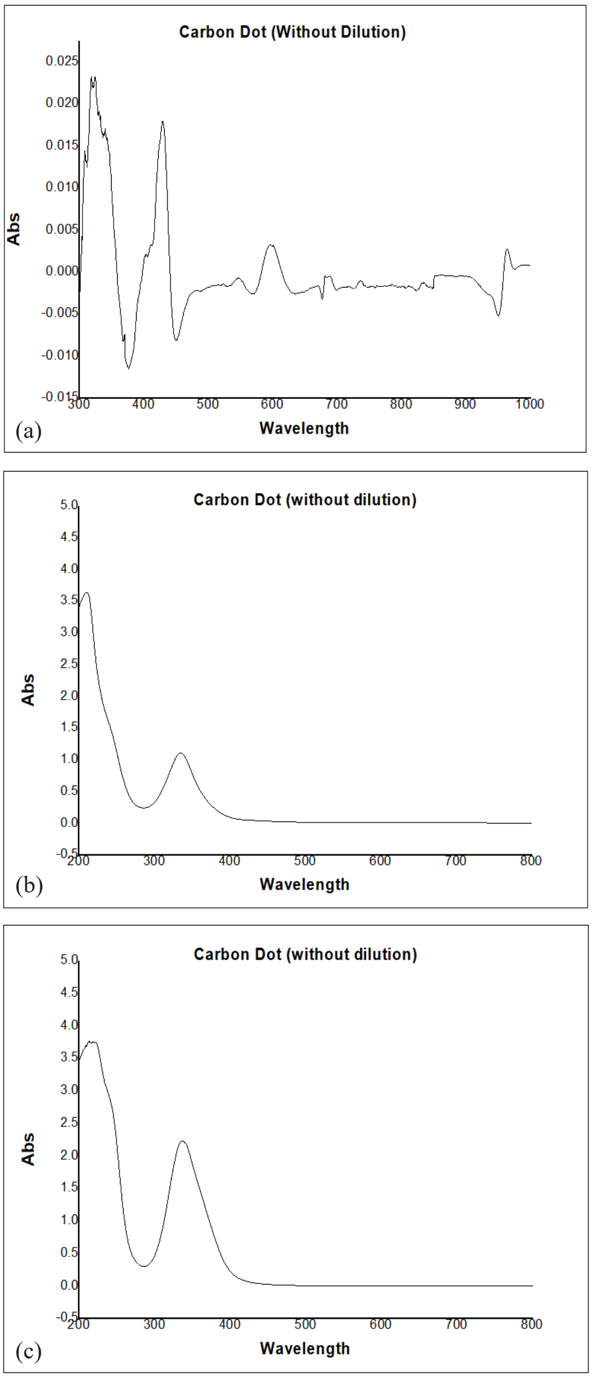


Figure 2) *UV-Vis spectra of Carbon Dots by using different precursors (A) Ammonium thiocynate, (B) Ethanolamine and (C) Urea.*

The graph also indicates little bit dissociation energy after 650 nm wavelength. It has maximum intensity around 350 nm to 400 nm wavelength. The blue fluorescence absorbs at its highest intensity at 380 nm wavelength. But it is also depend on the parameters of the spectroscopy. Similarly, (Figure 3) shows the fluorescence spectra of the CDs.

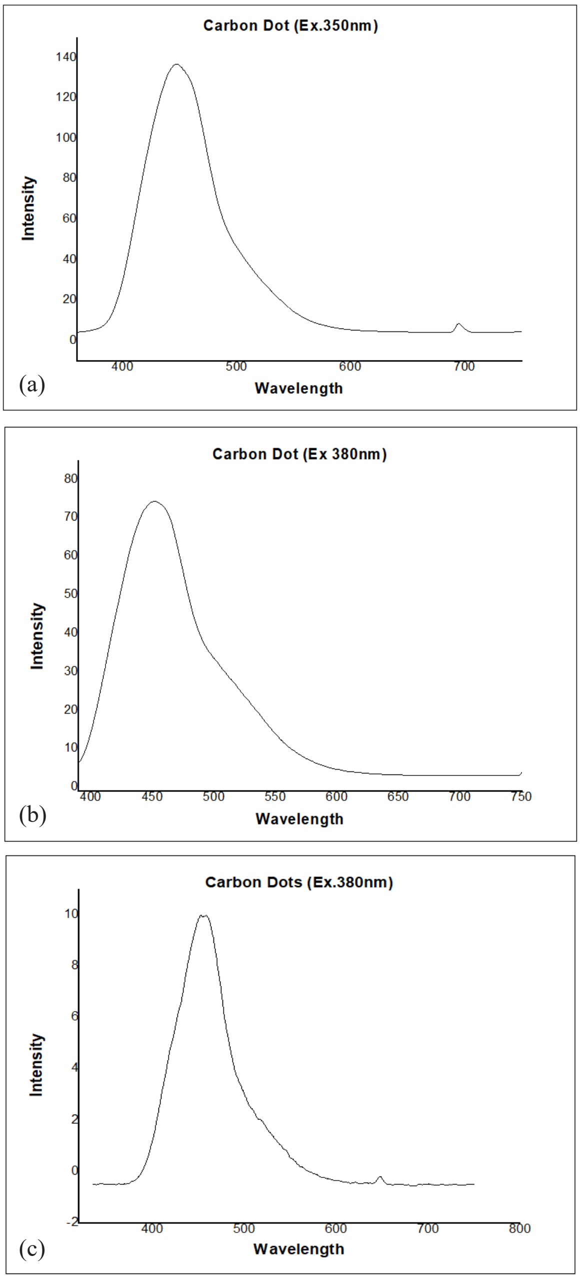


Figure 3) *Fluorescence spectra of Carbon Dots by using different precursors (A) Ammonium thiocynate, (B) Ethanolamine and (C) Urea.*

Carbon Dots have their own excitation peak at 459 nm wavelength. After putting the excitation wavelength 350 nm in spectroscopy parameter, the typical and diverse fluorescence absorption shows at the 459 nm wavelength. In all the batches, the excitation wavelength has been same (350 nm) which is one of the parameters. After changing the excitation wavelength at 300 nm and 325 nm, the results are same as we found the result with 350 nm excitation wavelength. In contrast, the absorption peak of CDs in water is at 350 nm and the luminescence spectra between

400 nm to 500 nm show a maximum at 459 nm with strongest intense peak. The absolute QY of CDs in water is between 10-17% higher than of the pure CDs. When further increasing the absorption wavelength of CDs in water, the luminescence peak will become broader and shift to its original position. The CDs particle size is analysed by the AFM instrument. (Figure 4) shows the AFM image of CDs.

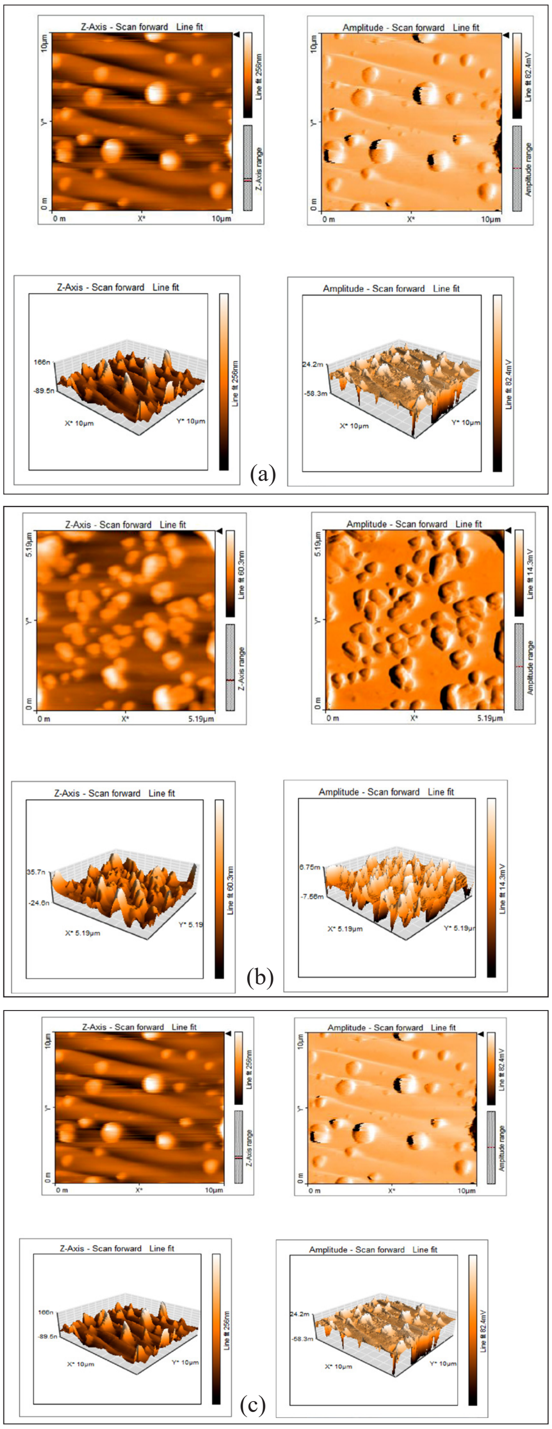


Figure 4) *AFM images of CDs by using different precursors (2D & 3D image) (A) Ammonium thiocynate, (B) Ethanolamine and (C) Urea.*

By using different precursors, the sizes of the carbon dots are being changed. So, the image shows the particle size on an average of CDs, which is in between 5 nm to 40 nm. The particle size was calculated by the Nano surf C3000 model software. The software can be easily detected the height of the particle, which was placed and dried at the top of the MICA plate. CDs have hydrophilic property, so some particles become clusters to joint with each other in atmosphere. The size can also be calculated from the 3D image of CDs, which shows the surface of the MICA plate and some CDs particles are placed on it.

Fluorescence quenching study

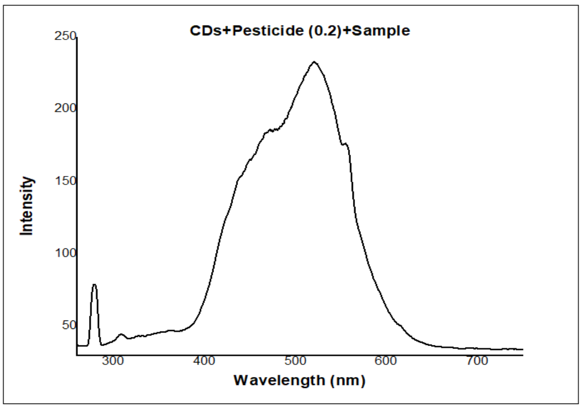
The process that decreases the fluorescence intensity of a sample refers to fluorescence quenching as of quenching there might be a variety of molecular interactions. In consideration to quenching there are a variation of examples which includes molecular rearrangements, excited-state reactions, energy transfer, ground-state complex formation, and collisional quenching. Study also relates the presence of collisional or dynamic quenching, which trends for the interaction of an excited state fluorophore with the quencher results in radiation-less deactivation of the fluorophore to the ground state, as per this the efficiency of dynamic quenching is hence sensitively dependent on the concentration of the quenching species [25,26]. Furthermore, the rate of collisional quenching can be used to calculate the quencher's diffusion coefficient. Numerous factors that are inclusive in data analysis, one of a kind is static quenching which occurs when the molecules from a complex in the ground state, *i.e.,* before excitation occurs, so as per its complexity it does have its own unique properties, such as being non fluorescent and having a unique absorption spectrum [27]. High optical densities or turbidity can result in decreased fluorescence intensities. Both static and dynamic quenching requires molecular contact between the fluorophore and quencher. Fluorescence quenching has been widely studied both as a fundamental phenomenon, and as a source of information about biochemical systems. Requirement of molecular is contacted for quenching results in the numerous applications of quenching. For example, quenching measurements can reveal the accessibility of fluorophores to quenchers. If the protein or membrane is impermeable to the quencher and the fluorophore is within the macromolecule, neither collisional nor static quenching can occur [28,23].

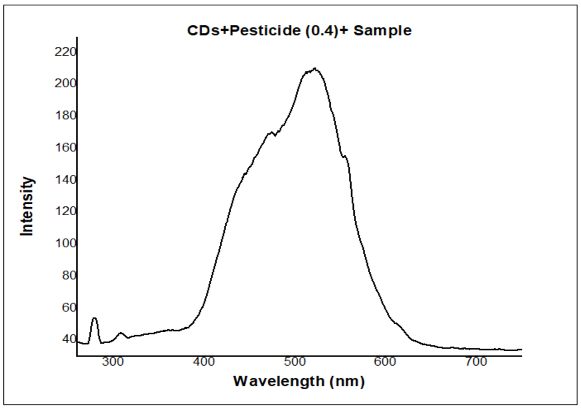
Fluorescent conjugated polymers have proven to be extremely useful and sensitive in chemical and biological sensor schemes [29]. The superior performance of these polymers is due to the "molecular wire" effect *i.e.,* the conjugated polymer backbone allows efficient electron delocalization and exciton migration over large distances, thereby creating amplified sensory responses compared to small-molecule-based sensors. Another advantage of using polymers as sensor materials is their modular nature; that is, the structure and sequence of the repeating units within polymers can be widely varied and changed, allowing the polymers to be customised to suit diverse targets and potentially achieve high selectivity [20,30,31]. In the late 1960s and early 1970s, solute fluorescence quenching reactions were 1st applied to biochemical problems after this there are been used in research tool for study of membranes, proteins, and other macromolecular assemblies. Quenching study is a diverse background which is used in small samples and is non-destructive, and they can be applied to fluorescence probe which are having an intrinsic or extrinsic property [32,33]. In order to resolve contributions or aid in the measurement of data, solute fluorescence quenching reactions can also be used selectively by altering the fluorescence properties in sample. To elaborate this specific point, consider different characteristics of fluorescence; quantum yields, excitation and emission spectral positions, anisotropy, the time dependence of the intensity and anisotropy decay, and the wavelength dependence of these parameters. Solute fluorescence quenching provides the experimenter with another variable (*i.e.,* another variable axis, in addition to time and wavelength) which may enable the resolution of fluorescence contributions [34].

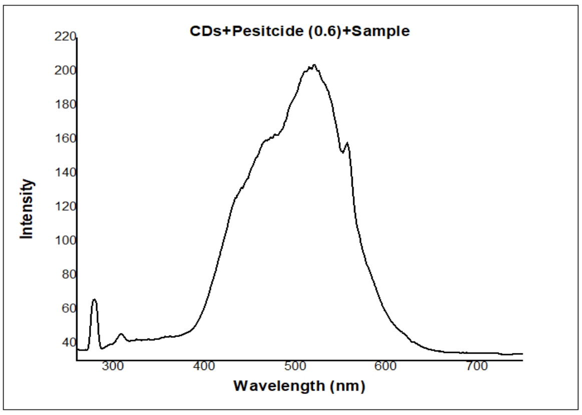
When a molecule receives light in the visible or ultraviolet spectrum, it is excited from its electronic ground state to an excited state. There will be reverting to its initial state by releasing out the stored energy form of heat and radiation in the visible or near-infrared spectral ranges. Single molecules are also been used to detect fluorescence with high sensitivity and also a significance range of uses are in the biochemistry and chemistry fields [35]. The emission of light by a substance that has absorbed light, or the other electromagnetic radiation is said to be the fluorescence and also the light energy produced by a particular type of chemical reaction where the excess chemical energy of the reactants is given off as light energy is preferred to be termed as phosphorescence. Sensitive fluorescence detection is based on the fact that the emitted light has longer wavelength than the extreme light that has been used for excitation, and thus can be suppressed by filters or monochromators. In addition to the change of electronic structure absorption which may lead to excitation of vibrational levels, which requires more energy or light of shorter wavelength, this explains what is the difference between absorption and fluorescence wavelength, which also can be known to be the stokes shift [36,37].

Fluorescence quenching study by carbon dots with the precursor Ammonium thiocyanate

By the hydrothermal process, we tend to synthesize carbon dots with the precursor ammonium thiocyanate. The process is mentioned in previous topic with the partial structural characterisation of carbon dots. Here (Figure 5) shows the fluorescence spectra of carbon dots with the pesticide ratio of 20, 40, 60, 80 ppm respectively.







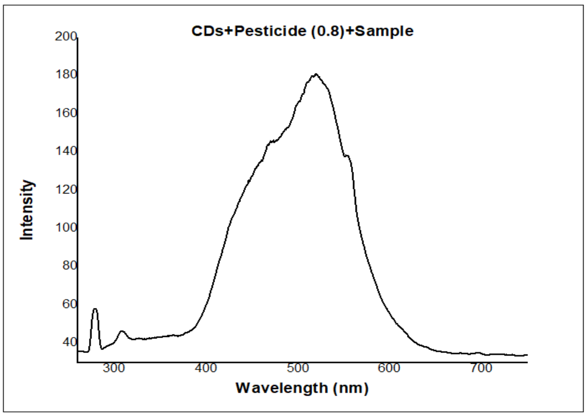


Figure 5) *Fluorescence spectra of carbon dots with synthesising precursor ammonium thiocynate including different ration of pesticides (20, 40, 60, 80 ppm).*

Carbon Dots have their own excitation peak at 459 nm wavelength. After putting the excitation wavelength 350 nm in spectroscopy parameter, the typical and diverse fluorescence absorption shows at the 459 nm wavelength. In contrast, the absorption peak of CDs is shifted at 500 nm from 459 nm in all relevant samples (20, 40, 60, 80 ppm) because of glyphosate pesticide. The pesticide is having some absence of electrons, so when the electrons of the CDs are exciting, the peptides with negative moiety has taken the electron from host. So, the intensity of the main solution has been decreased gradually after adding more amount of pesticide in the solution. The glyphosate pesticides were added with some decided amount of 20, 40, 60, 80 ppm in main solution, which has carbon dots synthesised by using ammonium thiocyanate precursor and apple-based fruit juice in it. (Figure 6) shows the all-fluorescence quenching spectra of the pesticide detection, which indicates the presence of some amount of glyphosate pesticide in it. Because of the isopropyl ammonium contain, the carbon dots fluorescent spectrum has two visible peaks and some of the minor diffraction peaks. Due to presence of phosphon in glyphosate, the minor diffraction peak was found from 260 nm to 300 nm wavelength, which is neglected.

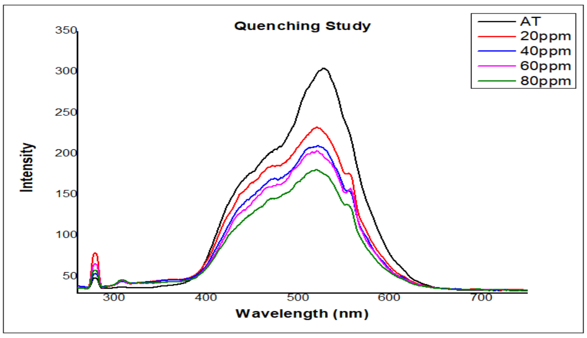
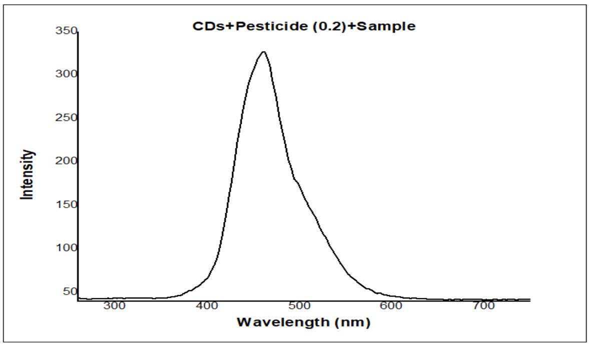


Figure 6) *Fluorescence quenching spectra of glyphosate pesticide.*

Here, the carbon dots are excited after implanting laser radiation on it, but the atoms of the glyphosate are taking charge from the atoms of the carbon dots to fulfil its molecular geometry. So, the intensity is being decreased after adding more amount of the glyphosate pesticide in main solution. Here the ratio of the glyphosate pesticide is fix from 20 ppm to 80 ppm with using different batches of the carbon dot, which synthesized by using different precursors.

Fluorescence quenching study by carbon dots with the precursor Etholamine

Similar to the above topic, the fluorescence quenching process is examined by using carbon dots, which is synthesised by ethanolamine precursor. (Figure 7) shows all fluorescence quenching spectra of the pesticide detection (20, 40, 60, 80 ppm), which indicates the presence of some amount of glyphosate pesticide in presence of ethanolamine precursor.



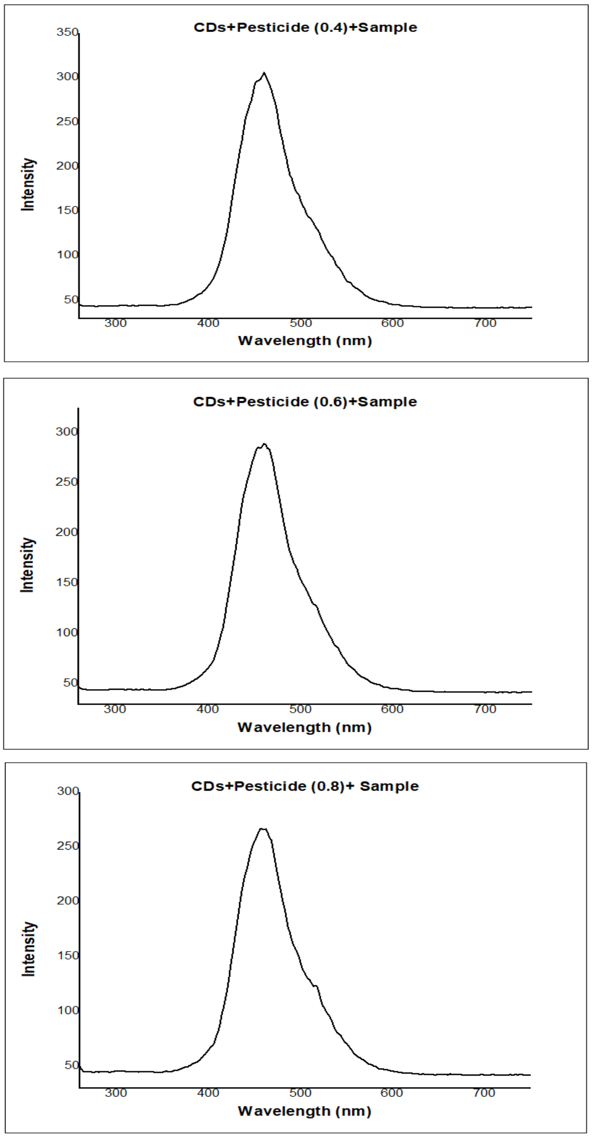


Figure 7) *Fluorescence spectra of carbon dots with synthesising precursor ethanolamine including different ration of pesticides (20, 40, 60, 80 ppm).*

Here, the fluorescence spectrum shows much fined diffraction curve at 459 nm. Here, the spectrum has shown perfect fluorescent of carbon dots without interfering any contain of ethanolamine precursor. After increasing glyphosate pesticide in the solution, the fluorescent spectra show minor distracting diffraction peak near 500 nm in all relevant samples (20, 40, 60, 80 ppm). Based on the results, ethanolamine precursor is good to synthesis fined crystalline carbon dots and also it is detecting pesticide in food beverages very accurately. After increasing the glyphosate, the intensity of the carbon dots is decreased without shifting any diffraction peak because of having negative moiety of the glyphosate pesticide, which attracts the electrons from the excited particles of CDs. (Figure 8) shows the fluorescence quenching study by the carbon dots, which is synthesised by the ethanolamine precursor. The quenching graph is gradually decreased by the increasing the value of the glyphosate pesticide in the apple-based juice solution, which easily detects the pesticide in apple-based juice.

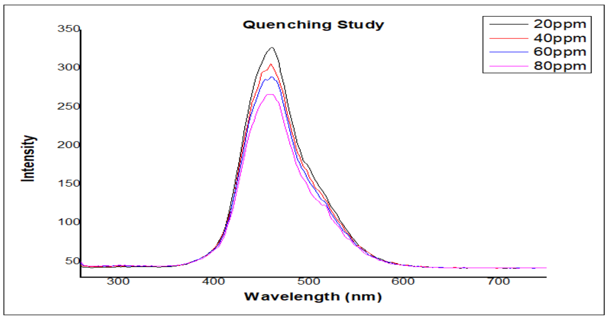
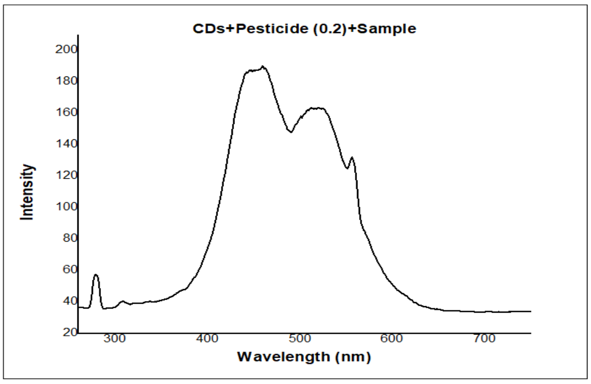


Figure 8) *Fluorescence quenching spectra of glyphosate pesticide.*

Fluorescence quenching study by carbon dots with the precursor Urea

Urea is another precursor to synthesise carbon dots with help of hydrothermal process. Here, the structural properties of the carbon dots have well, but for the detection it shows some of the distracting isolation peaks. (Figure 9) shows all fluorescence quenching spectra of the pesticide detection (20, 40, 60, 80 ppm), which indicates the presence of some amount of glyphosate pesticide in presence of urea precursor.



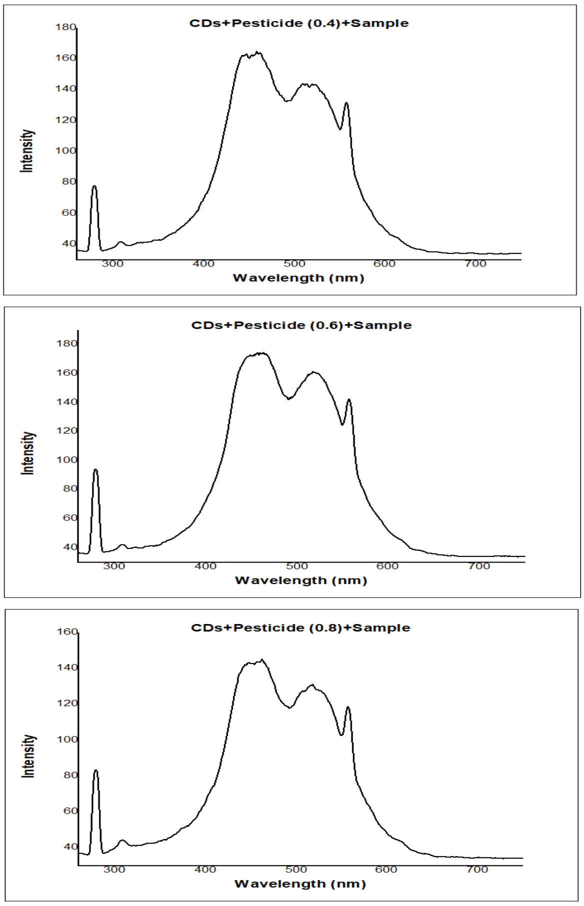


Figure 9) *Fluorescence spectra of carbon dots with synthesising precursor urea including different ration of pesticides (20, 40, 60, 80 ppm).*

Here the carbon dots has fluorescence peak at 459 nm or either it can be shifted to 500 nm or 400 nm. Means we can found the highest intensity of carbon dots between 400 nm to 500 nm. But here the presence of isopropyl ammonium and phosphon, the spectrum has various diffraction peaks before 400 nm in all relevant samples (20, 40, 60, 80 ppm). After neglecting those peaks, we did not get the perfect peak of the blue fluorescent diffraction peak near 459 nm. It is clearly shown three different diffraction peaks between 400 nm to 600 nm, which indicate carbon dots distracting isolation peaks including carbon dot luminescent peak. But the process is same, while adding the glyphosate pesticide in the main solution, the intensity of the luminescence is increasing-decreasing gradually from 180 to 140% intensity. (Figure 10) shows the quenching study of the glyphosate pesticide.

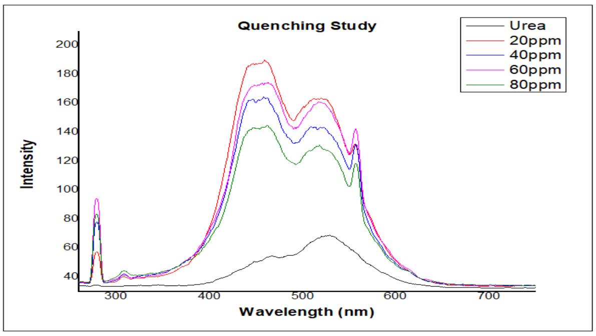


Figure 10) *Fluorescence quenching spectra of glyphosate pesticide.*

Here, all the fluorescence spectra have been compared with the standard fluorescence spectra. The standard fluorescence spectrum shows very poor result by indicating only 40-60% intensity of carbon dots. But adding sensor solution, it is gradually increasing up to the 60 ppm and then it is decreasing in 80 ppm value. By adding 20 ppm solution in carbon dots solution, the intensity reaches up to 180%, but after adding more 20 ppm, it is gradually decreased. By increasing the glyphosate ratio up to 60 ppm, it is again increased and by adding 80 ppm ration of pesticide, it is decreased. The spectra have not been contestant degradation. This result cannot be considered, but it is shown the presence of the glyphosate in the apple-based juice.

Conclusion

By one step synthesising carbon dots, the hydrothermal method is very accurate and precise. It gives the crystalline and much fined nanoparticles of carbon. By one day process of synthesising, having blue fluorescence carbon dots are ready for further characterisation. It has the range of 5 nm to 40 nm particle size with high intense luminescence peak at 459 nm with 380 nm absorption peak. With the help of all the precursors, the carbon dot solution made transparent. So, the transparent and fluorescence solution has many applications in bio-imaging, energy sector, security purpose (anti-counterfeiting), drug delivery, etc. By comparing three precursors with carbon source citric acid, it concluded that ethanolamine precursor is good for synthesis blue fluorescent carbon dots with fined crystalline structure over less than 40 nm (range 5 nm-40 nm, because of using different precursors) particle size. By the partial examination of structural properties of the carbon dots, it has been proved that the carbon dots are being synthesized with good quality of crystalline structure. But for the detection of the pesticide, ethanolamine precursor is accurate and precise. By analysing all results, it is clearly shown that with help of fluorescence quenching study, we can find out about the hazardous materials in food beverages. Here, different precursors are used to synthesise carbon dots. But by synthesising carbon dots with ethanol precursor is shown good results in detecting of pesticides. The fluorescence spectra have concluded that the carbon dots can detect pesticides, but the precursor urea and ammonium thiocyanate are not being good to detect or to fabricate sensor solution due to phosphon and isopropyl ammonium group present in the pesticides. It showed distracting diffraction peaks or other neglecting peaks in the spectra. Even the result is also not to be in considerable condition, due to not getting gradually decreased graph at the carbon dots luminescent intensity.

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