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Nitrogen Doped Carbon Dots: A Brief Review on Heavy Metal Ion Sensing

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Abstract

Carbon dots (CDs) are zero dimensional carbonaceous nanomaterials showing tremendous physicochemical and optical properties, synthesized from any organic precursors rich in carbon content. Due to their greater biocompatibility, non-toxicity, and tiny particle size up to 10 nm, CDs have numerous applications in the field of nano-sensing, bio-imaging, solar energy, light-emitting devices, photo-catalysis etc. The doping of CDs with any heteroatom can improve photoluminescence (PL) properties with great photo stability and enhanced sensing limits. Consequently doped CDs are in great demand for many advanced applications, including sensing heavy metal ions. Herein, Nitrogen doped CDs (NCDs) are reviewed in the light of bottom-up preparative methods to compare their optical and sensing properties towards detection of specific heavy metal ions. From this study, it is revealed that NCDs can detect specific heavy metal ions with higher selectivity by fluorescence quenching or enhancing. The PL quenching of NCDs was found to be more commonly reported for the detection of wide range of metal ion concentrations. It is revealed that NCDs propose a low-cost technology to detect toxic heavy metal ions in aqueous media, without use of any sophisticated instrumentation and specific organic ligand.

Keywords: Carbon dots, NCDs, heavy metal ions, photoluminescence.

1. Introduction

Over the last decade, "Carbon Dots" (CDs) have gained keen interest of researchers around the globe, due to their biocompatibility, excellent photo-stability, and superb optical properties with flexible surface functionality for desired applications in the fields of nano-sensing, bio-imaging, solar energy, light-emitting devices, photocatalysis etc. CDs are classified as zero dimensional carbonaceous nanoparticles discovered fortuitously [1] and synthesized profoundly via two major approaches- "Top-down approach" (TD) and the "Bottom-up approach" (BU). TD method is referred to as a decrease in bulk to nanometric scale. Mechanical friction, nanolithography, laser ablation, sputtering and thermal disintegration are the utmost popular nanoparticle synthesis process used. The TD method entails breaking down or miniaturizing bulk materials structures while maintaining their original integrity. Attrition is an excellent example of a top-down strategy for nanomaterial production where large particles are broken into smaller ones using arc-discharge, electrochemical or laser ablation techniques while in the BU method small particles combines to form nanoparticles using hydrothermal, pyrolysis, ultra sonication or microwave techniques as mentioned in Fig. 1. The "bottom-up" approach has gained more attention due to ease of working, simplicity of apparatus and efficient fabrication of nanoparticles, most common methods reported are carbonization of any carbon rich precursors through hydrothermal, microwave, and ultra-sonication treatments.

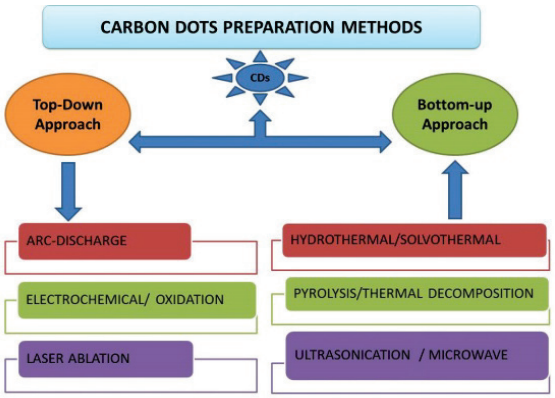

Fig. 1. Synthesis of Carbon Dots

Due to their greater biocompatibility, non-toxicity, and tiny particle size up to 10 nm, carbon dots have significant role in modern nanotechnology. The formation of any nanomaterial from small molecules to nanoparticles clusters is regarded as the most advantageous process. As a result, a bottom-up technique can be thought of as a synthesis method in which the basic elements are added to the substrates to generate nanostructures. CDs have nano dimensions in all three axes and are capable to show specific optical and electronic properties and thus are in great demand for many advance applications, in the field of nano-sensing, bio-imaging, solar energy, light-emitting

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devices, photocatalysis and many other including sensing heavy metal ions. The doping of CDs with any heteroatom can further improve sensing limits and enhance photoluminescence (PL) properties as proved by Wang *et al.* through research on selecting an effective and universal nitrogen-doping reagent in aqueous solution and reporting hexamethylenetetramine (HMTA) as a universal nitrogen-doping reagent because it was observed that the PL of CDs was always significantly enhanced by the introduction of HMTA, regardless of using organic acids or carbohydrates as carbon sources with quantum yield of 62.8% [2]. Herein, several bottom-up approaches have been reviewed that were adopted by scientists to prepare NCDs and used to detect specific heavy metal ion by fluorescence quenching/enhancing phenomena with higher selectivity as discussed in upcoming sections.

2. Bottom-up Preparation of Nitrogen Doped Carbon Dots

As mentioned above CDs can be prepared by both top-down and bottom-up approaches and due to ease of working, eco-friendliness, economical aspects and simple equipment set ups the bottom-up approach is most commonly used as shown in Fig. 2 [3 - 12]. Bottom-up (BU) approaches rely on the chemical synthesis of CDs using smaller organic molecules reacting together under some specific conditions like hydrothermal/solvothermal/decomposition/pyrolysis/ microwave heating/ultrasonic treatment etc. To introduce any hetero atom as dopant in the CDs, desired hetero atom containing molecule is selected and appropriate reaction conditions are made to form doped CDs. Doping atom may be a metal or non-metal but due to induced toxicity of metals, non-metals are preferred. Nitrogen Doped Carbon Dots can be formed easily because Nitrogen being the next atom to the Carbon shows comparative atomic radii, electron negativity, density, etc. and thus easily replace Carbon atom in any framework of organic molecules.

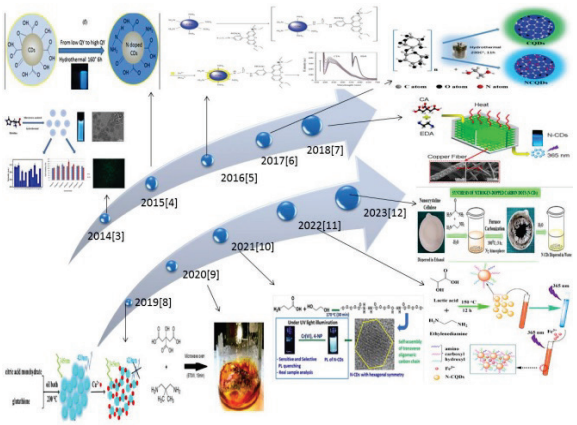

Fig. 2. Common Methods of Carbon dots Synthesis

For the formation of NCDs, most commonly used carbonaceous molecules are carbohydrates and organic acids, while nitrogen containing molecules are ammonia and it's derivatives, amino acids, urea, etc. The formation of NCDs may involve various steps like polymerization, dissociation, loading of dopant, etc. which depends upon the starting reactants used. The optical and physical properties of doped NCDs depend upon the synthesis method used and size and surface functionality of the particles. One-pot microwave assisted hydrothermal synthesis was carried out to prepare 2 - 5 nm sized NCDs with Nitrogen content of 18.5 - 26.13% and used to detect Fe +3 ions up to 10 nM [3]. Nitrogen can alter the electronic environment around the carbon which influences the fluorescence properties of NCDs. It is observed that with increasing concentration of dopant the fluorescence intensity is also increased, which is attributed to higher pyridinic Nitrogen contents [4]. A dual-signal-based ratiometric probe was introduced using nitrogen-doped carbon dots (NCDs) and rhodamine B isothiocyanate (RhB) to determine Fe^{3+} selectively in the linear range of 0.01 - 1.2 µM with lowest detection limit of 6 nM. In this method with increasing Fe^{3+} ions to the probe, FL intensity of the NCDs increased rapidly while the FL intensity of RhB remained constant [5]. Micro-crystalline cellulose and ethylene diamine was hydrothermally reacted to get NCDs showing quantum yield of 55% in acidic solution and able to detect Fe^{3+} at lowest detection limit of 0.2 nM [6]. A specially designed microreactor was developed using porous copper fibers to get NCDs with very high QY of 73% and used for selectively detect Hg^{2+} ions in the range of 0 ~ 50 µM Hg^{2+}ions concentration at the lowest limit of detection (LOD) of 2.54 nM. To prepare these NCDs ethylene diamine and citric acid are used as precursors and reacted in the microreactor, it was revealed that the elemental contents and surface functional groups of N-CDs are significantly influenced by the porosity of copper fibers [7]. Citric acid is most abundantly available cheap source of carbon and thus used as raw material for many synthesis processes like citric acid is mixed with glutathione to obtain NCDs for the detection of Cu^{2+}ions in real samples of tap/lake water, showing linearity in the concentration range of 0.20-200.0 µM with a lowest detection limit of 0.27 nM [8]. NCDs have been synthesized using green chemistry principles also, viz. - single step microwave-assisted synthesis was carried by taking citric acid as a carbon source and 2,2-dimethyl-1,3-propanediamine as nitrogen source. As prepared NCDs shown 51.2% QY and were used to detect Hg^{2+} ions in the range of 0 ~ 4.2 µM Hg^{2+} ions concentration at the lowest limit of detection (LOD) of 7.63 nM [9]. Highly crystalline NCDs were obtained by oligomerization of ethylene glycol with β-alanine at 170 °C for 30 min, to be used as a probe for the detection of Cr^{+6} ions in the range of

0.29 - 2.5 µM possessing high accuracy and precision comparable to inductively coupled plasma optical emission spectroscopy (ICPOES) like costly instrumental technique [10]. L-lactic acid and ethylene diamine are reacted hydrothermally to obtain NCDs with QY of 46% and applied for the detection of Fe^{3+} ions in a wide range of concentrations up to 200 µM and with LOD of 1.89 µM [11]. Pyrolysis of nano crystalline cellulose, urea and ethylene diamine was carried out at 300 °C for 02 hours under Nitrogen to obtain NCDs with QY of 29%, used to detect Hg^{2+} ions within 10 min. in the range of 0 ~ 100 µM with lowest limit of detection (LOD) of 59 µM [12]. It is cleared from all above examples that hydrothermal approach is the most common technique adopted by many researchers due to easy handling, eco-friendliness and inexpensive way to form NCDs. Pyrolysis of starting reactants is also performed by scientists while some researchers considered greener energy sources viz. microwave and ultrasound treatment to form NCDs in quicker manner. Among all the methods studied here, it is observed that highest QY of 73% is obtained in a specially designed copper fiber microreactor system. Thus, some minor adjustments in synthetic apparatus can lead to produce highly fluorescent NCDs for multitasking purpose and quenching of that can be used to detect hazardous toxic metal ions.

3. Characterizations of NCDs

To ensure the properties of the NCDs, these were characterized by various spectral techniques like Fourier transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) absorption spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and fluorescence spectroscopy. Fig. 3 is showing fundamental techniques used for the characterization of NCDs. Most commonly used different spectral methods are as follows:

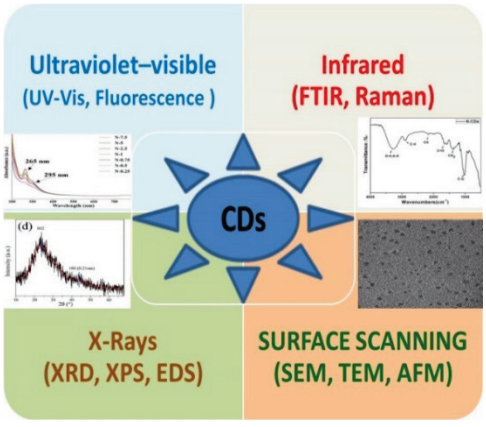

Fig. 3. Characterization Methods for Carbon dots

3.1. UV- Visible/ Fluorescence Spectroscopy

The optical properties of NCDs are studied by UV-vis and fluorescence spectra. UV- spectra are generally used to identify the specific olefin or unsaturated bonding groups viz. various bands related to n-π* or π- π* transitions can be observed for C=O or C=C kinds and the value of maximum wavelength *(λ)* for absorption is determined. As per the nature of bonding and energy provided to the system under study, the fluorescence measurements are measured with fluorescence spectrophotometer by taking the samples in quartz fluorescence cuvette of 10 mm optical path length. Then sample is excited at various wavelengths *(λ)* ranging from 290 - 800 nm and on the basis of emission spectra obtained with maximum absorbance wavelength *(λ_{max})* for that sample has to selected for example Xu, *et al.* placed the samples in a 10 mm optical path length quartz fluorescence cuvette excited at 350 nm, and get the emission spectra for 360 - 660 nm to obtain maximum emission as shown in Fig. 4.

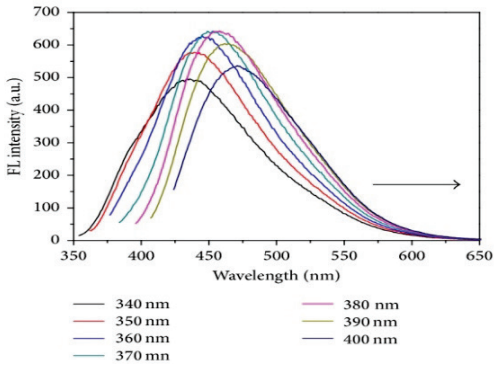

Fig. 4. Emission spectra Nitrogen doped Carbon dots

The excitation and the absorption spectra of any fluorescent molecule should be identical as excitation is equivalent to absorption and after absorption the molecule reaches the excited state belonging to the amount of energy absorbed. In this way using both spectra quantum yield (QY) of the NCDs has to be calculated by using a standard i.e. mostly Quinine Sulphate (QY 54%). The fluorescence quenching or enhancing studies are used to detect any pollutant in environment to find out the ability of NCDs as biosensors in various fields.

3.2. FTIR-Spectroscopy

The Fourier-transform infrared spectroscopy (FTIR) investigations are used to study the surface functionality of the prepared NCDs. It can be recorded by Spectrometric Analyzer in the range from 400 to 4000 cm^{-1} using potassium bromide (KBr) as background at room temperature. The appearance of various band on different vibrational frequencies confirmed the presence of that specific groups viz. broad peak in the 3200 - 3500 cm^{-1} region is assigned to the stretching vibration of O−H and N−H groups, while a peak at 1720 cm^{-1} is attributed to the vibration absorption of C=O and confirm the presence of hydroxyl, carboxyl, and amino groups on the surface of NCDs, as shown in Fig. 5 [5].

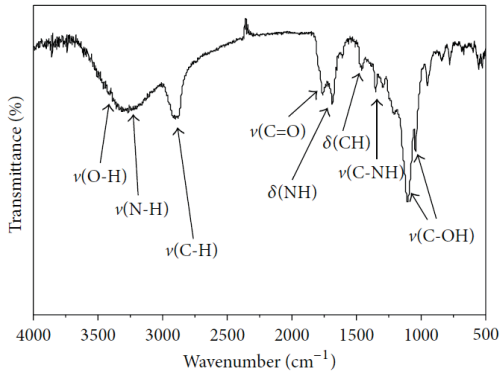

Fig. 5. FTIR spectra Nitrogen doped Carbon dots

These functional groups are responsible for the higher solubility of NCDs in water and making covalent, coordinate, electrostatic or weak bonds with the pollutants present in the air, water or soil.

3.3. X-Ray Studies

X-Ray Diffraction (XRD) patterns are obtained by an X-ray Diffractometer using copper Cu-Kα radiation (0.154nm) as the X-ray source. The recorded range of diffraction angle may be chosen from 2° to 110°, and the scanning rate may be set as desired rate or mostly at 4°- 6° per min. to obtain the positions of the diffraction peaks, which are matched with International Centre for Diffraction Data (ICDD) or reviewing the literature to understand the amorphous or crystalline properties of NCDs. X-ray Photoelectron Spectroscopy (XPS) are recorded by monochromatic X-Ray source Al Kα excitation (1486.6 eV). X-ray Photoelectron Spectroscopy (XPS) were used for the elemental compositions of NCDs showing three peaks at 284.6, 399.4, and 531.5 eV, corresponding to C1s, N1s, and O1s, respectively which confirms the formation of nitrogen doping on carbon dots as shown in Fig. 6 a and b. At high resolution the nature of binding of nitrogen viz. pyridinc, pyrolinic, quaternary etc. may also be confirmed by XPS.

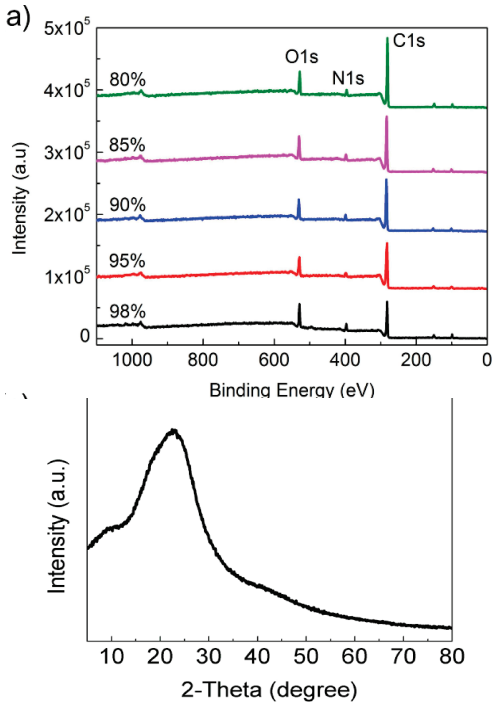

Fig. 6. a, b. XPS and XRD spectra Nitogen doped Carbon dots

3.4. Particle Size Analysis

Particle size analyses (PSA) are performed to verify the images of TEM and zeta potentials along with surface charge calculations are performed by PSA. Dynamic light scattering (DLS) measurements and surface charge on NCDs are determined to know about the ability of NCDs to mitigate any pollutants. Higher negative charges on NCDs favours for the removal of positively charged pollutants like heavy metals.

3.5. Transmission Electron Microscopy Studies

Transmission electron microscopy (TEM) is used to characterize the surface morphology of the prepared NCDs and identify the particle size. It can be used to find out the nature well dispersed particle of NCDs and to observe the phenomenon of agglomeration. Shape of the NCDs can also be understood by magnifying the power using high resolution TEM (HRTEM) as shown in Fig. 7 a and b, additionally histogram of size distribution may be plotted to find out average size of the prepared NCDs[7]. Mostly spherical, hexagonal, flake-shape, graphitic, and grapheme like structure has been seen.

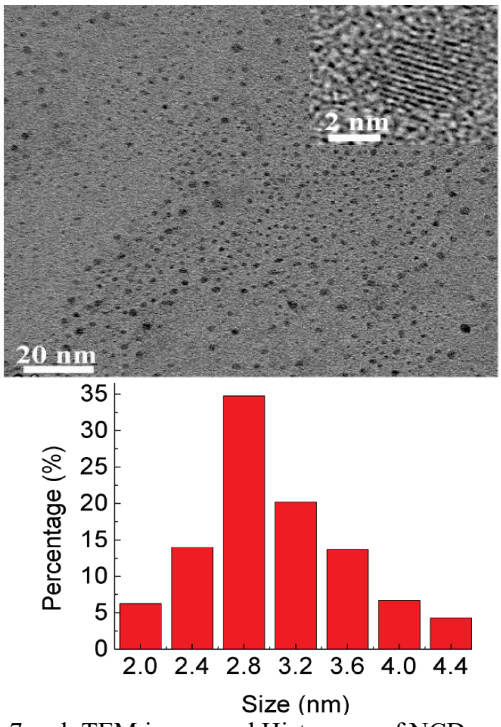

Fig. 7. a, b TEM image and Histogram of NCDs

3.6. Scanning Electron Microscope Studies

The surface morphology and porosity pattern of the NCDs are studied with scanning electron microscope (SEM). The SEM gives the magnified images of the size, shape, composition, crystallography, and other physical and chemical properties of any specimen.

3.7. Atomic Force Microscopy

The surface topography and 3-dimensional morphology of NCDs is studied by atomic force microscopy (AFM), which tells about the dispersive nature of particles and size distributions along with possible number of layers. Samples for AFM may be prepared by dip-coating on any smooth substrates like mica and dried properly to carry out analysis within one day. Mica substrates are generally utilized due to their low roughness which allowed identification of NCDs as isolated NCDs.

3.8. Photo Stability of the NCDs

The stability of the prepared NCDs are detected by studying the effect of exposure to ultraviolet light, the effect of ionic strength and the effect of pH. The NCDs solution is exposed constantly to ultraviolet light of wavelength of 365 nm for selected time periods of time to find out any significant change in the intensity of fluorescence during that period of time. Similarly change in the intensity of fluorescence is measured without NaCl and with NaCl, as well as by changing the pH from neutral to acidic or basic.

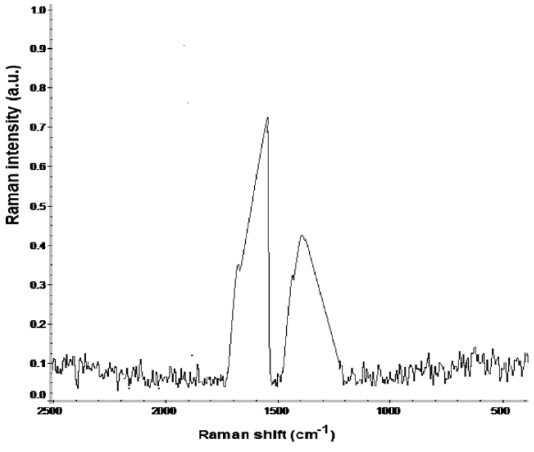

Fig. 8. Raman Spectra of NCDs

3.9. Raman Spectroscopy

The Raman spectrum of NCDs is recorded with maximum emission wavelength to find out the G-band which is related to sp2 hybridization showing the amount of graphitization in the NCDs and the D-band linked to sp3 hybridization defects and functionalization present in the NCDs. The higher G/D ratio indicates that the synthesized NCDs are mostly composed of graphitic structure with a certain number of defects and functionalization of NCDs. For example- Fig. 8 representing the Raman spectra at excitation wavelength of 532 nm with two distinct peaks at 1370 (D-band) and 1602 cm^{-1} (G-band), respectively and intensity ratio of the peak of the G band to the D band is found to be 1.7, indicating the doping of nitrogen in CDs. [9]

4. Application of NCDs for Sensing Heavy Metal Ions

Heavy metal ions have toxic impacts on our environment and are lethal to living organisms; hence it is an urgent need to detect and mitigate their higher concentration in our surroundings. Anthropogenic activities, such as industrial production of batteries, dyes, paints, varnish, mining, agricultural herbicides, etc. can release huge amounts of these metal ions into our ecosystems. One of the most important sensing applications of CDs corresponds to the recognition of heavy metals with environmental concerns. These metals can cause carcinogenic impacts on living organisms and bio-accumulate in the cells with magnification on the ecosystem. Various agencies like World Health Organization (WHO) and Bureau of Indian Standards (BIS) have recommended the limits of heavy metal in water. Above the permissible limits of any of these metal ions the water may be lethal to our eco-system, hence it is essential to know their values in various water sources to safeguard the lives. There are many tedious and costly laboratory/ instrumental techniques to detect theses toxic ions viz. electrochemical methods, inductively coupled plasma mass spectrometry (ICPMS), atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES). However, these methods have several serious problems, including complicated sample processing, longer detection times, and high cost, which make their applications limited hence CDs have emerged as an efficient low-cost and handy alternative to those costly techniques. The negative zeta potential charges on CDs make their surface suitable to detect heavy metal ions and open up the way for mitigation of toxic pollutants. Their amazing PL properties, sensitivity and selectivity; moreover considering their non-toxicity and biocompatibility they can perform this job without generating a negative impact on the surroundings. Some recent studies reported that the use of doped viz. NCDs could detect heavy metals like Mercury (Hg^{+2}), Copper (Cu^{+2}), Iron (Fe^{+3}) and Chromium (Cr^{+6}) ions, as summarized in Table 1 showing informative details of Nitrogen doped Carbon dots

Table 1. Nitrogen doped Carbon dots

S.No.	Precursors	Method	Detection Range & Heavy Metal Ion Mechanism	QY(%) & Size (nm)	Reference
1.	Histidine	MWS	0-10 nM Fe^{+3} Quenching PL	8.9% 2-5nm	[3]
2.	Sodium Citrate and Ethylenediamine	HTS	0.1-45µM Fe^{+3} Quenching PL	32 % 3-11 nm	[4]
3.	Polyethylene glycol diamines and Ascorbic acid	HTS	0.1-1.2 µM Fe^{+3} Enhancing PL	15 % 2.7-5.9 nm	[5]
4.	Cellulose and Ethylenediamine	HTS	10-500µM Fe^{+3} Quenching PL	55 % 3.2 nm	[6]
5.	Citric acid and Ethylenediamine	MRS	0-50 µM Hg2+ Quenching PL	73%. 3 nm	[7]
6.	Citric acid and Glutathione	TDS	0.2-200µM Cu +2 Quenching PL	44% 4.5nm	[8]
7.	Ctric acid and 2,2-dimethyl-1,3-propanediamine	MWS	0-4.2 µM Hg2+ Quenching PL	51.2 % 40-60 nm	[9]
8.	Ethylene glycol and β-alanine	TOS	0.29-2.5 µM Cr^{+6} Quenching PL	14.3 % 1.07-.2.12 nm	[10]
9.	L-Lactic acid and Ethylenediamine	HTS	1.89-200µM Fe^{+3} Quenching PL	46 % 1-4 nm	[11]
10.	Cellulose Urea and Ethylenediamine	PyS	0-100 µM Hg2+ Quenching PL	29 % 2-5 nm	[12]

HTS=Hydrothermal Synthesis; MRS=Microreactor Synthesis; TDS= Thermal Decomposition Synthesis
MWS=Microwave Synthesis;TOS=Thermal Oligomerization Synthesis; PyS= Pyrolysis Synthesis

The specific zeta potential, a parameter used to determine the surface charge of colloids and nanomaterials distributed in a liquid, may be developed on the surface of NCDs by varying some factors like-pH of the solution and thus tunable negative surface charge can attract positively charge pollutants like toxic heavy metals. The mechanism of quenching/enhancing fluorescence for detection is still not completely understood, however as briefly reviewed in this article, electrostatic attraction, complex formation with the functional groups available on the surface, covalent or coordinate linkage, weak inter-ionic interactions, etc. may be responsible for the decrease or increase in the fluorescent intensity [12] of the NCDs as shown in Fig. 9.

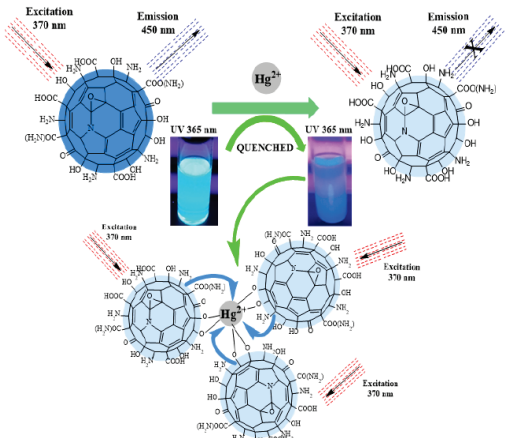

Fig. 9. Mechanism of quenching fluorescence

Sufficient data availability on detection of heavy metals with NCDs, lowest detection limit and linearity of detection in wide range on analysis of heavy metal ion concentrations are represented in current review for supporting the proposed mechanism. The development of greener, low-cost synthetic strategies of NCDs may open ups the easy gate-ways for reliable methods to detect toxic and hazardous pollutants. Although tremendous progress has been made for decreasing the lowest detection limit values in aqueous solution for heavy metal detection, there are still some barriers to overcome, which include pathways having lesser time taking strategies for faster preparative methods, low cost purification and separation techniques, cheaper and easier methods for characterization and reproducibility at bulk level which can provide cost effective and handy solution to detect and mitigate heavy metal pollution.

5. Conclusion

The Nitrogen-Doped Carbon dots (NCDs) can be synthesized in number of facile ways using bottom-up approaches in ecofriendly and economical manner. Many researchers used hydrothermal synthesis while others have adopted microwave, ultrasound, pyrolysis, oligomerization, and thermal decomposition synthesis methods. The prepared NCDs exhibit a broad range of specific properties, such as shiny multicolour fluorescence varying from blue to red emissions, showing very high quantum yield (73%QY), low cytotoxicity, greater biocompatibility, easy fabrication etc., that make them a suitable vehicle for serving in numerous applications including heavy metal ion sensing. The properties of NCDs are studied by instrumental analysis viz. Fourier transform infrared (FTIR), Ultra-violet-visible (UV-Vis), Florescence, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM) etc. to prove the formation of NCDs with desired characteristics.

NCDs are low-cost excellent fluorescent materials which may find expanded applications in many environmental fields of wastewater pollutant detection, catalysis, drug delivery, pH sensing, LEDs, medical bio-imaging, chemical sensing etc. This review suggests that NCDs propose a low-cost technology to detect toxic heavy metal ions in aqueous media, without use of any specific organic ligand or sophisticated and costly instrumentations.

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