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GREEN SYNTHESIS OF N-DOPED CARBON NANODOTS FROM WALNUT HUSK AND THEIR APPLICATION FOR DETECTION OF FE^{3+} ION IN SERUM AND IN WATER SAMPLES.

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Abstract

Carbon Nano dots (CNDs) with a size bellow 10 nm is a recently developed class of carbon-based nanomaterial's, initially N-NCDs were incidentally discovered as a fluorescent impurities by scrivens in 2004 during the refining process of single walled carbon based nanotubes, the carbon nano dot's received a tremendous debate because of their low cytotoxicity, environmental friendly, excellent biocompatibility, high photo stability and good water solubility. And having a wide range of application in various field such as fluorescent sensing, photo catalysis, biological imaging, opto- electronic devices, medical diagnosis, and in drugs delivery etc. In this particular work we have formed green nitrogen doped carbon nano dot's (N-CNDs) by utilizing walnut husk as a carbon precursor and ethylenediamine as a nitrogen precursor (Nitrogen doping enhance the optoelectronic properties and quantum yield of N-CNDs) with the use of an eco – friendly and simple hydrothermal method with a quantum yield of 45.6 %. the characterizations of N-CDs was done by applying different spectroscopic techniques, for example transmission electron microscopy (TEM) measurements show quasi – spherical shape of N-CNDs having an average particle of 3.1nm in diameter, FT – IR, RmS, X – rays diffraction, and X – rays photoelectron spectroscopy were used to analyzed the structural composition of nitrogen doped carbon nano dots (N-CNDs), the as prepared N-NCDs shows high fluorescent property with a very strong emission fluorescence spectra at 470nm upon excitation at 387nm. It was research observed that N-CNDs show high sensitivity and selectivity towards iron ion sensing in aqueous medium, so we have used it for the detection of Fe^{3+} in real samples such as in human blood serum and in tap water. A good liner relationship ranging from 0 – 600 µM were observed between Fe^{3+} ion concentration and Fo/F, with correlation coefficient (R^{2}) 0.9943 µM, the LOD (limit of detection) were 0.084 µM which is better than most of the

reported values.

INTRODUCTION

Carbon nano dots (N-CNDs) with a size bellow 10 nm is a recently developed class of carbon-based nanomaterial's Other members of the carbon based family include nanofibers,[1] nanotubes,[2, 3] graphene,[4, 5] and fullerenes[6] etc. initially N-CDs were incidentally discovered as a fluorescent impurities by scrivens in 2004 during the refining process of single walled carbon nanotubes[7, 8] the carbon dots have received a tremendous attention because of their wide range of application in various field of such as fluorescent sensing, [9] catalysis,[10, 11] biological imaging,[12] opto- electronic devices,[13, 14] medical diagnosis,[15] Biomedicine[13, 16] in drug delivery [17] and so on. Heave metal based quantum dots are excellent fluorescent nanomaterial because of their high photoluminescence quantum yield large molecular extinction coefficient good bio imaging properties nano probe sensing and high photo stability[18-20], however the metal based nanodots are harmful for human and other living organism and also polluted the environment[21] due to their non-biodegradable nature while carbon quantum dots (CQDs) have low cytotoxicity,[22, 23] environmental friendly, excellent biocompatibility, high photo stability good water solubility[24-27] and are mostly used as a fluorescent probe for the examination of various pollutants hence carbon nano dots are better alternative for conventional metal synthesized quantum dots and fluorescence organic dyes[28], fluorescent carbon quantum dots can be synthesized by two well established approaches' namely Top-Down approach and Bottom up approach[29, 30]. The top down approach consist of Arc-discharge[21] electrochemical oxidation[31] Laser- ablation[32] etc. where large structural carbon precursor such as graphite oxide, carbon nanotubes, graphite and activated carbon are broken down into small pieces to form carbon quantum dots (CQDs) however most of Top – Down approaches required costly equipments, complex time consuming processes and surface passivation, which limit their widespread use. The Bottom –up approach include micro wave[33] solvothermal [34, 35] ultrasonic[36] and hydrothermal method[37-39]. Where direct carbonization or polymerization of carbon precursor take place, the Bottom –up approach are most widely used as compared to Top-Down approach because of its simplicity and environmentally friendly nature, especially the Hydrothermal method of bottom- up approach attracted more attention due to their simplicity, mass production eco-friendly and cost effectiveness behaviour. Raji Atchudan et al have synthesized CNDs from banana peel through hydrothermal method, having average partical size of 5nm with quantum yield of 20 %. Hydrothermal method are mostly applied to synthesized carbon nano dots (CNDs) from low cost and easily accessible green carbon sources, different chemical materials such as urea [40] citric acid [41] polyethylene imine [42] ethylene glycol [43], boronic acid [44] and many organic solvent can be used to synthesized carbon nano dots (CNDs) however the carbon dots synthesized from these conventional chemicals are costly and involve toxic products. while the green renewable natural carbon source such as plants biomass for carbon dots synthesis are most commonly used because of its cost effectiveness, easily accessible, renewable and environmental friendly nature. Various parts of plants can be used to synthesized carbon dots (CDs) for example plants waste such as water million peel[45], orange peel[46], banana peel,[47] walnut shell,[48] Fruit juices such as Lemon juice [49, 50] papaya juice[51] orange juice other parts includes Flower Leaves, roots, seeds, stem and husk[52]. Plant parts are natural precursor for CDs synthesis and having several benefits. As they are easily accessible, eco-friendly renewable and high in abundance.

2.2 Material and Method

Synthesis of Pure Walnut Husk Carbon Dots (CDs)
Pure carbon dots were synthesized via a hydrothermal method using walnut husk as a carbon precursor. Briefly, walnut husk was shade-dried and ground into a fine powder. A total of 1.5 g of the powder was dispersed in 20 mL of deionized (DI) water and magnetically stirred for 10 min to obtain a

homogeneous suspension. The mixture was then transferred into a 25 mL stainless-steel autoclave and heated at 200 °C for 8 h in an electric oven. After natural cooling to room temperature, the resulting brown solution was centrifuged twice at 12,000 rpm for 15 min. The collected supernatant was filtered through a 0.22 µm syringe filter to remove large particulates. The filtrate was further purified by dialysis (MWCO 1000 Da) against DI water for 48 h, with water replacement every 6 h, to eliminate residual impurities and soluble ions. The obtained carbon dot solution was stored at 4 °C for further use.

2.3.2 Synthesis of Nitrogen-Doped Carbon Nanodots (N-CDs)

Nitrogen-doped carbon nanodots were synthesized using a similar hydrothermal approach, with ethylenediamine (EDA) as the nitrogen source. In brief, 1.5 g of walnut husk powder and 400 µL of EDA were added to 20 mL of DI water and stirred for 10 min to form a uniform mixture. The suspension was transferred into a 25 mL Teflon-lined autoclave and heated at 200 °C for 8 h. After cooling naturally to room temperature, the product was centrifuged, filtered (0.22 µm), and purified by dialysis (MWCO 1000 Da) under the same conditions as described for pure CDs. The final N-CDs solution was stored at 4 °C for further characterization. To optimize the synthesis conditions, key parameters including walnut husk amount (0.5–3.0 g), EDA dosage (50–500 µL), reaction temperature (160–220 °C), and reaction time (3–14 h) were systematically varied, while the remaining experimental procedures were kept constant.

2.3. SYNTHESIS OF CARBON NANODO

Figure 17. Schematic representation of the formation of fluorescence CQDs from walnut huskvia hydrothermal method.

2.4 N-CNDs QUANTUM YIELD MEASUREMENT (QY)

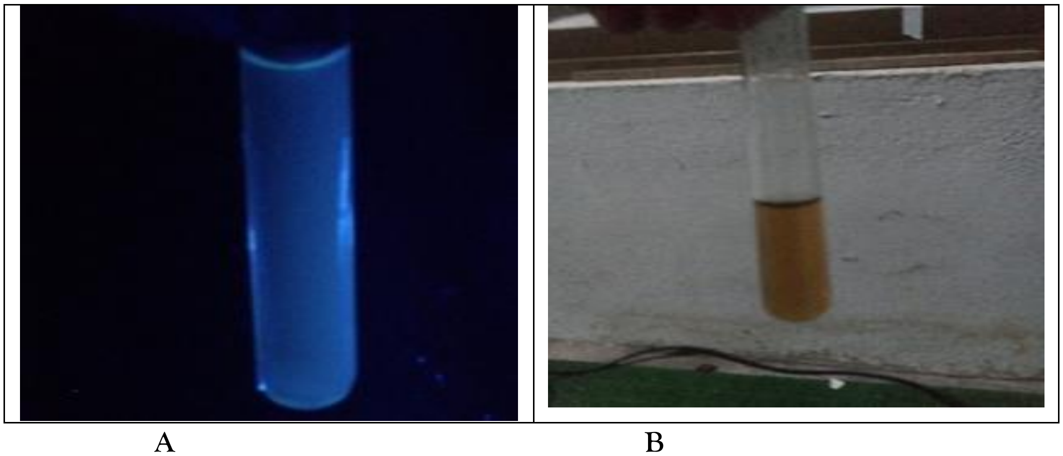
The well established relative method was adopted to calculate the quantam yield (QY) of N doped carbon nanodots (N-CDs). An aqueous solution of quinine sulfate was dissolved in 0.1 M H_2SO_4 (At 360 nanometer its QY was equal to 54 %) are taken as a standared. To avoid reabsorption effect the optical absorbance of both N – CDs and quinine sulfate solution must be kept below 0.1 at the excitation wavelenth, the quantum yield measurement of N – CDs were obtain by using a well known equation given below.

$$Q_x = Q_q \times I_x / I_q \times As / A_x \times n^2_x / n^2_q$$

2.5 Sensing (Detection) and analysis of Fe^{3+} ion:

For Fe^{3+} sensing, 1000 µM stock solutions of various metal ions were prepared and mixed (1:1, v/v) with N-CDs, followed by shaking for 10 min at room temperature. The fluorescence intensity was recorded using a spectrofluorimeter at an excitation wavelength of 360 nm. Among all tested ions, Fe^{3+}

caused the maximum fluorescence quenching, demonstrating high selectivity of N-CDs toward Fe^{3+} detection. A good linear response was observed in the concentration range of 0–600 µM with a low limit of detection (LOD) of 0.083 µM. Practical applicability was confirmed in tap water and blood serum samples, where coexisting metal ions showed negligible interference, and fluorescence quenching was clearly visible under UV (365 nm) and daylight conditions.



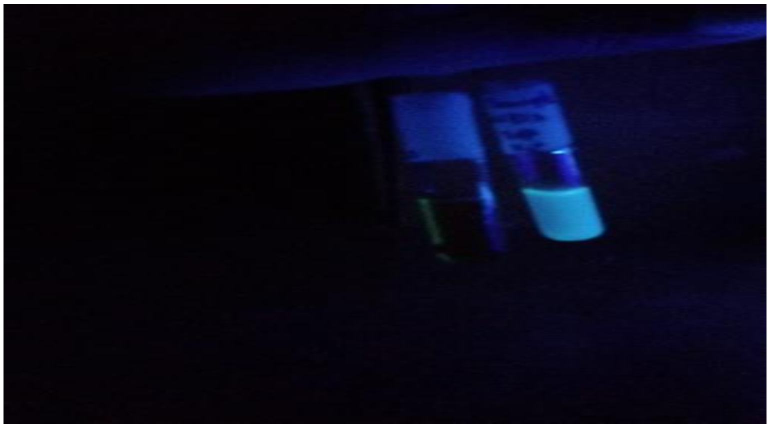


Figure1: (A) N-CDs at a wavelenth of 365 nm (UV) and (B) Day light uv- vis

Figure .2: Comperission of the N-CNDs in the presence and absence of Fe^{3+} ion using 365nm wavelenth UV light. Lift side tube contain fe^{3+} ion in N-CDs solution(quenching occur) while ther is no Fe^{3+} ion in right side N-CDs solution, (no qenching)

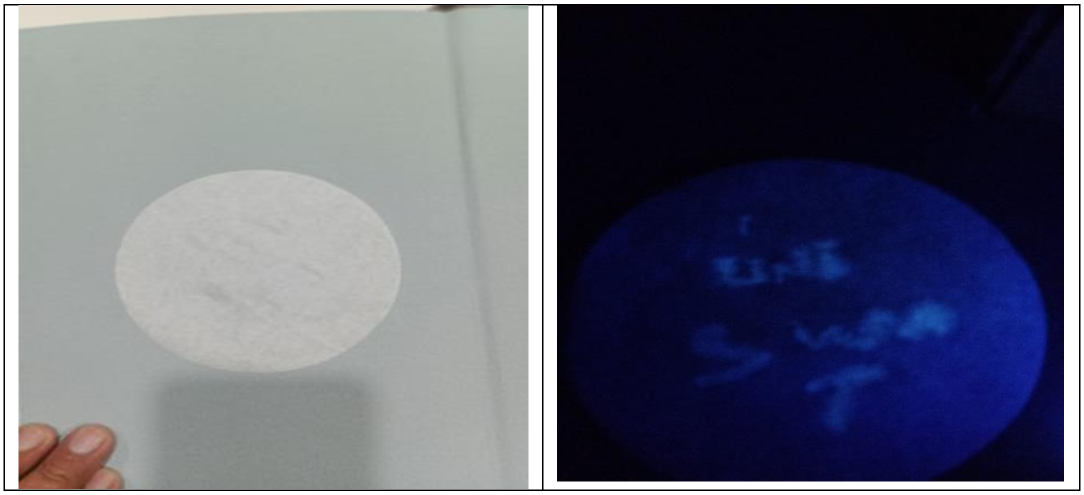


Figure 20. (a) Photograph under day ligh (b) Photograph under UV ligh

3.1. Procedure of N-CNDs Synthesis

Walnut husk waste was utilized as a green carbon source to synthesize nitrogen-doped carbon nanodots using a one-step hydrothermal method. Ethylenediamine served as the nitrogen dopant, while reaction parameters were systematically optimized. Fluorescence intensity increased with walnut husk amount up to 2 g but decreased beyond this value. Similarly, 400 µL of ethylenediamine produced maximum fluorescence emission. Optimal synthesis conditions were 2 g walnut husk, 400 µL en, 20 mL DI water, 200 °C temperature, and 8 h reaction time.

Parameter	Variation Studied	Observed Effect on Fluorescence	Optimized Value
Carbon source	Walnut husk amount	Fluorescence intensity increased up to a certain amount and then decreased	**2 g**
Nitrogen dopant	Ethylenediamine (en) volume	Maximum fluorescence obtained at an optimal volume	**400 µL**
Solvent	Deionized water	Provided suitable reaction medium	**20 mL**
Reaction temperature	Hydrothermal temperature	Enhanced carbonization and fluorescence at higher temperature	**200 °C**
Reaction time	Hydrothermal duration	Optimal nanodot formation at specific time	**8 h**
Synthesis method	One-step hydrothermal method	Efficient synthesis of N-doped carbon nanodots	Optimized

Heteroatom (Nitrogen) Doping (Summary)

Heteroatom doping is an effective strategy to enhance the optical and electronic properties of carbon nanodots. Among various dopants, nitrogen is preferred due to its atomic size similarity with carbon and five valence electrons. Nitrogen incorporation modifies surface states and improves chemical reactivity. It also enhances fluorescence intensity and quantum yield. Therefore, nitrogen doping plays a crucial role in improving N-CND performance.

3.3. Morphological and Structural Characterization of N-CNDs (Summary)

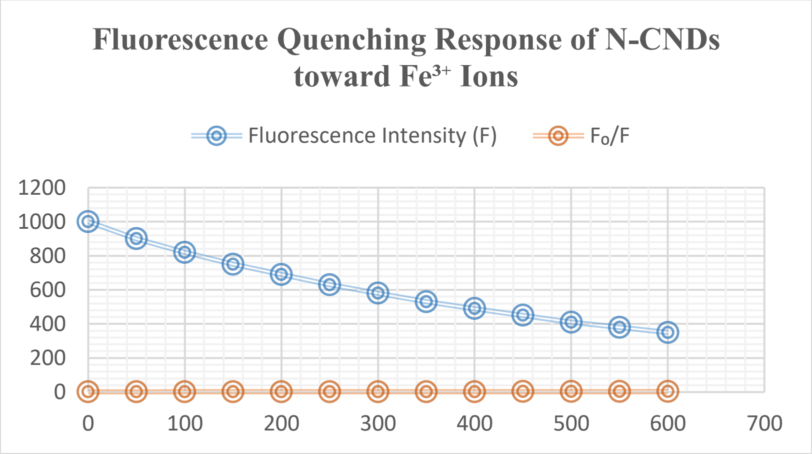
TEM analysis confirmed that N-CNDs are nearly spherical, uniformly distributed, and well dispersed in water with an average size of ˜3.1 nm. FT-IR spectra revealed abundant oxygen- and nitrogen-containing functional groups on the surface. Raman and XRD analyses indicated a partially graphitic yet predominantly amorphous carbon structure. XPS results confirmed the presence of carbon, oxygen, and nitrogen with successful nitrogen doping. These surface functionalities enhance solubility, fluorescence, and sensing ability of N-CNDs.

3.4. Optical Properties of N-CNDs (Summary)

UV–Vis spectra of N-CNDs showed characteristic π–π* and n–π* absorption transitions. The nanodots emitted bright sky-blue fluorescence under UV light, indicating strong photoluminescence. Excitation-independent emission behavior suggested surface-state-controlled fluorescence. A slight red shift in emission maxima was observed with increasing excitation wavelength. Fluorescence intensity was also found to be concentration dependent.

3.4.1. Metal Ion Sensing (Summary)

N-CNDs were evaluated for selective metal ion detection using fluorescence quenching studies. Among various tested ions, Fe^{3+} caused the strongest quenching effect. A linear relationship between F_0/F and Fe^{3+} concentration was observed from 0 to 600 µM with a high correlation coefficient. The limit of detection was calculated as 0.083 µM. These results demonstrate high sensitivity and selectivity of N-CNDs toward Fe^{3+} ions.



3.4.2. Fe^{3+} Ion Detection in Real Samples (Summary)

The applicability of N-CNDs was verified in tap water and blood serum samples. Filtration removed insoluble impurities before analysis. Coexisting metal ions showed negligible interference in Fe^{3+} detection. Strong fluorescence quenching was observed only in the presence of Fe^{3+} ions. Visual and spectroscopic results confirmed reliable Fe^{3+} sensing, highlighting the potential of N-CNDs for real-sample applications.

Conclusion

In summary a green Nitrogen doped carbon nanodots (N-CNDs), were successfully prepared through an eco friendly, simple low cost hydrothermal method using green walnut husk as a precursor for carbon and ethylenediamine as a source for nitrogen with a quantum yield of 45.6 %. nitrogen doping enhance the optical and QY of N-CNDs. The TEM measurement shows qusi – spherical shape of N-CNDs having an average partical size of 3.1nm in diameter, FT – IR and XPS spectroscopy confirm the existance of – OH, NH_2, – CO – and – COOH functional group on the outer surface of N-CNDs. UV – VIS spectra shows the presence of 2 absorption pecks at 345nm attributed to n – π* transition and at 236nm assigned to π – π* transition, our as synthesized N-CNDs are used for the sensing of Fe^{+3} ion in DI water and in practical samples tap water and blood serum, a good linear relationship ranging from 0 – 600 µM was recorded with (R^2) = 0.9943, the (LOD) were 0.083 µM.

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