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Fluorescent N-doped Carbon Dot-Copper and Silver Nanocomposite - An effective uric acid sensor

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Green fluorescent Nitrogen doped Carbon Dots (N-CDs) was synthesized by solvent free pyrolysis technique . Using the synthesized N-CDs, for first time we report the synthesis of Blue fluorescent Nitrogen doped silver and copper carbon dot nano composite using a Simple, Solvent free Green method. The N-CDs function as reducing agent to reduce Ag^{+} and Cu^{2+} ions to Ag^{0} and Cu^{0} which leads to the formation of composite. The synthesized N-CDs and nano composites were applied as Uric Acid(UA) sensor. N-CDs and the composites function as a flurophore in the recognition of Uric acid.Both the N-CDs and the composites were characterized using UV-Vis, FTIR, SEM-EDX, TEM and PL spectroscopic techniques. The UV-Vis and FTIR response of N-CDs in comparison with N-doped silver and N-doped copper carbon dot composites confirms that the surface functional groups on N-CDs have been used in the formation of silver and copper carbon dot composite Thesurface morphology and elemental composition of synthesized carbon dots and its nanocomposites were identified using SEM-EDAX analysis. High resolution transmission electron microscopy (HR-TEM) analysis shows that N-Cdot are spherical in shape with an average size of 15.23 nm, Cu/N-Cdot composite is found to be spherical and the size to be in the range of 18.02 nm and Ag/N-Cdot composite is found to be spherical and the size of the composite is found to be 16.40 nm. The PL spectra was also observed for Cu/N-Cdots, Ag/N-Cdots. With addition of Uric acid there was a quench in fluorescence which is immediately visible by our naked eye The quench in fluorescence is due to the synergistic effect between the fluorescence Inner Filter Effect (IFE) and the static quenching effect, with a Lower detection limit (LDL) of 4µM thus functioning as a highly rapid UA biosensor.Simple naked eye observation and the absence of catalytic effect by the metal ion in the detection of UA are the merits of the present study.

Keywords: Copper/N-doped carbon dot, N-doped carbon dots, Silver/N-doped carbon dots, Uric acid sensor

Heteroatom doped Carbon dots provide effective binding site and incorporates surface modifications such that they function as effective Fluorescent sensors.The ratio of the hetero atom to the carbon content can be varied depending on the type of synthesis which also alters the optical property of Doped CDs. Compared to Non doped CDs , the doped CDs function as effective sensors since their excitation wavelength shifts from 260-320 nm to higher wavelength and the emission band may shift to green, yellow and red. If the functional groups of the heteroatom doped CDs do not function as an effective sensor, they can be modified by the addition of biopolymers, organic moieties through substitution, elimination, condensation, dehydration, carbonization reactions to obtain specific sites for binding[1]. The fluorescence nature of NCDsis tuned based on the surface groups and also by using various precursors for synthesis[2-4]. Because of the attractive properties of N-CDs likephoto stability,ease of solubility in aqueous medium, tunable photoluminescence, biocompatibility they are used as suitable candidates in the field of sensors, bioimaging, catalysis etc[5].The recognition of biomolecules has become a vital need in the field of medicine, food, environment etc. Uric Acid (UA) being a major catabolite of purine metabolism in the human system[6]has a pKa of 5.75. Since it is present in body fluids like serum and urine it functions as an important biomarkerin clinical diagnosis for diseases such as gout, hyperuricemia or Lesch-Nyan Syndrome[7]. The normal level of uric acid in serum is between 0.13 and 0.46 mM (2.18-7.7 mg dL–L)[8] and 1.49-4.46 mM (25-74 mg/dL) in urine[9]. Abnormal levels of UA in body fluids leads to cardiovascular, neurological, hypertension, and renal insufficiency related diseases. As a result, its determination and diagnosis are a prerequisite for physiological investigations. Recognition of UA is carried out using various analytical technique like Chromatography[10], electrochemistry[11,12] and spectroscopic methods[13-15]. NCDs due to their

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multicolour photoluminescence(PL) makes them as suitable candidate in the field of sensors.Recently doped CDs with metal and noble metals are used as fluorometric sensors[16-18] have been used in the recognition of biomolecules, here we reportthe synthesis of N-CDs and their silver and copper composite . The composites were synthesized using solvent free pyrolysis method, characterized using UV-Vis, SEM-EDX, HR-TEM and PL spectroscopy.The synthesized N-CDs and its composites function as a flurophore and based on the Inner Filter Effect and quenching phenomenon they successfully function as an UA sensor.

Materials and Methods

All the chemicals used in the study were of extra pure analar grade and hence without further purification they were used for the analysis. Ethanolamine was purchased from Sisco Research Laboratories, Hydrogen peroxide (H_2O_2) 6% solution from PS Laboratories, Uric acid from Scientific Chemicals, KH_2PO_4 from Nice Chemicals, Na_2HPO_4 from Nice Chemicals, Cupric sulphate from Chemspure, Silver Nitrate from Spectrum Chemicals and Reagents. All solutions were prepared using Distilled water.Phosphate buffer of pH 6.8 was used for the sensor studies towards recognition of UA.

Synthesis of N-CDs, Copper /N-Carbon dot nanocomposite (Cu/N-CDs) and Silver /N-Carbon dot nanocomposite (Ag/N-CDs)

N-CDs were synthesized as reported in earlier literature[19,20]. For the synthesis of Cu/N-CDs, in a clean dry beaker 3 mL of ethanolamine was taken and 4.5 mL hydrogen peroxide was added, to that 10 mL of 5 mM copper sulphate solution was added and heated at 150°C for 7 min. The solution was completely vaporized and a black residue was obtained which is copper/N-Carbon dot nanocomposite, the residue shows blue fluorescence under UV radiation confirming the formation of Cu/N-C-dots[21]. It was also confirmed with SEM-EDX analysis.The residue is used for further studies.For the synthesis of Ag/N-CDs in a clean beaker 3 mL of ethanolamine is added to 4.5 mL hydrogen peroxide, to that 10 mL of 5mM silver nitrate solution was added and heated at 150°C for 7 min. The solution was completely vaporized and a black residue was obtained which is Ag/N-CDs. The residue shows blue fluorescence under UV radiation confirming the formation of silver/ N-C-dots[22]. For the sensor studies 0.01 g of N-CD and its composite were used.

Characterization Studies

UV-Vis studies were conducted using Elico SL20 Double Beam UV-Vis spectrophotometer in the range of 200-800 nm. Surface functionalized Carbon Dots are stable in water through the formation of an extensive hydrogen bonding network[23]. Fourier Transform-Infrared Spectroscopic Analysis was carried out using KBr pellet method in the range 400-4000 cm^{-1} using SHIMADZU IR AFFINITY 1. Surface morphological studies and elemental analysis was carried out using FEI Quanta 200 F High Resolution Scanning Electron Microscopy with Energy dispersive X-ray diffractometer. HRTEM analysis of the sample was done using TEM-2100 plus High-Resolution Tunneling Electron Microscopy. Very small amount of N-CDs, was dissolved in ethanol, sonicated for 15 min and 10 micro litre of the sample was taken and dispersed on the copper grid. The copper grid was further dried in hot air oven for 30 min at 60°C and analysis was carried out. The same procedure was carried out for the composites too for HR-TEM analysis. Photoluminescence Analysis (PL) was performed using Jascospectro-flurometer FP-8300.

Protocol used for the Detection of UA using N-CDs, Cu/N-CDs and Ag/N- CDs

To 0.01g of N-CDs, 10 mL of phosphate buffer was added and stirred for 15 min to get a homogeneous solution. The pH of the solution was maintained at 6.8. Further, to this solution 1 mL additions of 1 mM of uric acid (UA) was added and stirred for a few min. With every 1 mL addition, the change in absorbance was measured using UV- Vis, change in fluorescence was measured using PL techniques The same procedure was repeated for different concentrations of UA *i.e.*, 1 Milli molar, 10 Milli molar, and 100 Milli Molar. Similarly for the detection of UA using Cu/N- CDs and Ag/N-CDs 0.01 g of each was taken separately and the above procedure was repeated.

Results and Discussion

Synthesis of N-CDs

During the synthesis of N doped Cdots, color change was observed. *i.e.*, from bright yellowto orange red which indicates the polymerization step is in progress and finally a dark colored solution is obtained which confirms that the carbonization process is complete[23]. On complete vaporization of the residue N-Cdots was obtained (Fig. 1A). It was collected , stored at room temperature and used for

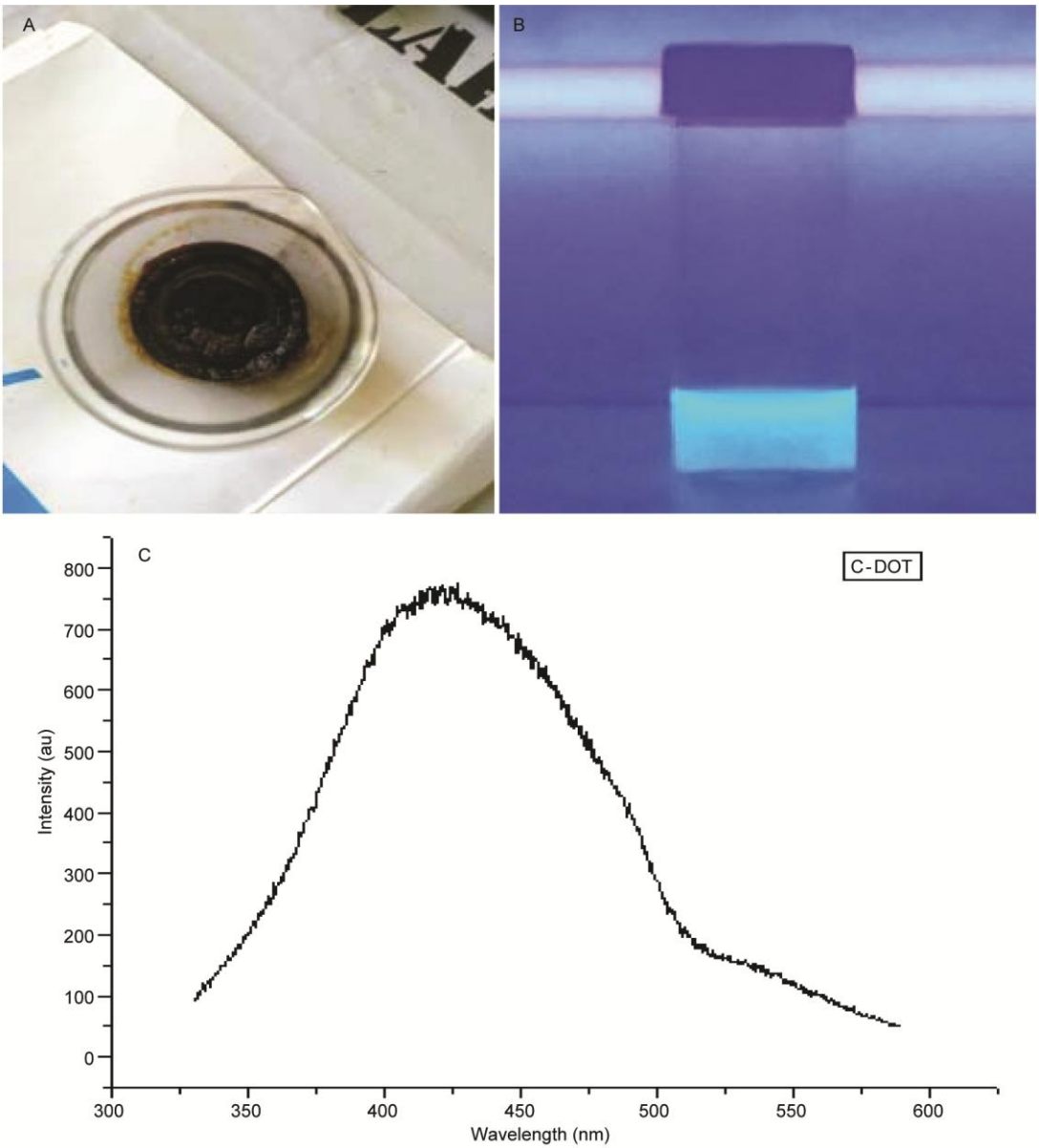


Fig. 1 — (A) synthesized N-CDs; (B) N-Cdot under UV light; and (C) PL response of N-Cdot

characterization and application studies. The N-CDs obtained was found to be highly soluble in aqueous solution conforming a high content of surface polar organic groups containing carbon, nitrogen and oxygen[24]. A bright GREEN fluorescence was observed (Fig. 1B) for N-CDs which is due to the increased amount of surface passivation caused due to hydrogen peroxide which is in accordance to earlier reports[23]. The green emission (longer wavelength) is due to the intrinsic state emission (electron-hole recombination)[25]. The photoluminescence spectra of N-CDs shows peak intensity at 750 au at cm^{-1} (Fig. 1C). This behavior is attributed to the surface state affecting the band gap of the carbon dots[26] *i.e.* the fluroscence originate from the transition of lowest unoccupied molecular orbital (LUMO) to the highest occupied molecular orbital (HOMO). The HOMO-LUMO gap depends on the size of the N-CDs and stronger luminescence is observed in smaller C-dots. The polygonal structure of N-CDs from the SEM-EDAX value also confirms arm chair edge and hence reason for increased luminescence[27]. The N-CDs were found to be stable in solution at room temperature for more than 2 months and also exhibited fluorescence. This stability is due to the amorphous nature of N-CDs[28].

Synthesis of Copper/C- dot composite

Figure 2A shows the synthesis of Cu/N-C-dot. It is observed that in the process of synthesizing the composite the colour changes from blue to yellow and then to reddish orange and finally to black solution, conforming the formation of Cu/N-Cdot composite. From (Fig. 2B) it is observed that there is a blue luminescence is observed compared to N-doped carbon dot *i.e.* absorbance is shifted towards shorter wavelength. The photoluminescence spectra of Cu/ N-Cdot show peak intensity occurs at 900 au as shown in (Fig. 2C). A blue shift compared to that of N-CDs. Large number of amine groups and oxygen rich groups on the surface of carbon dots function as receptors[21] and capture the copper ions through metal coordination to form a complex whose absorption band overlap partially with the emission spectra of carbon dots. The blue fluorescence may be because the Cu^{2+} ions may be attached to the carboxyl groups and hydroxyl groups of N-CDs and not to the nitrogen moiety. Thus no quench in fluorescence is observed[29]. Cdots also function as reducing agent as they are excellent electron acceptors and electron donors[30]. The hydroxyl and carboxyl groups on the surface of CQDs play an important role such that N-CDs

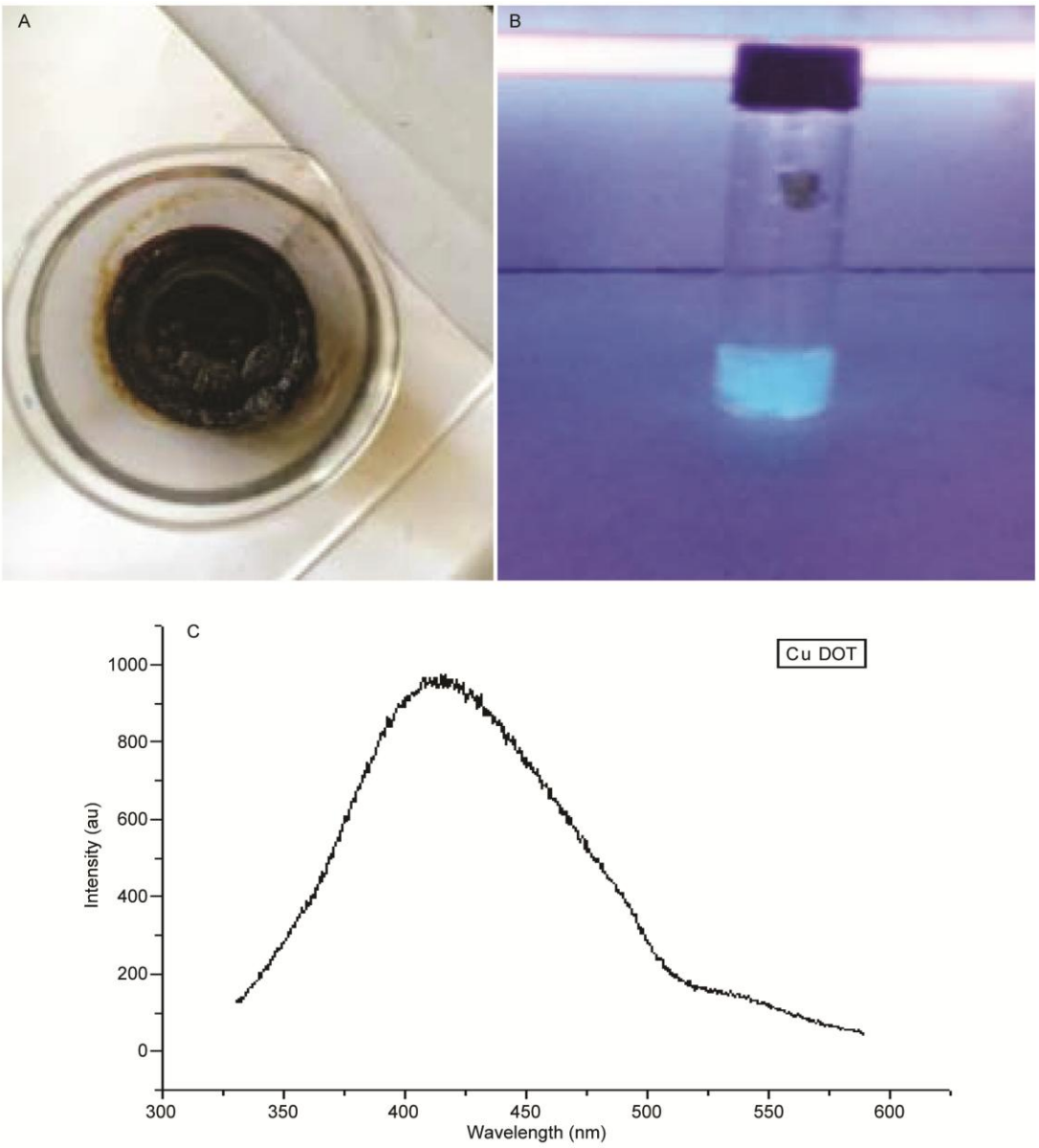


Fig. 2 — (A) Cu/N-CDs; (B) Cu/N-CDs under UV radiation; and (C) PL response of Cu/N-CDs

function as reducing agent and also lead them to be the nucleation centres for the growth of metallic nanoparticles[31]. Herein, N-CDs function as the reducing agent like citrate, ascorbates and sodium borohydride.

Synthesis Ag-Carbon dot composite

From Figure 3A shows the synthesis of Ag/C-dot composite. From Figure 3B it is observed that there is a blue luminescence compared to the green luminescence observed in N-doped carbon dot composite solution, which is due to inner filter effect[32]. The photoluminescence spectra of Cu/N-Cdot show peak intensity at 850 au in (Fig. 3C), a shift towards lower wavelength compared to N-CDs. Large number of carboxyl, hydroxyl and oxygen rich groups on the surface of carbon dots function as receptors and capture the silver ions through metal ion coordination forming a complex whose absorption band overlap partially with the emission spectra of carbon dots[33]. This results in the blue fluorescence[34]. The C-dots have the ability to directly reduce of Ag^{+} to Ag^{0} without the addition of any external reducing agent. By just incubating Ag^{+} with C-dot for 5 min, Ag NP's grow on carbon dot. This involves the oxidation of amine or phenolic hydroxyl group on

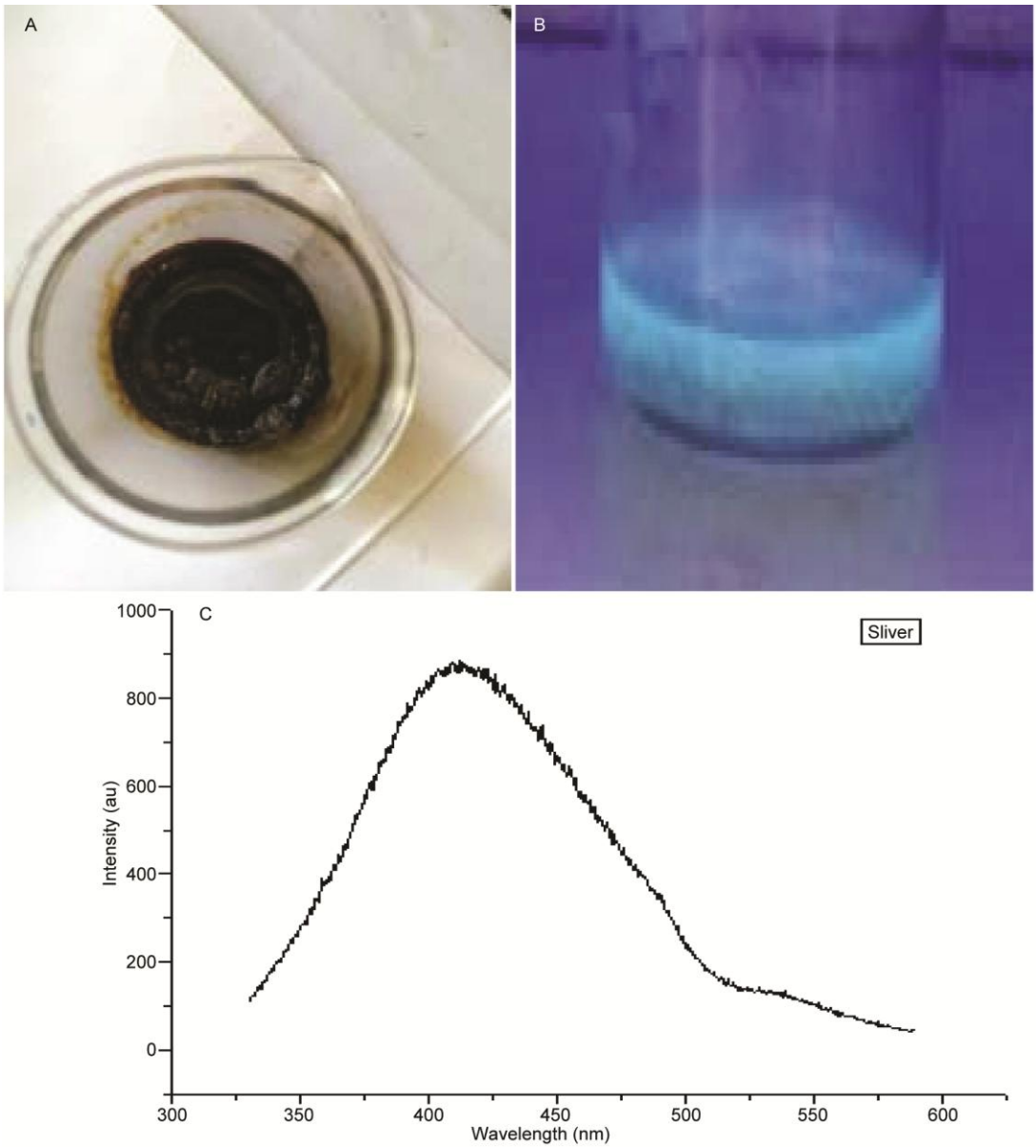


Fig. 3 — (A) Ag/N-CDs; (B) Ag/N-CDs under UV radiation; and (C) PL response of Ag/N-CDs

aromatic ring of C-dot. The C-dot functions as a reducing and stabilizing agent for the formation of Ag/N-Cdot composite.

UV-Visible, FTIR, XRD, SEM- EDX, TEM response of the synthesized N-Cdot,Cu/N-Cdot, Ag/N-Cdot

The UV response observed at 350nm in (Fig. 4A) confirms the formation of N-Cdots[35].Thus N-CDs dots with higher oxidation degree poses bright luminescence, the highfluorescence emission of N-CDs is attributed to the radiative recombinationof their surface-confined electrons and holes[29]. Figure 4B & C shows UV-Vis absorption spectrum for Cu/ N-C- dot composite and Ag/N-C- dot composites with a shoulder at 282 nm and 268 nm. On comparing the UV response of the composite with N-CDs, it is observed that the absorbance peak is shifted to lowerwavelength which confirms the formation of composite, this is further confirmed with thechange in fluorescence from green to blue.As N-CDs reduce the Cu^{2+} and Ag^{+} salt, nucleation gets initiated at the surface sites which host the electrons, thusthe electron–holerecombination process on N-CDs surface is disrupted, leading to blue fluorescence *i.e.* a shift towards shorter wavelength[30].

On comparing Figure 5B with 5C it is observed that the intensity of the peak in the N-CD composite is reduced compared to pure N-CDs. The peak between 3000- 3500cm^{-1} which is due to the –OH stretching is reduced and found to be broad in (Fig. 5B) on comparing with pure N-CDs Weak peaks ranging from 2500-3000 cm^{-1} are due to C-H stretching vibration of CH_2 and CH_3 groups. The strong peak at 1400 cm^{-1} in (Fig. 5B) corresponds to C-N stretching. The peak at 1360 cm^{-1} confirms the symmetric stretch of N-O due to the oxidized amido group. The peak at 1600 cm^{-1} shows the C=O stretching of the amide bond. The peak at 1271 cm^{-1} is the stretching vibration of the benzene ring linked to tertiary amine or aryl ether =C-O-C. The peak at 1060 cm^{-1} confirms the symmetrical stretching vibration peak of aryl ether. The peak at 782 cm^{-1} corresponds to C-H stretch. On comparing the FTIR response between N-Carbon dot, silver/N-Cdot and copper/N- carbon dot composites it is clear that the in plane and out of plane bending vibrations of OH at 1300-1500 and at 650 cm^{-1} and the intensity of C=O vibrational absorption at 1670 cm^{-1} is decreased. This confirms that the surface functional groups on N-carbon dots have been used in the formation of silver and copper carbon dot composite.

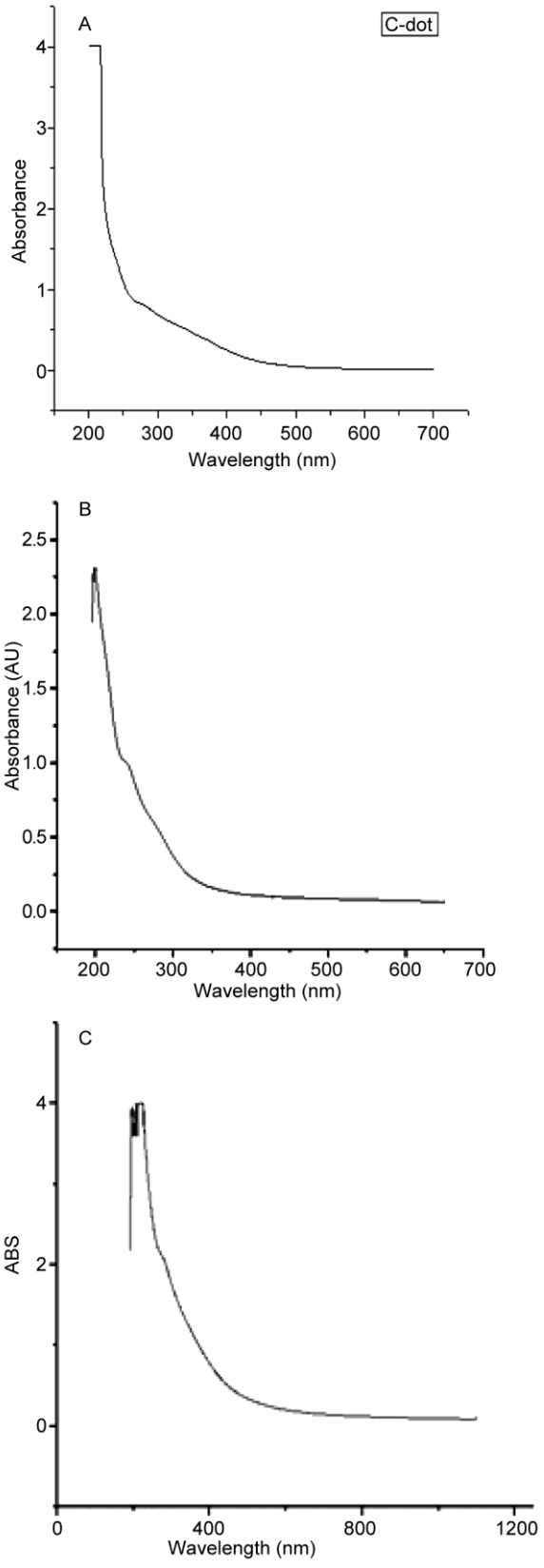


Fig. 4 — (A) UV-Vis spectrum of N-doped CDs; (B) UV-Vis spectrum of Cu/N-CDs; and (C) UV-Vis spectrum of Ag/N-CDs

Figure 6A shows the SEM image of N-doped Carbon dots. The C-dots are found to be polygonal in shape *i.e.* arm chair edge. The dimensions of C-dot were found to be in the range between 22 and 72 nm which is also confirmed from XRD studies. The EDAX response, (Fig. 6B & C) depicts the elemental composition of N-doped Carbon dot. The weight percentage ofcarbon was found to be 62.32

Figure 6D shows the SEM image of Cu/ N-Cdot composite. It is found that the composite are spherical in shape. Its dimensions were found to bein the range of 84 and 105 nm. Figure 6D & E represents the elemental composition of Cu and N-CDs using EDX. The weight percentageof carbon and copper was found to be 58.98 and 3.14

SEM image of Ag/C-dot composite (Fig. 6G) illustrates its dimensions to be in the range between 62 and 142nm. Figure 6H & I depicts the elemental composition of Ag/C-dot Nano composite using EDAX. The weight percentage of silver and carbon was found to be 13.77 and 51.53.

From the HR-TEM image of N-CDs (Fig. 7A) it is observed that the particles are spherical in shape and fitting the histogram to a Gaussian model with mean diameter of 15.23 $\pm$ 2 nm. Figure 7B shows the HR-TEM image of Cu/N-Cdot Composite. They are also found to be spherical and fitting the histogram to a Gaussian model the mean diameter is 18.2 nm. Figure 7C shows the Ag/N-Cdot Composite which are found to be spherical and fitting the histogram to a gaussian model the mean diameter of the composite is found to be 16.40 nm.

Detection of Uric acid using N-Cdots,Cu/N-Cdot nanocomposite, Ag/N-Cdot nanocomposite

Figure 8A shows the quench in fluorescence with additionof 1mM uric acid, It is also confirmed by the decrease in absorbance observed from Fig. 8(B &C) UV-VIS spectraand photo colorimeter response *i.e.* The quench in fluorescence observed with addition of UA when compared to N-CD is because of the synergistic effect between the fluorescence Inner Filter Effect (IFE) and the static quenching effect.Withe every addition of UA the absorbance decreases and similarly a quench in fluorescence is also observed. Thus N-C dot is able to function as a flurophore to sense UA . The quench in fluorescence is observed immediately with the addition of UA. The decreasein fluorescence is observed easily by the naked eye.

Figure 9A shows decrease in intensity of blue fluorescence with every 1 mL addition of 1mM uric acid *i.e.*, fluorescence is found to be quenched, which is also confirmed by the decrease in absorbance measured using UV-Vis spectra results which support the above findings (Fig. 9B & C). Thus, similar to N-C dot the quench in fluorescence is because of the synergistic effect between the fluorescence internal filtering effect and the static quenching effect. At the surface sites that host the electrons, the electron–hole recombination process were disrupted which led to fluorescence quenching effect. The quench in absorbance is observed immediately with addition of UA to the composite solution.

Detection of Uric acidusing Ag/N-Cdot nanocomposite

Figure 10A shows a blue fluorescence of Ag/N-C dot observed in presence of UV radiation in presence of 1 mM UA. The hydroxyl and carboxyl groups in the surface of N-Cdot serve as reducing and stabilizing centres for the reduction of Ag^{+} to Ag^{036}. On comparing the fluorescence with Ag/N-Cdot, it is observed that with every 1 mL addition of 1 mM uric acid, there is a quench in fluorescence, which is also confirmed by the decrease in absorbance measured

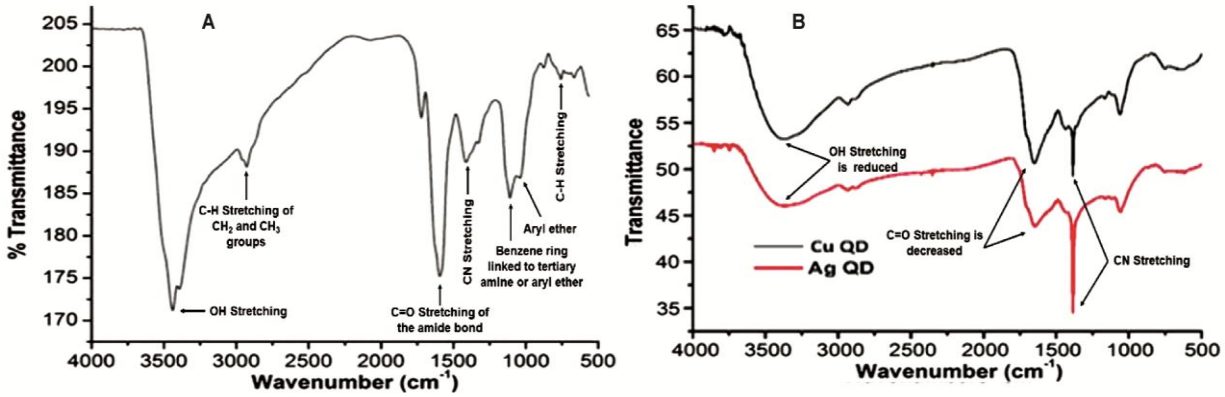
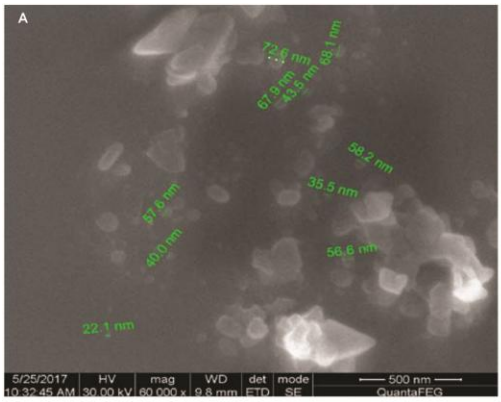
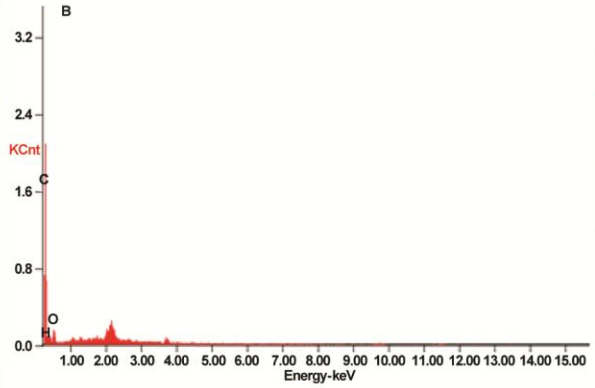


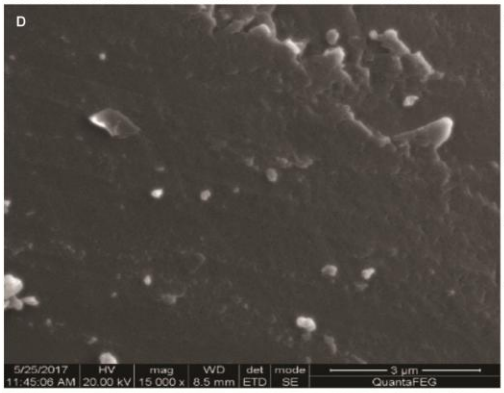
Fig. 5 — FTIR spectrum of (A) N-C dots; and (B) Ag/N-CDs and Cu/N-CDs

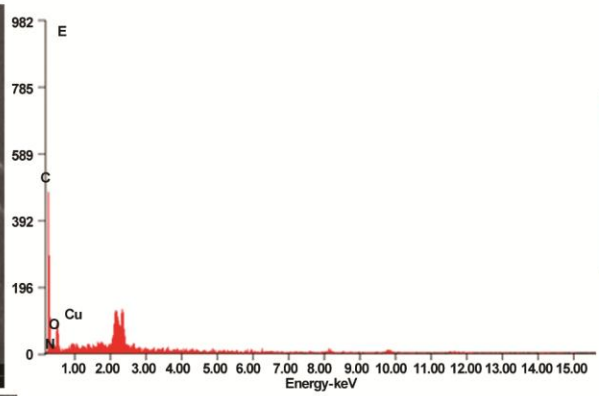




C

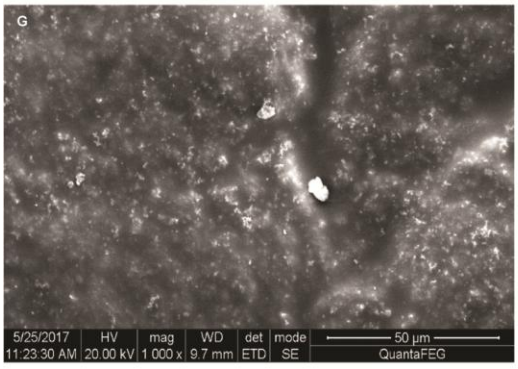
Element	Wt%	At%
CK	62.32	66.95
NK	23.23	21.40
OK	14.45	11.65
Matrix	Correction	ZAF

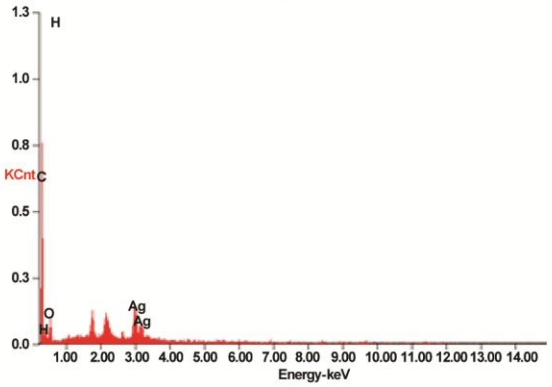




F

Element	Wt%	At%
CK	58.98	65.60
NK	17.80	16.98
OK	20.08	16.76
CuL	03.14	00.66
Matrix	Correction	ZAF





I

Element	Wt%	At%
CK	51.93	64.09
NK	16.93	17.92
OK	17.37	16.10
AgL	13.77	01.89
Matrix	Correction	ZAF

Fig. 6 — (A) SEM Image of N-CDs (B & C) EDAX response of N-CDs; (D) SEM image of Copper/N-CDs (E & F) EDX response of Copper/N-CDs; (G) shows the SEM image of Ag/N-CDs; and (H & I) EDX image of Ag/N-CDs

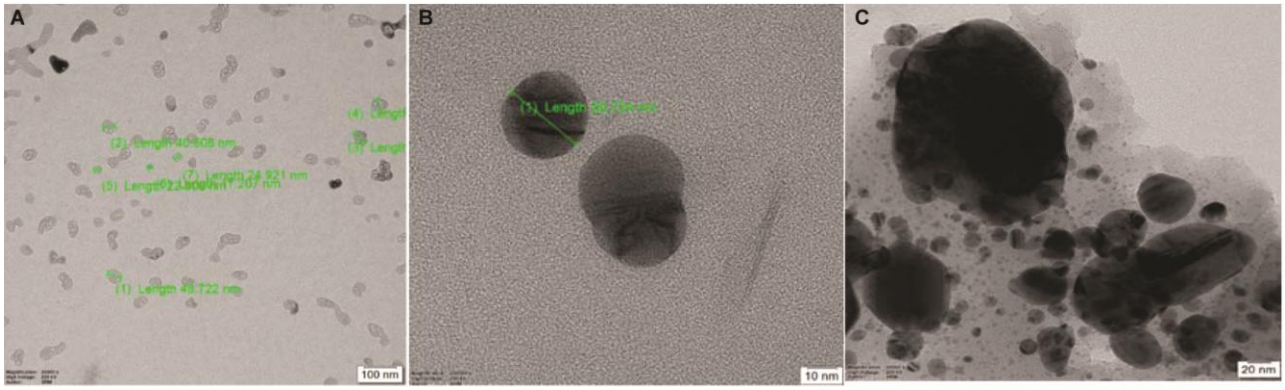


Fig. 7 — High-Resolution Transmission Electron Microscopy image of (A) N-CDs; (B) Cu/N-CDs; and (C) Ag/N-CDs

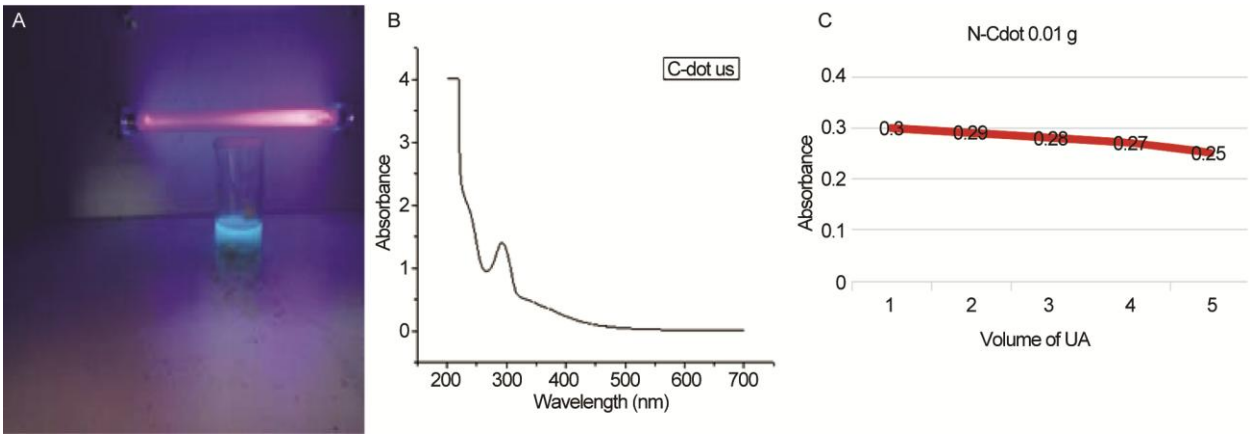


Fig. 8 — (A) N-C-dot +UA (1 mM); (B) UV-Vis response for N-Cdot with 1 mM UA addition; and (C) Change in Absorbance with 1 mL additions of UA

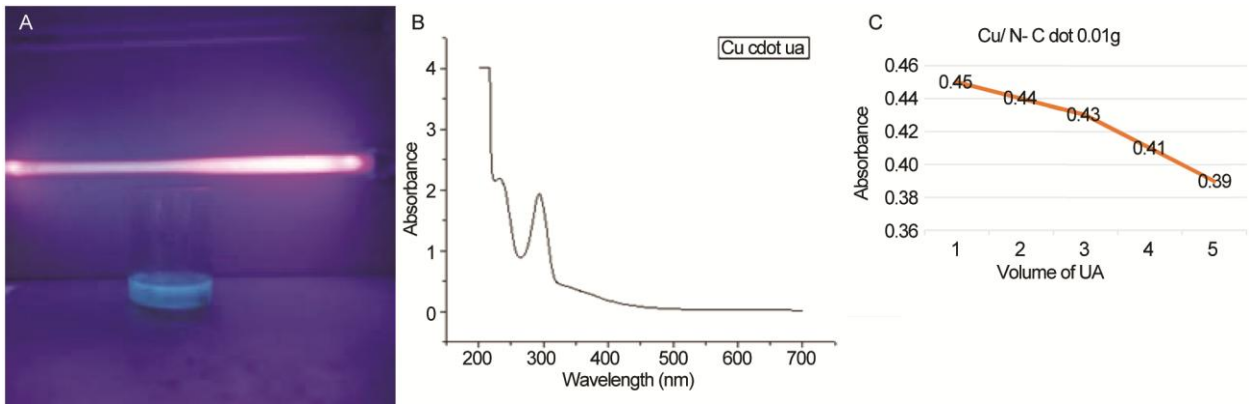


Fig. 9 — (A) Cu/N-Cdot +UA (1 mM); (B) UV-Vis response of Cu/ N-Cdot+ UA (1mM); and (C) Change in Absorbance with additions of 1 mM UA

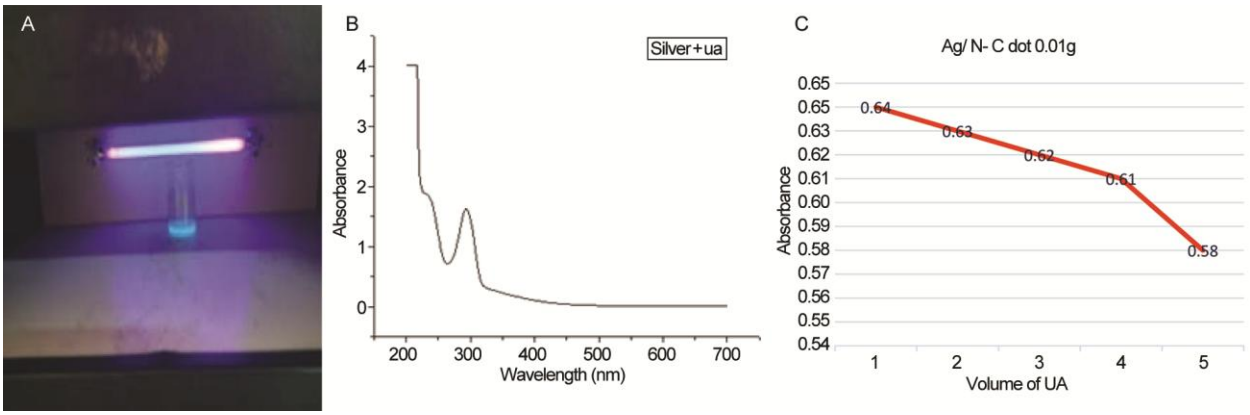


Fig. 10 — (A) Ag/N-Cdot +UA (1 mM); (B) UV-Vis response of Ag/N-Cdot+ UA (1 mM); and (C) Change in absorbance with additions of 1 mM UA

using UV-Vis spectra results (Fig. 10B & C).The surface sites that host the electrons, the electron–hole combination processes on N-CDs were disrupted because of the electrostatic and hydrogen bonding[37-40] formed between the UA and N-Cdots, leading to fluorescence quenching effect. The quench in absorbance is observed immediately with addition of UA to the composite and similarly in all the cases it is observed that the quench in fluorescence was found to have a linear decrease in absorbance with increase in addition of UA.

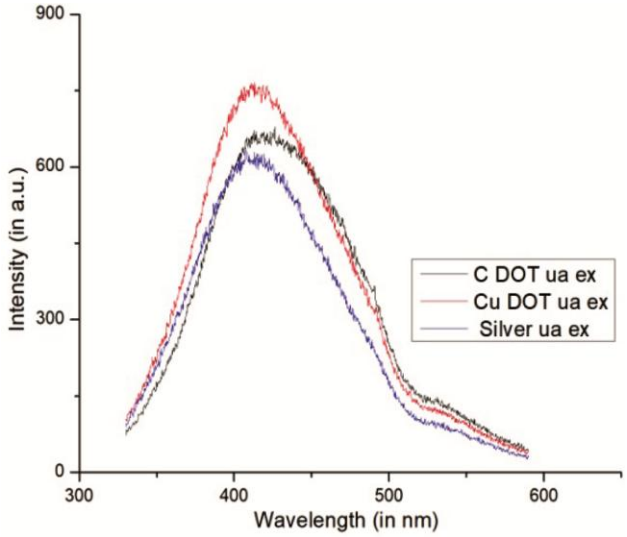


Fig. 11 — Detection of UA by N-CDs, Cu/N-CDs,Ag/N-CDs

Comparison of N-C dot, Cu/ N-Cdot and Ag/N-Cdot as an effective UA biosensor

The PL response in Figure 11 compares all the three systems towards sensing of UA. It is observed that all the three systems exhibit quench in fluorescence on addition of UA, the quench in fluorescence is considered as an analytical signal towards recognition of UA. No catalytic effect is observed on UA due to the presence of metal ions Thepka of uric acid is 5.75 and it is a weak acid. As a result of this uric acid gets easily ionized and bonds with the surface groups present on N-Cdots, Cu/ N-Cdots and Ag/N-C dot leading to quenching in fluorescence. The interaction of molecules or ions with the surface groups of the C-dots can affect the recombination of electron hole[41-44]. A lowest detection limit towards sensing of UA is found to be 4 µM. The response by the system is also rapid. Thus N-Cdot, Cu/N-Cdot, and Ag/N-Cdot function as an effective and rapid UA biosensor.

Conclusion

In this work N-CDs, Cu/N-CDs and Ag/N-CDs were synthesised using the precursor's ethanolamine and hydrogen peroxide. The synthesized Nitrogen doped carbon dots and its nano composites were characterized using UV-VIS, FTIR, SEM-EDX, TEM and PL spectroscopic techniques. The UV-Vis peak for N-C-dots is observed at 350 nm and a peak is observed at 268 and 282 nm for Cu/N-Cdot and Ag/N-Cdot composites respectively.. This shift in peak towards lower wavelength compared to N-doped C dots due to surface plasma resonance confirms the presence of Cu^{2+} and Ag^{+} on the surface of N-doped C dots.With addition of copper and silver ions to N-CD matrix a blue luminescence is observed instead of Green obtained for pure N-C dots. On comparing the FTIR response between N-Carbon dot, N doped silver and N doped copper carbon dot composites it is clear that the in plane and out of plane bending vibrations of OH at 1300-1500 and at 650 cm^{-1} and the intensity of C=O vibrational absorption at 1670 cm^{-1} is decreased in the composites compared to N-Carbon dot confirming that the surface functional groups on carbon dots have been used in the formation of silver and copper carbon dot composite.The elemental composition of synthesized N-CDs and its nanocomposites were identified using EDAX analysis. SEM and HR-TEM studies shows the gives the surface morphologies of prepared samples in nanometer dimensions. It shows that N-Cdot and its composites are spherical in shape The PL spectra was observed for N-Cdots, Cu/N-Cdots, Ag/N-Cdots .PL response for the synthesiesd Cu/N-Cdot, Ag/N-Cdot in presence of UA shows a shift towards higher wavelength compared to that of N-CDs and no catalytic effect is observed in presence of metal ions thus functioning as a very good system towards UA sensing. A lowest detection limit towards sensing of UA is found to be 4 µM. The response by the system to sense UA is very fast thus N-Cdot, Cu/N-Cdot, and Ag/N-Cdot function as an effective UA biosensor.

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Conflict of interest

All authors declare no conflict of interest.

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