Effect of Cu-doped carbon nanoparticle (CuCNP) on *Vignaradiata*

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Carbon dots or carbon nanoparticles (CNPs) are unique class of nanocarbons with unique photoluminescence property. Considering the biocompatibility of CNPs, versatile biological applications have been documented including augmentation in photosynthesis. Significant attention has also been paid towards the electron donor-acceptor property of CNPs, industrial importance was increased too. Despite some of the scattered efforts with CNPs on plant system, less attention has been paid on the photo-physiological properties of plant system and downstream compatibility study in agricultural sector using doped CNPs. Here in we showcase facile synthesis of Cu doped CNPs as model component of metal doped CNPs and its preliminary study on plant system. Our study shed the light of effectivity in plant system for downstream compatibility in agricultural sector.

Keywords: Carbon nanoparticle, Copper, Fluorescent, Chlorophyll

1. Introduction:

Carbon dots or carbon nanoparticles (CNPs) are a fascinating class of quasi-spherical nanocarbons with unique photoluminescence property. It exhibits size and excitation wavelength (λ_{ex}) dependent photoluminescence (PL) behavior and are arguably biocompatibility to be used for biological purposes especially in imaging as biomarkers [1]. Simple and low-cost precursors for their synthesis further adds value to the process. They are highly water soluble and could be easily surface functionalized with various organic, inorganic, polymeric or biological species for versatile applications. CNPs with their well-defined nearly isotropic shapes together with ultrafine dimensions, tunable surface functionalities and sheer variety of simple, fast and cheap synthetic routes provide an interesting alternative to other nanocarbons like fullerenes, nano-diamonds in host of applications. Amongst many of the known properties, CNPs act as a good electron donor as well as good electron acceptor too. Of late, continuous efforts have been made to develop doped CNPs with good electrochemical properties. For instance, phosphorus doped CNPs can be used as good semiconductors because of strong air-stable properties [2]; and can also be used as efficient metal free electrocatalyst [3]. Meanwhile, boron doped CNP exhibits excellent oxygen reduction reaction (ORR) activity, stability and immunity towards methanol crossover and co-poisoning [4]. Even nitrogen and boron co-doping in CNP shows better ORR performance [5]. Taking the advantage of unique PL

properties of CNPs, detection of hazardous materials, like carcinogenic dyes or iodide ions, have been made using doped CNPs [6, 7]. Therefore, industrial importance of metal doped CNPs have increased enormously, however the downstream compatibility or doped CNPs/plant interactions has not been addressed yet properly.

Photosynthesis is considered to be a biological process which converts the light energy into chemical energy by means of a series of complex systems involving electron donor acceptor pathways. Therefore, it was anticipated that CNPs could have a significant impact towards photosynthesis through its electron donor-acceptor properties. Keeping this perspective in mind, Chandra et al. have demonstrated the augmentation of photosynthesis through energy transfer without toxicity within plant system [8]. Despite some of the scattered efforts with CNPs on plant system, less attention has been paid on the photo-physiological properties of plant system and downstream compatibility study in agricultural sector using doped CNPs. It was previously noted that copper nanoparticles (CuNPs) had shown promising effect on *Vigna radiata*. Integrating all these know how, we anticipated that copper doped CNPs (CuCNP) might have a beneficial response to the plant system and downstream agricultural sector, without any kind of toxic symptoms. Therefore, CuCNPs were synthesized using microwave assisted technique and its efficacy was tested on model plant system *Vigna radiata*. The results were critically compared with commercially available copper salt, CS as well. Our result highlighted that CuCNP

was found to elevate the growth and development in the plant system, without significant detrimental effects.

2. Materials and methods:

2.1. CuCNP synthesis

CuCNP was synthesized according to the established protocol with little modification [9]. Briefly, 10 mg of starch was dissolved in 100 mL of water; 5 mL of this solution was added to 30 mL of PEG-200 solution. To it 5 mL of CuSO_4 solution (1×10^{-3} M) was added. The resultant solution was heated in a domestic microwave oven at 450 watt until a characteristic brown color solution was formed indicating the formation of CuCNP.

2.2. Plant material and growth conditions

Mung bean seeds (*Vigna radiata* var. *Sonali*) were purchased from Berhampur Pulse and Oil Research Centre. Seeds (20 seeds/replicate; 3 replicates in total) were washed in deionized double distilled water and surface sterilized with 5 % sodium hypochloride solution for about 20 min. Then the seeds were washed thoroughly with deionized double distilled water and imbibed with the treatment solutions (control; CuCNP: 0.05 mg /L, 0.1 mg /L, 0.5 mg /L, 1 mg/L; CS 0.05 mg /L, 0.1 mg /L, 0.5 mg /L, 1 mg /L) in dark for 4-6 h, prior to the germination.

2.3. Morphological parameters

Root and shoot length, fresh and dry weight of CuCNP and CS treated plants were measured. Dry weight was recorded by drying the plants at 80 °C for 24 h.

2.4. Estimation of Photosynthetic Pigment content

Control and treated leaves were centrifuged twice with 80 % alkaline acetone at 6000 X g for 20 min and chlorophyll content were estimated with the supernatant spectrophotometrically [10] in terms of μg chlorophyll/ g fresh tissue. Carotenoid was estimated according to the method of Davies with little modification [11,12] and the data were expressed in terms of OD / g fresh wt.

2.5. Data analysis

The data were analyzed by using a two-way ANOVA (multivariate) with PASW statistics 18 software. Means were separated by using the Tukey-Kramer (HSD) test, at $P = 0.05$.

3. Results and Discussion:

Change in color under microwave irradiation was the primary indication of CuCNP formation. The color of the solution turned to golden yellow and gradually to brown starting from a colorless solution. Formation of CuCNPs were noted by UV-Vis measurement (Figure 1a). A characteristic peak was observed at 220 nm indicating the formation of CuCNPs from their aqueous dispersion. It was interesting to note that, aqueous dispersion was brown in color, however under the exposure of UV light, CuCNP

Figure 1. (a)UV-Vis spectra, (b) PL spectral pattern of copper doped carbon nanoparticles.

dispersion exhibited a bright green coloration. This motivated us to check for the PL properties of CuCNP. PL property of CuCNP was measured using its aqueous dispersion, while experiment, the excitation wavelength was varied from 340 nm to 410 nm. Interestingly, CuCNP exhibited excitation dependent emission and intensity. Once again, this excitation dependent PL emission and intensity is a characteristic feature of carbon dots reported in literature. Meanwhile, maximum PL emission was noted at 370 nm excitation wavelength as shown in Figure 1.b. The origin of high PL properties of doped carbon dots is a matter of debate till now. We believe presence of defect sites while incorporation of functionality, and shallow trapped excitons plays pivotal role for its high PL property. Meanwhile different trapped excitons may get excited at different excitation wavelength and fluoresce yielding an excitation dependent emission and intensity for the same. Since CuCNPs exhibited maximum emission at 370 nm excitation wavelength, the rest of the experiments were conducted at the same excitation wavelength. Another important phenomenon noted is the marginal quench in PL intensity in CuCNP compared to the pristine CNP at 370 nm excitation wavelength. This could be due to the doping of Cu on to CNP and is again consistent with literature, where quenching was noted with the doping of metal on to a fluorophoric material.

CuCNP was extremely hydrophilic in nature, which was

Figure 2. (a) Comparison of PL spectral pattern, (b) HR-TEM image of copper doped carbon nanoparticles (inset: SAED pattern).

evident with the ease of dispersion in a wide variety of polar solvents such as water, ethanol, NMP, acetonitrile etc. FTIR analysis also confirmed its hydrophilic nature with the presence of surface hydroxyl groups. Morphology of CuCNP was confirmed using high resolution transmission electron microscopic (HR-TEM) analysis, which confirmed extremely small particle size distribution of 2-5 nm (Figure 2.b). The particles were spherical and distinctive in nature, without any kind of agglomeration. Meanwhile, selected area electron diffraction (SAED) pattern confirmed its crystalline nature. In this context, presence of Cu as elemental composition in electron diffraction pattern associated with HR-TEM confirmed the doping of Cu onto CNP. Altogether, well characterized, hydrophilic, fluorescent Cu doped CuCNP was ready at this stage for their biological effect evaluation.

Synthesized CuCNPs were then tested against mung bean plant and compared with commercial Cu salt CS at same experimental dosages. CuCNP at four different dosages (0.05 mg/L, 0.1 mg/L, 0.5 mg/L and 1 mg/L) were tested against the plant system and also compared with CS. Although CuCNP did show positive impact on plant system in terms of root length, shoot length, fresh weight and dry weight. Plants were healthy, even no toxicity had been shown in higher doses as compared to CS. All the data are

Figure 3. (a) Effect of CuCNP and CS on chlorophyll contents of 15 days treated mung bean plants. (b) Effect of CuCNP and CS on carotenoid content of 15 days treated mung bean plants. Data represents mean $\pm$ SE (n, no. of samples = 3). Within each type of treatment mean pigment content ($\pm$ SE, n=3) followed by the same upper case letter is not significantly different for a particular dose, within each dose mean pigment content followed by the same lower case letter is not significantly different; Tukey-Kramer HSD test. (Detailed statistical analyses in Appendix 2, ESI).

shown in Table 1. Detailed statistical analysis is shown in supplementary information. Considering the results, we could outline that CuCNPs negated the toxic responses of CS in terms of growth and development. Pigment contents were also estimated using CuCNPs and compared with CS too. In both experiments i.e., chlorophyll and carotenoid content analysis, pigment contents were increased in comparison to CS. Pigment content (chlorophyll and carotenoid) analysis is shown in Figure 3. These data were statistically analyzed, and analyses were given in SI.

Table 1: Effect of CuCNP and CS on root and shoot length, fresh and dry weight of 15days treated mung bean plants. Data represents means $\pm$ standard errors (no. of samples = 25). Capital letter changes along the row owing to significance of respective pairwise treatments, while the small letter changes along the column. (Detailed statistical analyses in SI).

Treatment	Control	0.05mg/L	0.1mg/L	0.5mg/L	1mg/L
Root length(cm)					
CS	4.64 $\pm$ 0.853Aa	3.67 $\pm$ 0.334Ba	3.52 $\pm$ 0.303Ba	2.74 $\pm$ 0.36Ca	2.03 $\pm$ 0.29Da
CuCNP	4.64 $\pm$ 0.853Aa	5.8 $\pm$ 0.22Bb	4.5 $\pm$ 0.26Cb	3.53 $\pm$ 0.33Db	2.67 $\pm$ 0.44Eb
Shoot length(cm)					
CS	11 $\pm$ 0.678Aa	10.56 $\pm$ 0.518Aa	9.33 $\pm$ 0.502Ba	8.28 $\pm$ 1.2Ba	7.58 $\pm$ 0.67Ca
CuCNP	11 $\pm$ 0.678Aa	12.03 $\pm$ 0.48Bb	10.48 $\pm$ 0.64Cb	8.07 $\pm$ 0.41Da	5.69 $\pm$ 0.93Eb
Fresh weight(g)					
CS	0.159 $\pm$ 0.007Aa	0.152 $\pm$ 0.012 Ba	0.149 $\pm$ 0.001Ba	0.107 $\pm$ 0.013Ca	0.086 $\pm$ 0.005 Da
CuCNP	0.159 $\pm$ 0.007Aa	0.164 $\pm$ 0.004 Bb	0.159 $\pm$ 0.001 Bb	0.147 $\pm$ 0.002Cb	0.128 $\pm$ 0.012Db
Dry weight(g)					
CS	0.018 $\pm$ 0.002Aa	0.013 $\pm$ 0.003Ba	0.011 $\pm$ 0.006Ca	0.009 $\pm$ 0.001Da	0.006 $\pm$ 0.001Ea
CuCNP	0.018 $\pm$ 0.002Aa	0.022 $\pm$ 0.002Bb	0.019 $\pm$ 0.002Cb	0.013 $\pm$ 0.002Db	0.01 $\pm$ 0.002Eb

These results highlighted that, CuCNP showed promising effect in the plant system, and some of the basic physiological parameters are well within the safety limits. Currently, metal doped CNPs have attracted industrial attention. Therefore, our objective was to check the impact of metal doped CNPs (here CuCNP was used as a model) on agricultural sector, where the industrial effluent could have a significant role to play. This experiment therefore shed the light of biocompatibility of CuCNP in agricultural sectors, and some of the industrially important metal doped CNPs could follow the same trend as well.

4. Conclusion:

In a nut shell, CuCNPs were synthesized in a microwave assisted facile technique. Synthesized CuCNPs were hydrophilic in nature, demonstrated excitation dependent PL property. Preliminary study on mung bean plant demonstrated its biocompatibility and it showed promising effect on the plant system. Considering the current attention paid towards the metal doped CNPs, this study would further highlight the downstream safety aspects on agricultural sector for metal doped CNPs. We believe detailed systematic study on plant system would further unlock the potential of metal doped CNPs in agricultural sector in near future.

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Supplementary Information

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