

RESEARCH ARTICLE

Green Synthesis of Copper Quantum Dots using *Rubia cordifolia* Plant Root Extracts and its Antibacterial Properties

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Abstract

This study introduces a novel idea of quantum dots preparation using plant materials. The copper nanoparticles in the form of quantum dots were synthesized using aqueous extract of the root of the plant *Rubia cordifolia*. The prepared quantum dots were characterized using UV-Visible double beam spectrophotometer, SEM, TEM, AfM and fluorescence microscopy. The synthesized quantum dots (QDs) were spherical, rough surfaced particles in the size of 22.68 nm. AfM showed that these quantum dots are having green fluorescent nature. In this study, antibacterial efficiency of Cu quantum dots was also investigated. Colony forming capability assay and diameter of inhibition zone measurement showed a good antibacterial action of CuQDs against tested pathogenic bacteria viz., *Klebsiella pneumoniae*, *Shigella* spp., *Escherichia coli*, *Plesiomonas shigelloides*, *Streptococcus aureus* and *Pseudomonas aeruginosa*.

Keywords: *Rubia cordifolia*, copper quantum dots, capability assay, inhibition zone, antibacterial action.

Introduction

Nanomaterials have a long list of applicability in improving human life and its environment. Modern science has just started to explore quantum dots in the 21st century. Quantum dots (QDs) are nanocrystals of inorganic semiconductor that emit light in all colours of the spectrum depending on their size (Drbohlavova *et al.*, 2009; Zhai *et al.*, 2011). The size of QDs decreases as they get closer to the blue-end of the spectrum and increases as they proceed to the red end. They have unique properties such that they can even be tuned beyond visible light, into the infra-red or into the ultra-violet spectrum. They were able to confine conduction band electrons, valence band holes or excitons in all three spatial directions (Murray *et al.*, 2000). The QDs are valuable tools in biotechnology, most especially in cellular imaging (Ghasemi *et al.*, 2009) and labeling. They are believed to be an excellent alternative to conventional fluorescent dyes used in imaging (Chomoucka *et al.*, 2011; Kumar *et al.*, 2012). Nanocrystals of CdTe and CdTe/ZnTe are reported to be the most popular biolabels in imaging and direction (Gulia and Kakkar, 2013). Luo *et al.* (2011) reported the antibacterial efficiency of CdTe quantum dots. Jin *et al.* (2009) had reported the efficacy of zinc oxide quantum dots against food borne pathogenic bacteria. In the present study, Copper QDs were synthesized using the root extracts of the plant *Rubia cordifolia*. It is a "Novel" approach. The prepared Copper QDs were characterized and visualized by UV-visible spectrophotometer, SEM, TEM, AfM and fluorescence microscopy. Antimicrobial activity of QDs was tested against selected pathogenic bacterial strains.

Recent work on surface modification of QDs has led to the development of new generation of probes for trace able targeted gene delivery (Qi and Qao, 2008). CdSe QDs-amphipol technology for intracellular drug delivery and real time imaging of siRNA in to cancer cells with significantly reduced cytotoxicity had been reported (Qi and Qao, 2008). Low cytotoxic ZnO QDs based non-viral vectors with dual functions of delivering plasmid DNA and labeling cells also had been developed (Gulia and Kakkar, 2013). Numerous quantum-dots based drug delivery systems explained became the long term fluorescence stability of the QDs is attractive for visualization of drug distribution *in vivo* (Gulia and Kakkar, 2013). Hence, CuQDs synthesized by biological process can be well investigated as multifunctional smart drug delivery nano carriers.

Materials and methods

Collection and preparation of Plant materials: *Rubia cordifolia* roots were purchased from an Ayurveda shop in Nagercoil, Tamil Nadu, India. Twenty grams of the plant material were weighed and washed thoroughly with double distilled water. Then the roots was cut into fine pieces and boiled with 100 mL of sterile double distilled water at 95°C for 20 min. The extract was filtered through Whatman No.1 filter paper. Finally, the extract was cooled and stored at room temperature.

Biosynthesis of copper quantum dots (CuQDs)

Preparation of Fehling's solution: Fehling's solution was prepared by mixing equal parts of the following solutions.

Solution 1: This was prepared by dissolving of copper (II) sulfate penta hydrate (6.9 g 0.02 mol) in distilled water (100 mL).

Solution 2: This solution was prepared by dissolving potassium sodium tartarate tetrahydrate (34.6 g) and sodium hydroxide (12 g) in distilled water (100 mL).

Preparation of Cu₂O quantum dots: In the preparation, 10 mL of the prepared plant extracts was added to 10 mL of Fehling's solution. After 10 min, the colour of the solution turned from blue to brick red, indicating the formation of cuprous oxide nanoparticles (Fig. 1). Then, the solution was continuously stirred in room temperature for 4 d. The product was washed with distilled water three times with equal time intervals and finally the product was washed with 100% methanol. Then, it was dried at room temperature for further studies.

Fig. 1. Green synthesis of copper nanoparticles.



Characterization: Synthesized QDs were characterized using UV-Visible Spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Atomic force Microscopy (AfM). The prepared samples were analyzed for the rate of absorption in UV/Visible region using of UV-visible Spectrophotometer in the range of 200 to 1000 nm. The surface morphology of the CuQDs was observed using SEM. TEM observation revealed the size, shape and morphology of the QDs. A specimen for TEM sample was made by casting a drop of suspension on a carbon coated copper grid and the excess solution was removed by tissue paper and allowed to air dry at room temperature for overnight. AfM images were obtained by scanning the mica surface in air under ambient conditions using AfM, operated in the static force mode. AfM measurements were obtained using scan Asyst air probes. The spring constant (nominal 20 nN) and deflection sensitivity has been calibrated (but not the tip radius the nominal value has been used; 2 nm). The advanced Fluorescence Microscope and UV light source were used to analyze the light emitting capability of synthesized nanoparticles.

Antimicrobial activity: Antibacterial activity of green synthesized QDs was tested against eight bacterial isolates viz., *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Plesiomonas shigelloides*, *Shigella* spp., *E. coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus* using agar well diffusion method.

Nutrient Agar plates were inoculated with 100 μ L of standardized inoculums (1.5×10^8 CFU/mL) of each bacterium (in triplicates) and spread with sterile swabs. Wells of 8 mm diameter were made in the agar plates containing the bacterial inoculums and the lower portion of the wells were sealed with a little molten agar medium. The prepared CuQDs (25, 50, 75 and 100 μ L volume) were poured into the wells. Standard Cu₂O was used as a negative control. The plates thus prepared were left at room temperature for 10 min to allow the diffusion of the extract into the agar medium. After incubation for 24 h at 37°C, the plates were observed. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9 mm zone was considered as inactive; 9-12 mm as partially active; while 13-18 mm as active and >18 mm as very active (Mariselvam *et al.*, 2013).

Results and discussion

The copper nanoparticles prepared were dark reddish brown in color in appearance. The CuQDs UV visible absorption spectra were in the visible range of 395 nm and near infrared region (Fig. 2). Surface morphology of the quantum dots was spherical (Fig. 3). The morphology and size of the synthesized copper quantum dots were further studied using TEM micrograph. TEM image is shown in Fig. 4. It is clear from TEM image that nanoparticles are spherical in shape and the size is 22-23 nm. AfM observation showed that the QDs synthesized were having surface roughness, surface stiffness and oxides growth in QDs (Fig. 5a-5f). In fluorescence microscopical observation, then the UV sources are focused on the prepared nanoparticles, the nanoparticles emitted green fluorescence and the fluorescence images confirmed reduction of copper oxides to CuQDs (Fig. 6 and 7).

Antimicrobial activity: The green synthesized CuQDs were tested against selected bacterial pathogens viz., *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Plesiomonas shigelloides*, *Shigella* spp., *E. coli*, *Streptococcus aureus*, *Vibrio alginolyticus* and *Pseudomonas aeruginosa* (Table 1). QDs inhibited the growth of all the bacterial pathogens remarkably. Hence, QDs had easily entered in to the cell and cellular content and interfered with the molecular mechanism leading to cell death and the bacteria are dead.

Fig. 2. UV-visible spectrum of synthesized QDs.

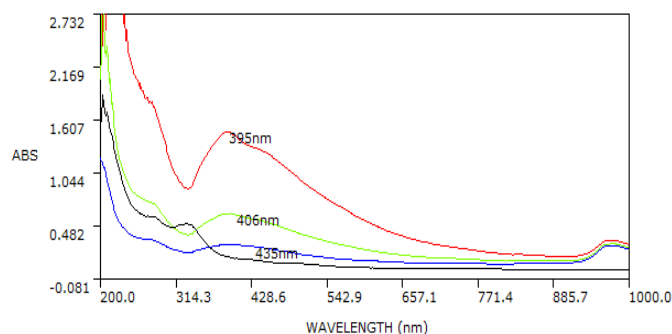


Fig. 3. SEM image of copper quantum dots synthesized.

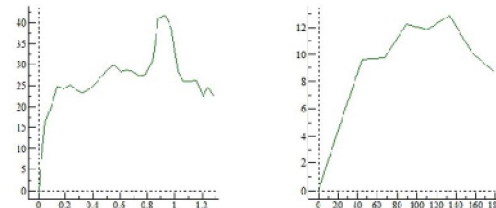
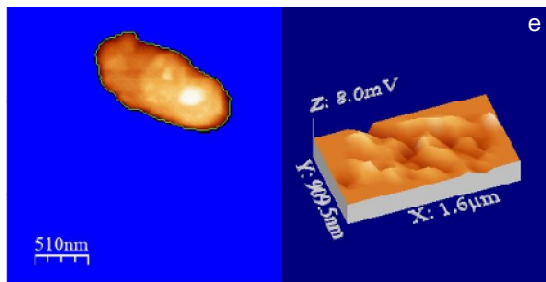
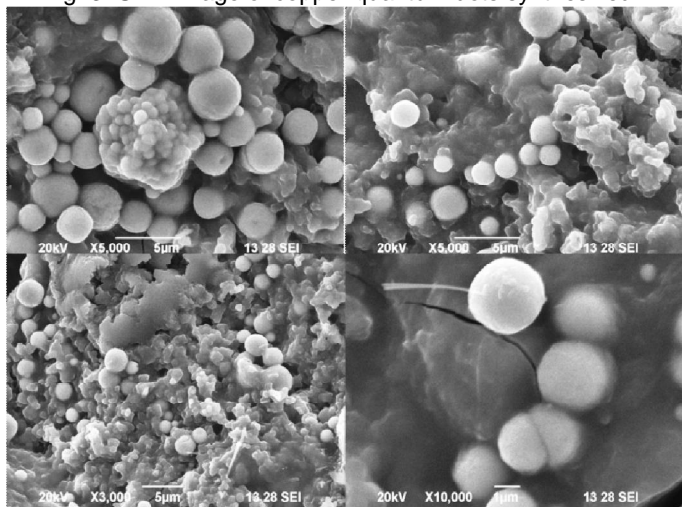


Fig. 4. TEM image of synthesized quantum dots.

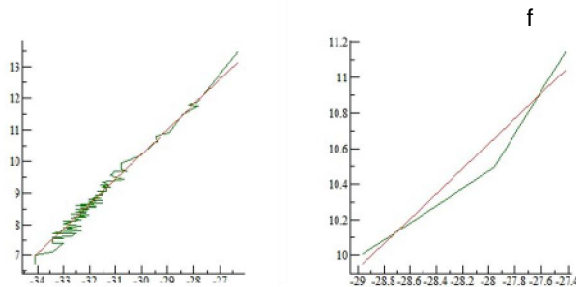
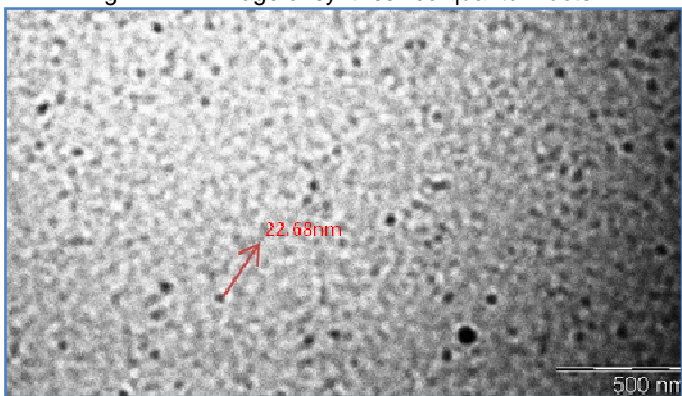


Fig. 5. AfM images of synthesized copper QDs.

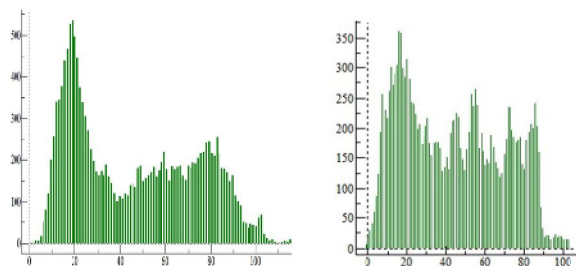
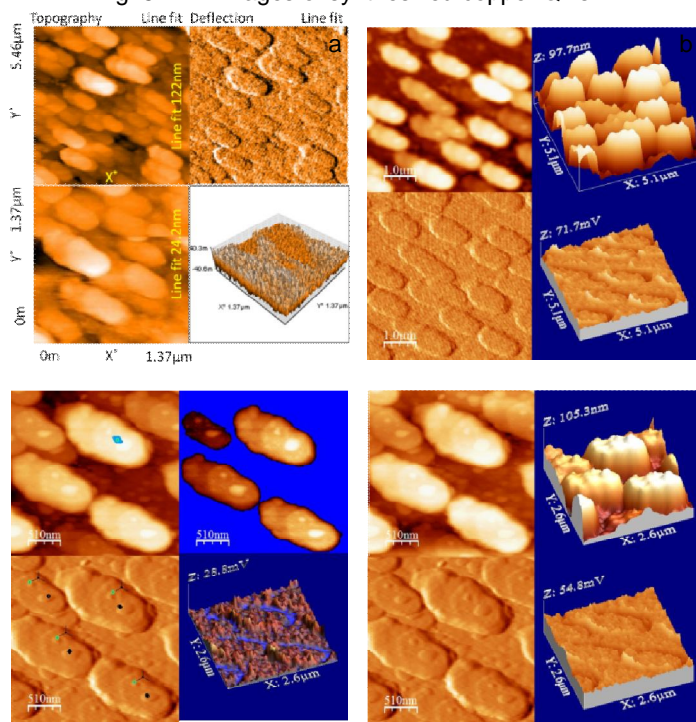


Fig. 6. Fluorescence Microscopical images of synthesized QDs.

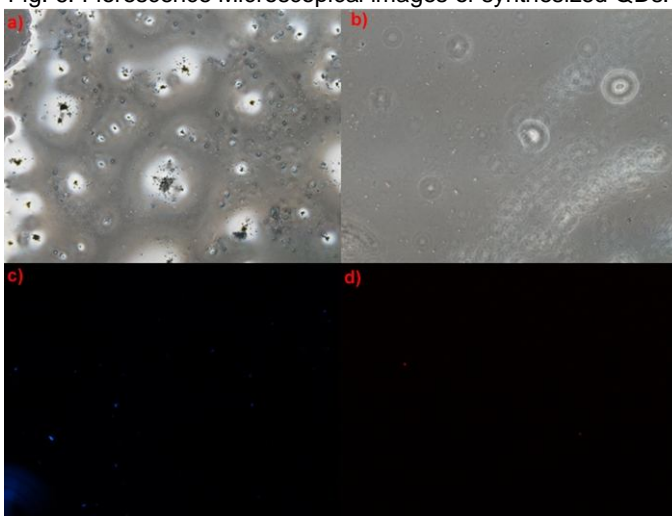
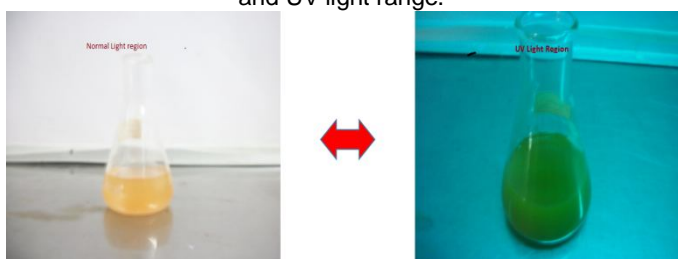


Table 1. Antimicrobial activity of green synthesized Copper QDs on selected bacterial pathogens.

Microorganisms	Zone of inhibition (mm)				Cu ₂ O (100 µL)
	25 µL	50 µL	75 µL	100 µL	
<i>Klebsiella pneumoniae</i>	11	20	24	25	21
<i>Vibrio parahaemolyticus</i>	0	0	0	0	20
<i>Plesiomonas shigelloides</i>	10	12	15	18	16
<i>Shigella</i> spp.	0	10	11	14	11
<i>Escherichia coli</i>	0	11	11	13	17
<i>Streptococcus aureus</i>	15	18	19	22	20
<i>Pseudomonas aeruginosa</i>	15	18	21	22	19
<i>Vibrio alginolyticus</i>	0	0	0	12	13

Fig. 7. Synthesized nanoparticles under normal and UV light range.



Conclusion

The use of fluorescent nanoparticles as probes for bio-analytical applications is a highly promising technique. The use of quantum dots in medicine is safe and it is an effective bio-sensitive material. As QDs are having the fluorescence, it can be used in various fields like biotechnology, healthcare, thin film coating, textiles and biomedical industries etc. (Choi *et al.*, 2008; Walling *et al.*, 2009). The size ranges for QDs had been reported to be between 2-20 nm (Kluson *et al.*, 2007). In the present study, the biologically synthesized copper quantum dots were in the size ranges between 22-23 nm (Fig. 4). Although there are reports on chemical synthesis of QDs, there is only a few works on the synthesis of QDs using plant extracts. The biologically synthesized QDs are very safe and eco-friendly when compared to the chemically synthesized nanoparticles. As the microorganisms are developing resistance to many antibiotics, the fluorescent copper nanoparticles can be used to prepare novel antibiotics also.

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