Int. J. Nano Dimens., 9 (1): 7-14, Winter 2018

ORIGINAL ARTICLE

Hydrothermal synthesis of Copper nanoparticles, characterization and their biological applications

Kondaiah Seku[1*]; Bhagavanth Reddy Ganapuram[2]; Babu Pejjai[3]; Girija Mangatayaru Kotu[2]; Golla Narasimha[4]

[1]Department of Chemistry, Malla Reddy Engineering College, Maisammaguda, Secunderbad, Telangana State 500100, India

[2]Department of Chemistry, Palamuru University, Mahabub Nagar, TS, India

[3]Department of Information Materials Laboratory (IMSL), School of Chemical Engineering, Yeuungnam University, Gyeongsam 38541, Republic of Korea

[4]Department of Virology, Sri Venkateswra University, Tirupathi, A. P., India

Received 27 July 2017; revised 18 October 2017; accepted 03 December 2017; available online 28 December 2017

Abstract

We report an ecofriendly novel method for copper nanoparticles (CuNPs) synthesis by hydrothermal processes using a carboxymethyl gum konagogu capping agent. The synthesized CuNPs characterized by Ultraviolet - visible Spectroscopy (UV-Vis), Fourier – transform infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM) and X-ray diffraction techniques (XRD). The XRD results showed a face-centered cubic structure with (111) as the prepared orientation. The CuNPs showed good antibacterial and antifungal activity against pathogenic strains like *E. coli, Bacillus cereus, Bacillus subtilis* and *Candida albicans, Candida parapsilosi, Aspergillus Niger* and *Aspergillus oryzae* respectively. A result of this study indicates that the CuNPs has remarkable potential antimicrobial property. It will be used in treating infectious diseases and also use full in biomedical application.

Keywords: *Antibacterial and antifungal activity; Characterization; Copper nanoparticles; Hydrothermal process.*

How to cite this article

Seku k, Reddy G B, Pejjai B, Mangatayaru Kotu G, Narasimha G. Hydrothermal synthesis of copper nanoparticles, characterization and their biological applications. Int. J. Nano Dimens., 2018; 9 (1): 7-14.

INTRODUCTION

Nanoscience and nanotechnology have been able to provide solutions to the society in various fields such as the environmental challenges viz. water treatment, sustainable chemical production, etc., as well as in areas like medicine, solar energy conversion, etc., [1, 2]. The properties of the materials at nano level scale are different from their same bulk size counterparts, due to quantum size effects and which is very significant to researchers due to their unique properties and wide-ranging application in a variety of areas including chemistry, physics, material science and biomedical science [3, 4]. Metal nanoparticles show outstanding optical, thermal, chemical, and physical properties which could be due to the combination of a large proportion of high energy [5] surface atoms relative to the bulk solid and the nanometer scale. The optical properties of nanoparticles depend on their size and shape as well as on the dielectric constant of the surrounding medium and inter particle distance [6].

CuNPs have greater attention in current years due to their novel electronic, optical, mechanical properties and catalytic, biological applications. Copper is a good alternative material for noble metals such as Au and Ag as it is extremely conductive and much more economical than them. CuNPs also have a high surface area to volume ratio, low production cost, antibacterial potency [7], and catalytic activity and magnetic properties as compared to precision metals such as gold, silver or palladium. Several methods were reported for the synthesis of CuNPs such as chemical reduction,

* Corresponding Author Email: *kondareddyseku@gmail.com*

electrochemical techniques, photochemical reduction and thermal decomposition, amongst them, most traditional and convenient method is the chemical reduction method. The main difficulty lies in their preparation and preservation as they oxidized immediately when exposed in air. Scientists are using different inert media such as Nitrogen, Argon [8] to overcome this oxidation problem also using reducing, capping or protecting agents for the reduction of copper salt used. Some reducing and capping agents is very expensive and also have toxic effects. To avoid the negative impacts of the chemical reduction methods, researchers are interested towards ''green methods'' for synthesis of nanomaterials using plant extracts, bio surfactants, natural gums, etc., in aqueous medium. The alternate of chemical synthetic methods without toxic, clean, and internationally approved eco - friendly methods [9, 10] are needed in current days for the synthesis of copper nanoparticles.

Eco- friendly synthesis of the CuNPs had significant importance in current years due to its simplicity and eco-friendliness. Several metal nanoparticles have been synthesized from various biological materials such as fungi, bacteria, leaf extract and fruit extract where metal salts are reduced into nano metal particles. In the reported research work modified carboxymethyl gum karaya used for the synthesis of gold nanoparticles [11]. Kondagogu gum is a polysaccharide ingredient and it is naturally available. This gum is easily obtainable, non-toxic, cheap, biodegradable, and biodegradable and it has potential applications in various fields.

Extraction of kondagogu gum from the bark of *Cochlospermum gossypium* (Bixaceae family) and the tribal people of Telangana was collected largely. The kondagogu gum primary structure is consist of sugars such as mannose, rhamnose, galactose, arabinose, glucose, galacturonic acid and gluconic acid with sugar linkage of (1 → 2) β – D-Gal p, (1 →4)β – D-Glc p, (1→ 6) β – D-Gal p, 4-O-Me-α-D-Glc p, (1 → 2) α-L-Rha [12] . It has effective surface activity due to the presence of fatty acids, high acetyl content and residual proteins in it.

Natural gums are polysaccharides, which have multiple sugar units linked together to create large molecules. Chemical modifications of natural gums have been employed to improve their properties as polymers. Carboxymethylation is one of the many strategies used for the functionalization of natural polymers. It is a widely employed modification method because of its ease of processing, lower cost of chemicals and versatility of the product obtained [13]. Generally increases the hydrophilicity and the solution transparency of the polysaccharides and makes it better soluble in an aqueous system by Carboxymethylation. Many natural gums such as Cassia Tora gum, gellan, guar gum [14] and tamarind kernel powder were investigated using the carboxymethylation process. Carboxymethylation of polysaccharides is a widely used conversion since it is simple and leads to products with a variety of properties.

The objective of this research work is to synthesis of CuNPs by hydrothermal processes using carboxymethyl gum kondagogu (CMGK). The synthesized CuNPs were characterized and studied their antimicrobial activities.

EXPERIMENTAL

Materials

All the test strains were procured from IMTECH, Chandigarh, India. Copper chloride, L-Ascorbic acid, monochloroacetic acid, sodium borohydride, tryptophan, yeast extract, Hydrazine Hydrate (HH) and bacterial grade agar–agar were purchased from Hi-Media Laboratories, India. All chemicals used were of analytical reagent grade. Gum Kondagogu was purchased from the Girijan Cooperative Corporation, Telangana, India. The solutions were made with Millipore water. All glassware and magnetic stir bars were cleaned in an aquaregia solution (3HCl : HNO_3) and then cleaned with Millipore water.

Synthesis of carboxymethyl gum of Cochlospermum gossypium

Gum kondagogu was functionalized to sodium carboxymethyl gum kondagogu by using chloroacetic acid. 1 gram of gum kondagogu was dispersed into a sodium hydroxide solution (45 wt %) with constant stirring for 40 minutes. 10 ml of mono chloroacetic acid (75 wt%) solution was added slowly for a period of half an hour to the above mixture under constant stirring. The reaction mixture was then heated to 70 ^{0}C for 40 minutes; the reaction mixture was cooled and suspended into 80% (v/v) methanol. The precipitate so obtained was then filtered and washed with glacial acetic acid till washing were neutral. The obtained product washed three times

with 60 ml portions of 80% (v/v) methanol, filtered and dried in hot air oven at 40^{0}C.The synthesis conditions were optimized [11].

Synthesis of copper nanoparticles (CuNPs)

Copper salts were used as basic precursors, carboxymethyl gum of *Cochlospermum gossypium* as a stabilizer, H-H as a reducing agent and L-Ascorbic acid as an antioxidant agent. NaOH was used as a catalyst and also to adjust the pH12. The CuNPs solution of concentration 0.6 % w/v was prepared in Millipore water. The Copper chloride (0.04M) and L-Ascorbic acid (0.001 M) solutions were prepared separately using Millipore water. The solutions of carboxymethyl gum of kondagogu as an L-Ascorbic acid were added to copper chloride solution under stirring. Then the solutions of HH (1M) and NaOH (0.01 M) were added to the mixed copper salt solution under stirring. The initial blue color of the reaction mixture eventually turned to brown-black color. Stirring was continued for another 1 hour to complete the reaction. The precipitate was washed twice with methanol after filtration and then dried to obtain copper nanoparticles. The schematic representation of CuNPs synthesis showed in Fig. 1.

Characterization of copper nanoparticles

The characterization of the synthesized CuNPs solution was recorded with Ultraviolet-visible- NIR spectrophotometer (Ultraviolet-3600, Schimadzu) at 200-700 nm scanning range and autoclaved gum used as a blank. The lyophilized CuNPs was used for FTIR analysis by KBr pellet method. A pure KBr pellet was used to subtract the spectra of the carboxymethyl gum and CuNPs. JASCO Fourier – transform infrared 4600 in the scanning range of 450–4000cm^{-1} used for recording spectrum. The powder – XRD technique was used to crystalline study of the CuNPs by XRD (Rigaku, Miniflex) method with Cukα (λ=1.5418 Å) radiation.

The prepared CuNPs morphology and size were examined by TEM. For TEM measurement the sample grid was prepared by placing a drop of aqueous CuNPs dispersion on the carbon-coated copper grid and consequently evaporating the water naturally overnight at ambient conditions. Measurements were completed on High-resolution transmission electron microscopy (HR-TEM) image was recorded using Tecnai G2 F20 S-Twin, USA system.

Antibacterial properties of CuNPs

The antibacterial activity of the ecofriendly synthesized CuNPs was carried out using the disc diffusion method. Gram-positive and Gram-negative bacteria, *Bacillus subtilis* and *Escherichia coli* were used as model test strains. Luria- Bertani (LB) agar medium was prepared and transferred to sterilize petri dishes. The medium was allowed to solidify and then the petri plates were spread with bacterial strains like *Bacillus subtilis* and *Escherichia coli*, *Pseudomonas aeruginosa* and *Escherichia coli* separately in a laminar air flow hood. Using micropipette, 5 and 10 μL of the CuNPs solution and 5 µL of CMGK solutions added to each well on both plates. These discs were air dried in laminar hood and incubated at 37 °C for 24 h. The zone of inhibition of bacteria was measured. The assays were performed in triplicate.

Antifungal Activity Studies of CuNPs

Antifungal activity of the green synthesized CuNPs was examined using the agar well diffusion assay method. The antifungal activities of CuNPs were tested against the fungi *Candida albican, Candida parapsilosi, Aspergillus oryzae* and A*spergilusniger,* on potato dextrose agar as the medium and miconazole as control.

In brief, 100 µL of a log phase cultures were seeded on PDA medium (Potato, Dextrose and agar) for fungus. After, solidification of all agar petri plates 8mm diameter five wells were formed by punching with sterile borer. In four wells 100 µL of CuNPs samples (0.2, 0.4, 0.6 and 0.8 µg/

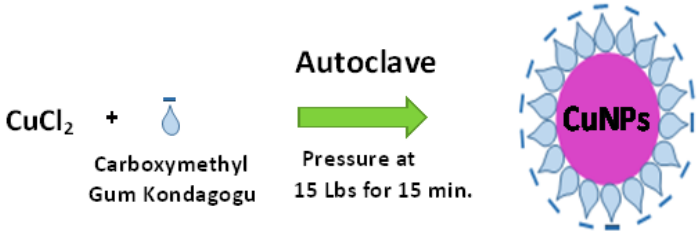

Fig. 1: The schematic representation of synthesis of copper nanoparticles (CuNPs).

mL) and in one well miconazole (400 µg/mL) as positive control were loaded. All Petri plates were incubated for 48 h at room temperature 28 + 4°C. The plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler. The mean value of such zones of inhibition was calculated by performing the experiments in triplicates.

RESULTS AND DISCUSSION

UV-Vis

UV-Visible absorption spectra of the CMGK stabilized copper nanoparticles prepared from copper chloride was shown in Fig. 2. The copper nanoparticles prepared displayed an absorption peak at around 558 nm. This peak was assigned to the absorption of copper nanoparticles [15, 16]. The broadness of the absorption peak was attributed to the size distribution of nanoparticles. This result was also in agreement with TEM observations. Based on TEM and ultraviolet observations, it was confirmed that prepared particles are copper nanoparticles. Since, no other measurable peak was observed in the spectrum that confirms that the synthesized products are copper only.

FTIR Spectrometry

FTIR spectra of CMGK and CMGK-capped CuNPs were recorded to study the interaction of functional groups of gum involved in the reduction of $CuCl_2$ and the stabilization of subsequently formed CuNPs. Fig. 3 shows the FTIR spectra of CMGK and CMGK capped CuNPs. The major peaks in the IR spectrum of CMGK peaks are observed at 3430, 2950, 1733, 1611, 1420, 1250 and 1040 cm^{-1} [curve (a) of Fig. 3. The broad absorption band at 3430 cm^{-1} and the narrow band at 1611 cm^{-1} are attributed to stretching and bending vibration modes of O–H bonds respectively. The peaks at 2948cm^{-1}, 1747 cm^{-1}, 1591 cm^{-1}, 1406 cm^{-1}, 1220 and 1026 cm^{-1} correspond to the asymmetric C–H stretch, the carbonyl stretching vibration, the asymmetric stretch of carboxylate [17], symmetrical stretch of carboxylate and acetyl group respectively. The peaks at 1149 and 1040 $cm^{-1}$are associated with the C–O stretching vibration of ether and alcohol groups [18]. Fig. 3 (curve b) shows the FTIR spectrum of CMGK capped CuNPs which shows the characteristic frequencies at 3365, 1745, 1592, 1410, 1225 and 1022 cm^{-1}. A shift in the peaks of the Fourier – transform infrared spectrum of CMGK capped CuNPs was observed from 3435 to 3365 cm^{-1} and 1609 to 1592 cm^{-1}. These shifts in the IR peaks suggest the binding of CuNPs with hydroxyl and carboxylate groups. Based on the peak shifts in the hydroxyl and carboxyl group, it can be inferred that both hydroxyl and carbonyl groups of gum are involved in the synthesis and stabilization of CuNPs.

XRD Studies

XRD was used to determine the crystalline structure of the nanoparticles. The XRD pattern of the CMGK stabilized copper nanoparticles was shown in Fig. 4. All the peaks in the XRD pattern can be indexed [18] to that of pure FCC metal Cu according to the literature pattern (JCPDS, File No 04-0836). The diffraction peaks observed at 2 theta values 44, 49 and 74 were indexed as (111), (200) and (220), respectively. The corresponding peak (111) plane is most intense compared to the other peaks in the spectrum. The broadening of

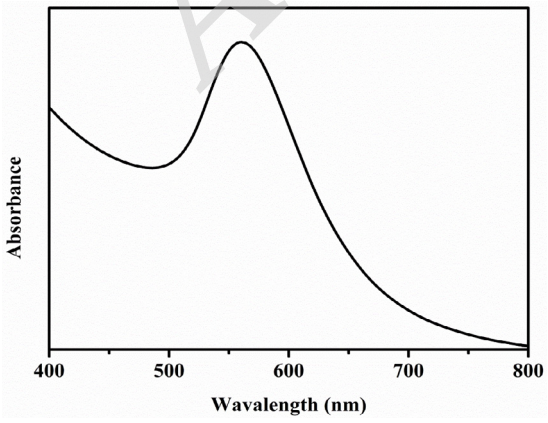

Fig. 2: UV visible absorption spectra of copper nanoparticles.

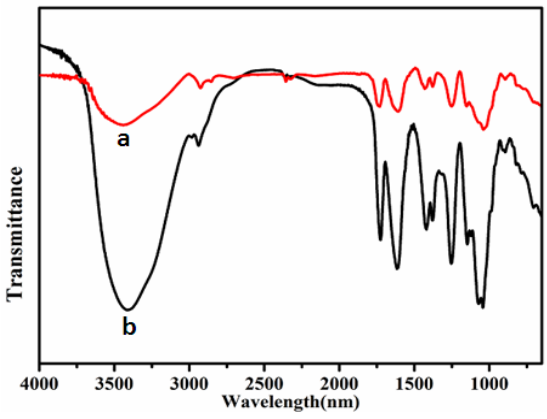

Fig. 3: FTIR spectrums copper nanoparticles a) carboxymethyl gum; b) CuNPs stabilized in gum.

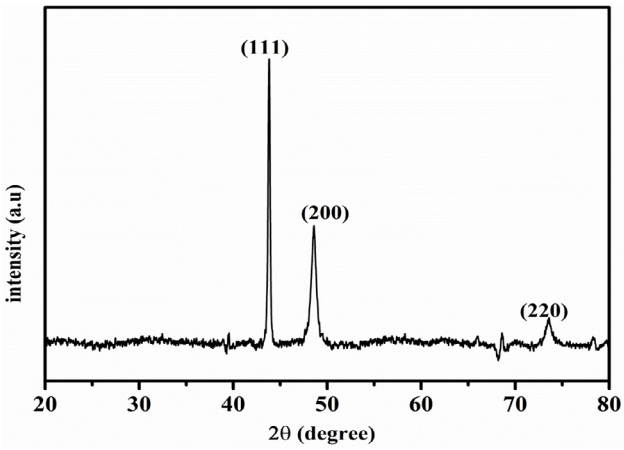

Fig. 4: XRD pattern of copper nanoparticles.

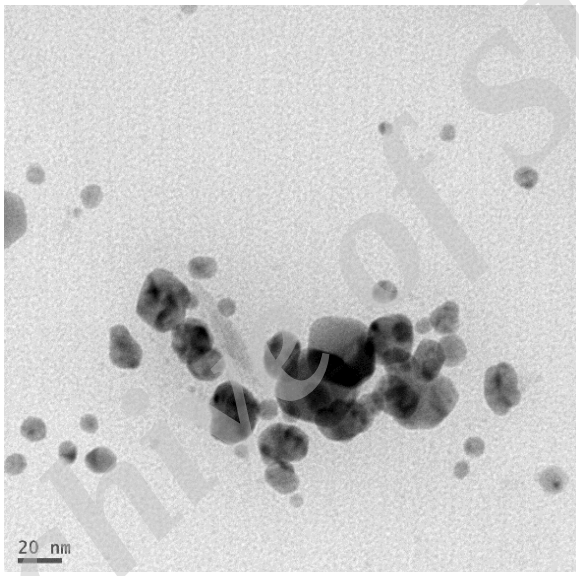

Fig. 5: TEM image of synthesized copper nanoparticles.

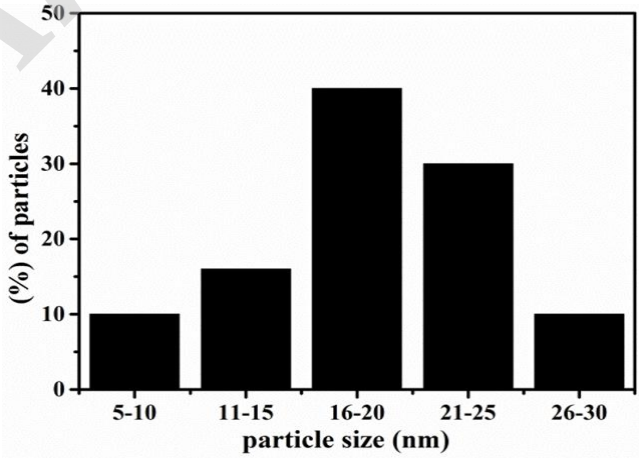

Fig. 6: Histogram of size distribution of copper nanoparticles.

these peaks [20] was mostly due to the effect of nano-sized particles. Thus the XRD pattern clearly shows that the synthesized CuNPs were very fine and crystalline. Crystallite size of synthesized CuNPs was calculated using the Scherer's formula from the XRD pattern and was found to be around 13.8 nm. The observations from the XRD analysis are in good agreement with the TEM analysis (14 ± 2 nm).

TEM

The green synthesized CuNPs were subjected to TEM to determine their size and shape. Fig. 5 shows that the prepared CuNPs are mainly spherical in shape. It is clear that the resultant CuNPs were completely revealing that the CMGK can protect Cu nanoparticles from aggregation effectively [18]. The histogram was constructed by considering 100 nanoparticles; it clearly suggests that the average size distribution is 14 ± 2 nm. Fig. 6 shows the particle size distribution (histogram). TEM image pattern confirmed that the prepared copper nanoparticles are spherical and single crystals. These results were also in agreement with XRD experiment results.

Antibacterial activity of CuNPs

Antibacterial activity [21, 22] of green synthesized were tested against gram negative (*Pseudomonas aeruginosa & E. Cole*) and gram-positive (*Bacillus cereus & B. Subtilis*) bacteria. The results of the study showed [23] that CuNPs possess distinct antibacterial activity against pathogenic bacteria at a concentration of 5 µg/ml. The CuNPs were compared favorably with a copper chloride solution, test solution and standard antibiotic ampicillin at concentration of 5µg/ml (Table 1). The CuNPs exhibited more activity than copper chloride and standard antibiotic, and the CuNPs were moderately to *B. subtilis, Bacillus cereus* and *E. Coli* with inhibition zone of 25, 25 and 26 mm. The CuNPs showed good antibacterial activity against *E. Coli, Bacillus cereus and Bacillus subtilis* and lower activity against *Pseudomonas aeruginosa.* All the bacterial strains showed good antibacterial activity with increasing of concentration of CuNPs. It can be concluded that the synthesized CuNPs showed significant antibacterial activity on both gram positive and gram negative bacteria [24].

Antifungal activity of CuNPs

Antifungal activity of CuNPs was studied using the agar well diffusion assay method. The antifungal activity of CuNPs against *Candida albicans*, *Candida parapsilosis, Aspergillus oryzae* and *Aspergillus niger* was investigated using antifungal drug miconazole as control. Different concentrations such as 20, 40, 60 and 80 µl of

Table 1: Antibacterial activities of AgNPs at different concentrations (Zone of inhibition in mm).

Organism	Zone of inhibition (mm) at different Concentrations (µl)			
	20	40	60	80
Ampicillin (control)	20	23	25	28
Bacillus subtilis	17	19	22	25
Bacillus cereus	15	19	21	25
Pseudomonas aeruginosa	11	13	15	17
Escherichia coli	19	22	25	26

Table 2. Antifungal activities of AgNPs at different concentrations (Zone of inhibition in mm).

Organism	Zone of inhibition (mm) at different Concentrations (µl)			
	20	40	60	80
Miconazole	19	23	26	28
Aspergillus niger	17	19	21	24
Aspergillus oryzae	15	17	19	20
Candida albicans	22	24	25	27
Candida parasilopsis parasilopsis	20	22	24	26

CuNPs were checked for antifungal activity [25], it showed a potent anti-fungal activity against fungal strains. CuNPs revealed higher antifungal activity with inhibition zone of 26 and 27mm (Table 2). By increasing concentration of CuNPs zone of inhibition also increases [26-28]. The CuNPs showed very good anti-fungal activity against *Candida albicans Candida parapsilosis* and showed moderate activity against *Aspergillus oryzae & Aspergillus niger.*

CONCLUSIONS

A facial, non- hazardous, cost - effective and a green approach for the synthesis of CuNPs was developed through the reduction of aqueous copper chloride solution using hydrazine hydride and carboxymethyl gum kondagogu as a reducing and stabilizing agent respectively. The synthesized CuNPs were stable in the air for about 30 days. The XRD pattern showed that the synthesized CuNPs were highly crystalline. The XRD results showed a face-centered cubic structure with (111) as the prepared orientation. The TEM images showed that the synthesized CuNPs were spherical in shape with an average size distribution of 14 ± 2 nm. The CuNPs exhibited good antibacterial and antifungal activity against pathogenic strains.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

ACKNOWLEDGMENTS

Author thankful to UGC, New Delhi, India for the financial support from UGC Minor Research Project Grant (Sanction letter: No: F. MRP–6210/15 (SERO/UGC). The Author wishes to thank the Principal and Managing of Malla Reddy Engineering College (Autonomous) for providing research facilities.

REFERENCES

[1] Ajeet K., *Swati* A., Amit S., Arnab de., (2012), Copper nanoparticulates in guar-gum: A recyclable catalytic system for the Huisgen [3+2]-Cycloaddition of Azides and Alkynes without additives under ambient conditions. *Green Chem.* 14: 3451-3452.

[2] Bhagavanth R. G., Ramakrishna D., Madhusudhan A., Venkatesham M., Veerabhadram G., (2015), Catalytic Reduction of p-Nitrophenol and Hexacyanoferrate (III) by Borohydride using green synthesized gold nanoparticles. *J. Chinese Chem. Soc.* 17: 420-428.

[3] Ashok K., Munish A., (2012), Carboxymethyl gum kondagogu: Synthesis, characterization and evaluation as mucoadhesive polymer. *Carbohyd. Poly.* 90: 637– 643.

[4] Kapoor S., Mukherjee T., (2003), Photochemical formation of copper nanoparticles in poly(*N*-vinylpyrrolidone). *Chem. Phys. Lett.* 370: 83–87.

[5] Gaber M., El-SayedY S., El-Baradie K., Fahmy R. M., (2013), Cu(II) complexes of monobasic bi- or tridentate (NO, NNO) azo dye ligands: Synthesis, characterization and interaction with Cu-nanoparticles. *J. Molec. Struc*. 1032: 185–194.

[6] Park B. K., Jeong S., Kim V., Moon J., Lim S., Kim J. S., (2007), Synthesis and size control of monodisperse copper nanoparticles by polyol method. *J. Colloid and Interf. Sci*. 311: 417-424.

[7] Chowdhury M. N. K., Beg M. D. H., Khan M. R., Fmina M., (2013), Synthesis of copper nanoparticles and their antimicrobial performances in natural fibres. *Mater. Lett.* 98: 26–29.

[8] Hatakeyama Y., Morita T., Takahashi S., Onishi K., Nishikawa K., (2011), Synthesis of gold nanoparticles in liquid polyethylene glycol by sputter deposition and temperature effects on their size and shape. *J. Phys. Chem. C.* 115: 3279–3285.

[9] Kapoor S., Mukherjee T., (2003), Photochemical formation of copper nanoparticles in poly(*N*-vinylpyrrolidone). *Chem. Phys. Lett.* 370: 83–87.

[10] Haitao Zh., Canying Zh., Yansheng Y., (2005), Novel synthesis of copper nanoparticles: Influence of the synthesis conditions on the particle size. *Nanotechnol*. 16: 3079–3083.

[11] Bhagavanth R. G., Rajkumar B., Ramakrishna D., Girija M. K., Veerabhadram G., (2017), Facile green Synthesis of gold nanoparticles with carboxymethyl cum karaya, selective and sensitive colorimetric detection of copper (II) ions. *J. Clust. Sci.* 28: 2873–2890.

[12] Rao C. N. R., Cheetham A. K., (2001), Science and technology of nanomaterials: Current status and future prospects. *J. Mater. Chem.* 11: 2887–2894.

[13] Valodkar M., Rathore P. S., NJadeja R., Thounaojam M., Devkar V. R., Thakore S., (2012), Cytotoxicity evaluation and antimicrobial studies of starch capped water soluble copper nanoparticles. *J. Hazard. Mater.* 201: 244–249.

[14] C˘alinescu I., Martin D., Ighigeanu D., (2014), Nanoparticles synthesis by electron beam radiolysis. *Cent. Europ. J. Chem.* 12: 774–781.

[15] Kalyanaraman R., Yoo S., Krupashnkara M. S., Sudarshan T. S., Dowding R. J., (1998), Synthesis and Consolidation of Iron Nanopowders. *Nanostruc. Mater*. 10: 1379-1392.

[16] Asim U., Shahid N., Naveed R., Muhammad Sh. R., Muhammad I., (2014), A green method for the synthesis of Copper nanoparticles using L-ascorbic acid. *Revista Matéria*. 19: 197-203.

[17] Bali R., Razak N., Lumb A., (2006), The synthesis of metal nanoparticles inside live plants. *Int. Conf. Nanosci. Nanotechnol.* 224-227.

[18] Zhou R., Wu X., Hao X., Li H., Raw W., (2008), Influences of surfactants on the preparation of Copper nanoparticles by electron beam irradiation. *Nuclear Instruments and Methods in Physics Research. Section B, Beam Interactions with Materials and Atoms*. 266: 599-603.

[19] Lee Y., Choi J. R., Lee K. J., (2008), Large-scale synthesis of copper nanoparticles by chemically controlled reduction for applications of inkjet-printed electronics. *Nanotech*. 19: 41-52.

[20] Chiang C. L., Chang R. C., Chiu Y. C., (2007), Thermal stability and degradation kinetics of novel organic/inorganic epoxy hybrid containing nitrogen/silicon/phosphorus by sol-gel method. *Thermochim. Acta*. 453: 97-104.
[21] Matthew A. A., Cameron W. E., Colin L. R., (2006), Green chemistry and the health implications of nanoparticles, the royal society of chemistry. *Green Chem*. 8: 417–432.
[22] Pornanong A., Nipaporn B., Juthamas R., Sanong E., (2014), Green synthesis of silk sericin-capped silver nanoparticles and their potent anti-bacterial activity. *Nanoscale Res. Lett*. 9: 1-8.
[23] Keya L., Lakshmi K. M., Masayuki S., Daisuke N. H., Takehiko S., Maheswarana H., (2012), Gold nanoparticles stabilized on nanocrystalline magnesium oxide as an active catalyst for reduction of nitroarenes in aqueous medium at room temperature. *Green Chem.* 14: 3164-3174.
[24] Rajkiran R. B., Veera B. N., Pratap R. K., (2015), Green synthesis and characterization of Carica papaya leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties. *Saudi J. Biolog. Sci.* 22: 637–644.
[25] Zain N. M., Staply A. G. F., Shama G., (2014), Green synthesis of silver and copper nanoparticles using ascorbic acid and chitosan for antimicrobial applications. *Carbohyd. Polym.* 112: 195–202.
[26] Argueta-figueroa L., Morales-Luckie R. A., Scougall- VilchisR J., Olea-Mej´ıa F., (2014), Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu–Ni nanoparticles for potential use in dental materials. *Prog. in Nat. Science: Mater. Int*. 24: 321– 328.
[27] Camacho-Flores B. A., Martínez-Álvarez O., Arenas-Arrocena M. C., Garcia-Contreras R., Argueta-figueroa L., de la Fuente-Hernández J., Acosta-Torres L. S., (2015), Copper: Synthesis techniques in nanoscale and powerful application as an antimicrobial agent. *J. Nanomater.* 2015: 1-10.
[28] Shankar S., Rhim J. W., (2014), Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles. *Mater. Lett*. 132: 307–311.