Synthesis and Characterization studies of Mesostructured Chitosan coated CuO Nanoparticles with Folic Acid

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Abstract

In this present work, we performed a facile solvothermal method for the active synthesis of chitosan (CS) coated copper oxide (CuO) nanoparticles. Further, conjugation of folic acid (FA) was done to generate FA-CS-coated CuO nanoparticles. The obtained final products were characterized to find the advancements in biological studies. The structural, functional, and optical analyses of the CS-CuO and FA-CS-coated CuO nanoparticles were characterized using X-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy (FTIR), UV-Vis spectroscopy. The thermal stability of synthesized nanoparticles was determined by thermogravimetric/differential thermal analyses (TGA/DTA). The crystallite size of nanoparticles was calculated to be 21.15 nm and 20.51 nm. Using Tauc's plot the value of the bandgap energy was estimated to be 3.36 eV and 3.09 eV respectively. The explored results of the above-mentioned characterization studies revealed that the synthesized NPs could possess the adequate potential to execute further processes on targeted drug delivery.

Keywords: Copper oxide, chitosan, folic acid, nanoparticles

1. Introduction

The majority of current research is concentrated in the area of nanotechnology. Science's basic building block is nanotechnology. It has received a lot of attention and is supposed to have a huge effect on biology and medicine. Metal oxide nanoparticles are developed in a wide range due to their low costs, non–toxic nature, and efficacy as therapeutics for all living organisms [1, 2)]. CuO is an interesting p-type material used in a variety of industrial and biomedical applications, among the different metal oxide nanoparticles. The Chitosan-cupric oxide nanoparticles were created using a sonochemical process. On cotton fabric, these nanoparticles have excellent antibacterial properties [3]. The study of nanomaterials has sparked a surge in

research interest. Madalina et al. recently published a collective report on the synthesis of CuO nanoparticles. According to the finding of this study, CuO nanoparticles have various applications depending on their various properties, such as optical, electrical, and magnetic properties as well as the synthesis methods (electrochemical, PEG – Dependant, sonochemical, sol-gel, and other synthetic methods) [4]. On different synthesized transition metal oxide NPs such as CuO, NiO, and Fe_2O_3, various characterization studies (XRD, TGA/DSC, UV-Vis, FTIR, and SEM) were carried out. When combined with different base fluids such as water, mobile oil, and engine oil, these nanoparticles showed a high rate of heat transfer and heat discharge [5]. Some researchers were involved in gathering the evidence for the toxicity of CuO NPs because they were used in various commercial applications such as gas sensors, heat transfer, nanofluids, photovoltaic cells, and antimicrobial agents [6]. Macro Leonardi et al. utilized an environmentally friendly approach in preparing CuO NPs which were encapsulated inside PEC NPs successfully to achieve novel hybrid nanoparticles. From the obtained results it was realized that CuO/PEG NPs have potential as alternative nano fertilizers [7]. Srijta Basumallick and Swadeshmukul Santra have researched chitosan-coated copper-oxide nanoparticles for efficient electrocatalytic reduction of CO_2. Chitosan has been selected as a coating agent because it is an excellent film-forming agent [8]. Chitosan is the only commercially available anticancer agent among the numerous anticancer agents, such as dextran, poly (lactic-co-glycolic acid), and poly (1-lactic acid). It is widely used because it is not only biodegradable but can also be chemically modified for drug encapsulation. An in-vivo study was carried out by Arindam Pramanik et al. for targeted delivery of Cu-organic complex to breast cancer. In this research, Cu was encapsulated in chitosan NPs and was conjugated with folic acid. The tumor volume was decreased and the mice's survival rate was improved, indicating that this nanocomposite had a significant effect [9]. A single-step reduction method was used to successfully formulate chitosan-coated copper oxide nanoparticles. Various characterization experiments on these built NPs revealed the therapeutic existence of novel biocompatible CS-CuO NPs. As a result of this study, a spark has been ignited in the formulation of biodegradable nanoparticles, allowing for the discovery of effective drug formulations [10]. Recent advances in chitosan and its derivative nanoparticles have been studied by M. Prabaharan. Nanoparticles treated with chitosan, one of the most widely used biopolymers, show great promise for tumor detection, targeted medication administration, and reduced toxicity [11]. Chitosan has the

potential to purify water by eliminating metal and microbiological pollutants. As the concentration of chitosan increases, the turbidity, TDS, electrical conductivity, and pH all decreased significantly [12].The addition of folic acid to the amine group of chitosan increases the polysaccharide's affinity for the folate receptor by a factor of ten, allowing nanoparticles delivery into cells through the folate receptor [13]. The delivery of FA-PEG-COL nanoparticles to targeted ovarian cancer gene therapy is critical. When compared to COL NPs, siRNA administration resulted in considerably higher suppression of proliferation. According to the findings, FA-PEG-COL NPs have a favorable tumor-targeting ability with minimal absorption [14]. Hadi Samadian et al. conducted a literature review to look into the great potential of folate-conjugated gold nanoparticles for targeted cancer therapy. They wanted to show off the latest developments in folate conjugated nanoparticles [15]. CuO nanoparticles have been synthesized using a variety of approaches, including physical, chemical, and biological processes. Green synthesis has a key role in biomedical applications due to its benign nature [16]. According to the literature, the environmentally friendly production of CuO nanoparticles from aloe Vera leaves extract is particularly effective in removing Congo red (CR) dye [17]. K. Subashini et al. made GEA-CuO nanoparticles from sterculia foetida leaf extract. Antibacterial and anti-cancer capabilities of these GEA-CuO nanocomposites have been demonstrated against bacteria and the lung cancer cell line A549 [18]. This research aims to propose the effective nature of the FA-CS-CuO nanoparticles in biomedical applications. Significantly, results of characterization studies reveal the successful encapsulation of Folic acid and Chitosan with CuO nanoparticles. This first step of present research work could be continued to examine the efficacy of produced NPs in the medical discipline. From the discussed details, we are convinced to step ahead with exploring the enhanced property of anti-cancer drug delivery.

2. Experimental details

2.1 Materials

Copper chloride ($CuCl_2.2H_2O$), chitosan $(C_6H_{11}NO_4)n$, ethylene glycol ($C_2H_6O_2$), and ethanol (C_2H_6O, 99%) were procured from Merck and used without further refinement. Folic acid ($C_{19}H_{19}N_7O_6$), polyvinylpyrrolidone $(C_6H_9NO)n$, and N-hydroxysuccinimide (NHS) ($C_4H_5NO_3$) were purchased from Sigma Aldrich. N-ethyl-N-(3- dimethylamino propyl) Carbodimide (EDC) ($C_8H_{17}N_3$), diluted acetic acid ($C_2H_4O_2$), and sodium acetate (CH_3COONa) were collected from

Himedia. Dimethylsulphoxide (C_2H_6OS) was obtained from Spectrum Reagents and Chemicals. All chemicals were used without further purification.

2.2 Preparation of Chitosan coated CuO NPs

CS-coated CuO nanoparticles were prepared by a simple solvothermal method. Typically, $CuCl_2.2H_2O$ (1.50 g), CS (0.75 g), NaOAc (3.68 g), and polyvinylpyrrolidone (PVP) (1.0 g) were fully dissolved into 70 mL of ethylene glycol under vigorous stirring for about 4 h at room temperature. This reaction mixture was autoclaved at 200^o C for 8 h. After it was filtered successively by Whatman No 1 paper and washed with dilute acetic acid (0.5 %) followed by deionized water. Finally, the obtained CS-coated CuO nanoparticles were dried in an oven at 60^o C for further use.

2.3 Preparation of FA-CS-coated CuO NPs

0.2 g of synthesized CS-coated CuO nanoparticles were dissolved in 100 mL dimethylsulphoxide (DMSO), then mixed with 0.1 g FA, 0.1 g NHS and 0.158 g EDC. This mixture was sonicated for 30 minutes and then stirred at room temperature overnight. After filtration, the modified FA-CS-CuO nanoparticles were alternately washed with deionized water and ethanol several times. The collected final product was dried at 100^o C for 6 h. The schematic representations of synthesizing CS-CuO and FA-CS-CuO nanoparticles were shown in figure A.

2.4 Instrumentations

2.4.1 X-ray diffraction analysis

The crystal structure was characterized by an X-ray diffractometer, (XRD-JEOL JDX – 3530) operating with a CuKα radiation source (λ=0.15456 nm).

2.4.2 Fourier transform infrared spectroscope analysis

FTIR spectra of synthesized nanocomposites were recorded by using Thermo Nicolet, NEXUS in the wavelength range of 400-4000 cm^{-1}.

2.4.3 UV-Vis spectroscopy

The optical properties of the samples were analyzed by a Jasco UV-Vis spectrophotometer, in the wavelength range from 200 nm to 800 nm.

2.4.4 Thermogravimetric analysis (TGA) and differential thermal analysis (DTA)

SIINT 6300, Japan TG/DTA was used to determine the thermal properties of the nanoparticles in an air atmosphere. As a guide, a platinum crucible was used with alpha alumina powder.

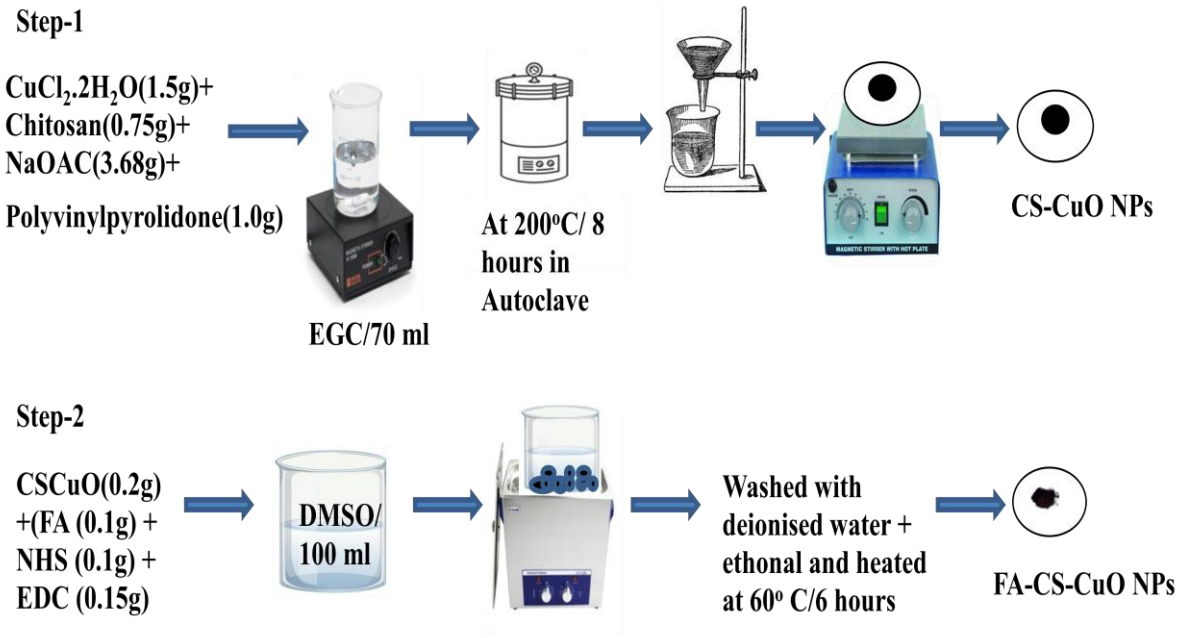

Figure A. Schematic representation of synthesizing process
Step-1 Synthesis of CS-CuO NPs ; Step-2 Synthesis of FA-CS-CuO NPs.

3. Results and discussion

3.1Characterization

The synthesized nanoparticles must be characterized to determine their structure, size, shape, and chemical composition. The following techniques were used to characterize the prepared Folic acid-Chitosan-Copper oxide NPs. XRD spectrums of synthesized Chitosan (CS)-Copper oxide NPs and Folic acid(FA)-Chitosan-Copper oxide NPs in Figures 1(a) and 1(b) respectively. The

XRD patterns show the characteristic peaks of nanocomposites at 2θ = 32.3º, 35.4º, 38.5º, 48.4º, 53.2º, 58.0º, 61.2º, 66.0º, 67.8º and 72º which corresponds to the diffraction planes (002), (111), (202), (020), (202), (113), (311), (220) and (311) of CuO respectively [19]. The broad and less intense peak was observed around 23º that reveals the presence of the amorphous nature of Chitosan.[20] The intensity of FA-CS-CuO nanoparticles decreased with the addition of Folic acid.

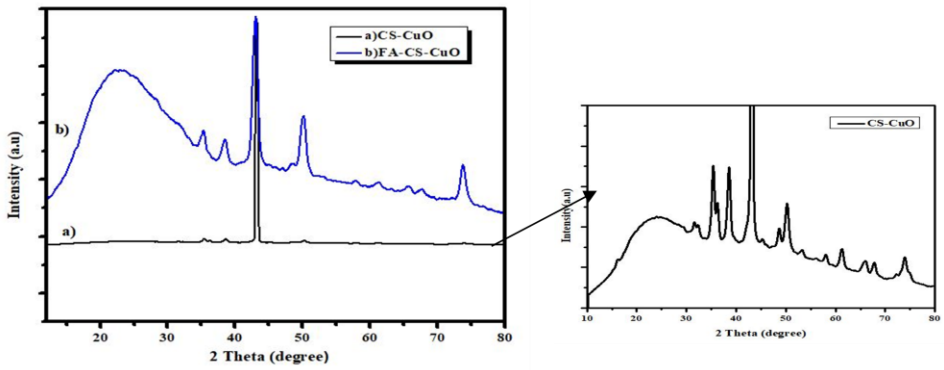

Figure 1. XRD pattern of a) CS-CuO NPs, b) FA-CS-CuO NPs

Furthermore, the presence of impurities on the prepared samples is also confirmed by the identification of a few other peaks in the XRD pattern. The broad peaks indicate the small size of the products. The obtained results endorsed the effective functionalization of FA-CS-CuO NPs. The average crystallite size of the nanoparticles was calculated using the Debye-Scherrer equation

$$D = K\lambda/\beta\cos\theta$$

Where D is the crystalline size, K is the shape factor (0.9), λ is the wavelength of Cu-Kα radiation (1.54Å), β is the full width at half maximum (FWHM) of the X-ray diffraction peak at 2θ and θ is the Bragg angle. The estimation of crystal sizes for CS-CuO and FA-CS-CuO as 21.15nm and 20.51nm respectively.

Functional groups of any organic and inorganic compound have been estimated by an effective tool such as Fourier transform infrared spectroscopy. FTIR spectrum of CS-CuO and FA-CS-CuO has been plotted in Fig. 2(a) and 2(b) respectively. The broadband at 3440 cm^{-1} in Fig. 2 indicates the presence of –OH groups [21]. The observed characteristic peaks around 2923 cm^{-1}

and 2849 cm^{-1}, 1255 cm^{-1} are assigned to the symmetric and asymmetric stretching vibrations of $–CH_3$ and the presence of chitosan [22, 8]. Small and weak absorption spectra at 615 cm^{-1} attributed to the presence of CuO nanoparticles [3]. The peak exhibited around 1112 cm^{-1} represents FA [22]. The bands observed at 617 cm^{-1}, 778 cm^{-1} were caused by the effective interaction of CuO with chitosan and the strong band obtained at 1640 cm^{-1} corresponds to –NH groups [1]. UV-absorption spectrum and Tauc's plots of CS-CuO (a,b) and FA-CS-CuO (c,d) were analyzed in detail.[Fig.3] Absorption peak has been achieved at 258 nm for CS-CuO NPs. On the other hand, strong and weak bands have been determined at 279 nm and respectively. The optical band gap energy (E_g) of samples was calculated using the theoretical relation as follows:

$$(\alpha h\nu)^n = A\,(h\nu - E_g)$$

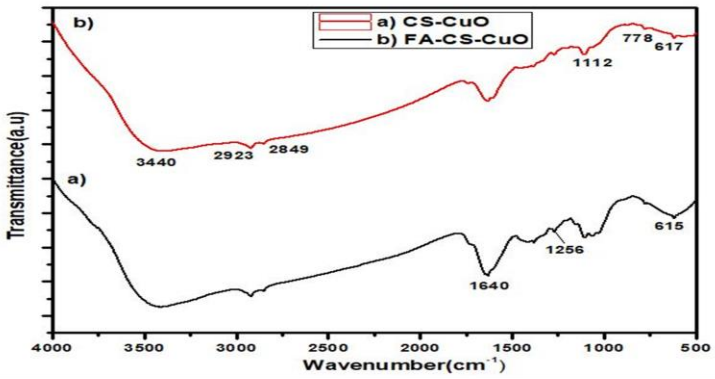

Figure 2. FTIR of a) CS-CuO NPs and b) FA-CS-CuO NPs

Where A is a constant, E_g is the energy bandgap of the material, α is the absorption coefficient, h is Plank's constant, ν is the frequency of light and the value of the exponent n depends on the type of the transition. For direct bandgap and allowed transition n=1/2; for indirect transition n=2; and directly forbidden transition n=3/2. From Tauc's plot [$(\alpha h\nu)^{1/2}$ vs hν] band gap energy was calculated to be 3.36 eV (CS-CuO) and 3.09 eV (FA-CS-CuO). Shifts in the absorption peaks and decrement in the value of band gap energy were the clear indication for the coating of folic acid with CS-CuO nanoparticles. Table 1 represents the resultant values of Crystallite size, Absorption, and bandgap of produced nanoparticles.

The synthesized CS-CuO and FA-CS-CuO nanoparticles were subjected to thermogravimetric analysis to determine the thermal behavior of the samples from room temperature up to 900°C. From Fig. 4 it is obvious that TGA curves of both the samples [4a) CS-CuO and 4b) FA-CS-

CuO] decomposed in a similar manner and degradation takes place in three stages. The initial weight loss (3%) in the range 34ºC-100ºC was attributed to the evaporation of water. Second weight loss occurs due to the decomposition of organic compounds between 300º C-470º C. The third major weight loss was recorded in the range 534ºC-764ºC for CS-CuO and 577ºC-725ºC for FA-CS-CuO nanoparticles with 26.54% and 30.3% respectively. The DTA plots of CS-CuO and FA-CS-CuO nanoparticles reveal an exothermic peak shift from 477.49ºC to 637.05ºC with a maximum of 561ºC [Fig. 5a] and 500.88ºC-733ºC with maximum at 615ºC [Fig. 5b].

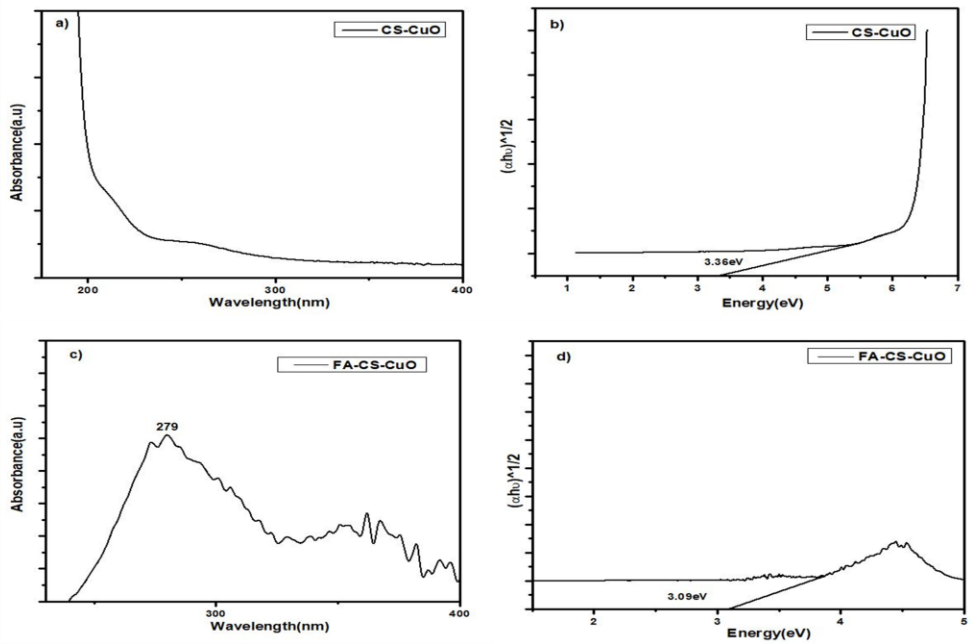

Figure 3. UV-Vis spectra (a,c) and Tauc's plot (c,d) of CS-CuO and FA-CS- CuO NPs

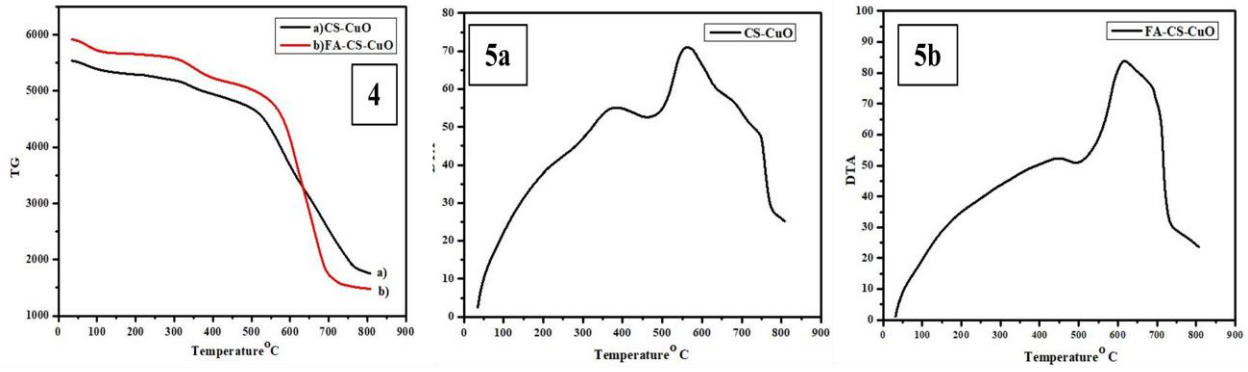

Figure 4. TGA curves of a) CS-CuO, b) FA-CS-CuO NPs ; Figure 5. DTA plots for a) CS-CuO, b) FA-CS-CuO NPs

Table 1: Crystallite size, Absorption and Band gap values of CS-CuO and FA-CS-CuO NPs

S.NO	Nanoparticles	Crystallite size	Absorption	Bandgap (eV)
1	CS-CuO	21.15 nm	258 nm	3.36 eV
2	FA-CS-CuO	20.51 nm	279 nm	3.09 eV

Conclusion

Metal oxide particles in nanosize have a powerful impact on the medical field. The synthesized nanosized CS-CuO and FA-CS-CuO NPs were subjected to physicochemical and structural characterization studies to unveil the potentials of anti-cancer drug delivery systems. XRD analyses revealed that CS-CuO and FA-CS-CuO NPs are in nanosize as to be 21.15 nm and 20.51 nm. FTIR characterization study proved the existence of significant functional groups which are supposed to be presented. Optical absorption and bandgap have been detected via UV-Vis spectroscopy as to be 3.36 eV (258 nm) and 3.09 eV (279 nm & 352 nm) for CS-CuO and F-CS-CuO NPs. 3 %, 26.54 %, and 30.3 % of weight losses have been recorded in three stages of decomposition which were examined through TG/DTA analysis. The results of this work instigated the researchers to take a further step in analyzing the distinct biomedical applications. From the observation of a reduction in crystallite size and band gap value, we can conclude that coating biocompatible polymers such as folic acid and chitosan with copper oxide nanoparticles is preferable for the delivery of the drug to cancer cells.

Acknowledgment

The authors are very grateful for the constant support provided by Rev. Dr. S. Mariadoss S.J, the principal of St. Xavier's College, Palayamkottai, and Rev. Dr. Alphones Manickam S.J, the secretary of St. Xavier's College, Palayamkottai.

References

1. Sathiyavimal, Selvam, Seerangaraj Vasantharaj, Thamaraiselvi Kaliannan, and Arivalagan Pugazhendhi. "Eco-biocompatibility of chitosan coated biosynthesized copper oxide nanocomposite for enhanced industrial (Azo) dye removal from aqueous solution and antibacterial properties." *Carbohydrate Polymers 241 (2020): 116243*.

2. Surendiran, A., S. Sandhiya, S. C. Pradhan, and C. Adithan. "Novel applications of nanotechnology in medicine." *Indian Journal of Medical Research* 130, no. 6 (2009).

3. Dhineshbabu, Nattanmi Raman, and Venkatachalam Rajendran. "Antibacterial activity of hybrid chitosan–cupric oxide nanoparticles on cotton fabric." *IET nanobiotechnology* 10, no. 1 (2016): 13-19.

4. Grigore, Madalina Elena, Elena Ramona Biscu, Alina Maria Holban, Monica Cartelle Gestal, and Alexandru Mihai Grumezescu. "Methods of synthesis, properties and biomedical applications of CuO nanoparticles." *Pharmaceuticals* 9, no. 4 (2016): 75.

5. Majumder, Sudip, Shikha Dhiman, Deepika Yadav, Niti Varshney, Preety Joon, and Subhra Das. "Synthesis and Characterization of Transition Metal Oxide Nanoparticles and Their Application as Heat Transfer Fluids." Vth International symposium on 'Fusion of Science & Technology, New Delhi, India, January 18-22, 2016.

6. Naz, Sania, Ayesha Gul, and Muhammad Zia. "Toxicity of copper oxide nanoparticles: a review study." *IET nanobiotechnology* 14, no. 1 (2019): 1-13.

7. Leonardi, Marco, Giuseppe M. Caruso, Sabrina C. Carroccio, Simona Boninelli, Giusy Curcuruto, Massimo Zimbone, Maria Allegra, Biagio Torrisi, Filippo Ferlito, and Maria Miritello. "Smart nanocomposites of chitosan/alginate nanoparticles loaded with copper oxide as alternative nanofertilizers." *Environmental Science: Nano* 8, no. 1 (2021): 174-187.

8. Basumallick, Srijita, and Swadeshmukul Santra. "Chitosan coated copper-oxide nanoparticles: A novel electro-catalyst for CO 2 reduction." *RSC Advances* 4, no. 109 (2014): 63685-63690.

9. Pramanik, Arindam, Dipranjan Laha, Sandeep Kumar Dash, Sourav Chattopadhyay, Somenath Roy, Dipak Kumar Das, Panchanan Pramanik, and Parimal Karmakar. "An in-vivo study for

targeted delivery of copper-organic complex to breast cancer using chitosan polymer nanoparticles." *Materials Science and Engineering: C* 68 (2016): 327-337.

10. Varukattu, Nipun Babu, Raju Vivek, Chandrababu Rejeeth, Ramar Thangam, Thondhi Ponraj, Alok Sharma, and Soundarapandian Kannan. "Nanostructured pH-responsive biocompatible chitosan coated copper oxide nanoparticles: A polymeric smart intracellular delivery system for doxorubicin in breast cancer cells." *Arabian Journal of Chemistry* 13, no. 1 (2020): 2276-2286.

11. Prabaharan, M. "Chitosan-based nanoparticles for tumor-targeted drug delivery." *International journal of biological macromolecules* 72 (2015): 1313-1322.

12. Al-Manhel, Alaa Jabbar, Asaad Rehman Saeed Al-Hilphy, and Alaa Kareem Niamah. "Extraction of chitosan, characterization and its use for water purification." *Journal of the Saudi Society of agricultural sciences* 17, no. 2 (2018): 186-190.

13. Sriram, Kamaraj, Palanisamy Uma Maheswari, Arasagounder Ezhilarasu, Kadhar Mohamed Meera Sheriffa Begum, and Gangasalam Arthanareeswaran. "CuO-loaded hydrophobically modified chitosan as hybrid carrier for curcumin delivery and anticancer activity." *Asia-Pacific Journal of Chemical Engineering* 12, no. 6 (2017): 858-871.

14. Li, Tony Shing Chau, Toshio Yawata, and Koichi Honke. "Efficient siRNA delivery and tumor accumulation mediated by ionically cross-linked folic acid–poly (ethylene glycol)–chitosan oligosaccharide lactate nanoparticles: For the potential targeted ovarian cancer gene therapy." *European Journal of Pharmaceutical Sciences* 52 (2014): 48-61.

15. Samadian, Hadi, Samira Hosseini-Nami, Seyed Kamran Kamrava, Habib Ghaznavi, and Ali Shakeri-Zadeh. "Folate-conjugated gold nanoparticle as a new nanoplatform for targeted cancer therapy." *Journal of cancer research and clinical oncology* 142, no. 11 (2016): 2217-2229.

16. Kumari, Puja, Pritam Kumar Panda, Ealisha Jha, Khushboo Kumari, Kumari Nisha, M. Anwar Mallick, and Suresh K. Verma. "Mechanistic insight to ROS and apoptosis regulated cytotoxicity inferred by green synthesized CuO nanoparticles from Calotropis gigantea to embryonic zebrafish." *Scientific reports* 7, no. 1 (2017): 1-17.

17. Batool, Madiha, Muhammad Zahid Qureshi, Farwa Hashmi, Nida Mehboob, and Abdul Salam Shah. "Congo red azo dye removal and study of its kinetics by aloe vera mediated copper oxide nanoparticles." *Indonesian Journal of Chemistry* 19, no. 3 (2011): 626-637

18. Subashini, K., S. Prakash, and V. Sujatha. "Polymer nanocomposite prepared using copper oxide nanoparticles derived from Sterculia foetida leaf extract with biological applications." *Materials Research Express* 7, no. 11 (2020): 115308.

19. Geetha, M. P., P. Pratheeksha, and Bhat K. Subrahmanya. "Development of functionalized CuO nanoparticles for enhancing the adsorption of methylene blue dye." *Cogent Engineering* 7, no. 1 (2020): 1783102.

20. Praffulla, S. R., and S. G. Bubbly. "Synthesis and characterization of Chitosan-CuO-MgO polymer nanocomposites." In *AIP Conference Proceedings*, vol. 1953, no. 1, p. 030168. AIP Publishing LLC, 2018.

21. Laha, Dipranjan, Arindam Pramanik, Sourav Chattopadhyay, Sandip kumar Dash, Somenath Roy, Panchanan Pramanik, and Parimal Karmakar. "Folic acid modified copper oxide nanoparticles for targeted delivery in in vitro and in vivo systems." *RSC advances* 5, no. 83 (2015): 68169-68178.

22. Jayaramudu, Tippabattini, Kokkarachedu Varaprasad, Radha D. Pyarasani, K. Koteshwara Reddy, Kanderi Dileep Kumar, A. Akbari-Fakhrabadi, R. V. Mangalaraja, and John Amalraj. "Chitosan capped copper oxide/copper nanoparticles encapsulated microbial resistant nanocomposite films." *International journal of biological macromolecules* 128 (2019): 499-508.