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Characterization of copper based chitosan nanoparticles synthesis by chemical method: Approach cytotoxic effects on MCF-7 cells

Ashwaq Abbass Fadhil a, Mais Emad Ahmed a,*

a Department of Biology, College of Science, University of Baghdad, Jadriya, Baghdad, Iraq

Abstract

Chitosan polymer is an essential supporting material for the synthesis of metallic nanoparticles (MNPs) because of its exceptional capping and stabilizing qualities, biocompatibility, biodegradability, eco-friendliness, polycationicity, and non-toxicity. To produce copper-based chitosan nanoparticles, choose a suitable chemical procedure hydrothermal method. Each approach offers advantages in terms of homogeneity, morphology, and particle size. This study has produced, described, and examined the bacteriological characteristics of metal nanoparticles based on chitosan, including copper nanoparticles (CS-Cu NPs). Chitosan-based metal nanoparticles have been created using the solution casting technique. The generated nanoparticles were characterized using FTIR, AFM, UV, ZP, and SEM examination. The TEM image illustrates that the materials created are nanomaterials, ranging in size from 1 to 100 nm. The CS-CuNPs, displaying a stable brick-red hue, showed an absorption peak at 214 nm, indicative of monodisperse nanoparticle formation and surface plasmon resonance. X-ray diffraction confirmed the face-centered cubic structure with peaks at 36.78°, 43.38°, 50.56°, and 74.26°, and an average particle size by AFM to 62.45 nm. FTIR analysis showed interactions between chitosan and copper, particularly around 3370 –3226 cm⁻¹, 1633 cm⁻¹, and 680 cm⁻¹ and The MCF7 cell line demonstrated the strongest anti-human breast cancer cell line properties of the nanocomposite when compared to the aforementioned cell lines. The aforementioned results suggest that the nanocomposite could be used to treat various forms of human breast cancer. Breast cancer had increased nanoparticle sensitivity. At a concentration of 25 μL/mL, Chi-Cu NPs demonstrated excellent cytotoxicity and anticancer activity, making them suitable for use as an anticancer agent.

Keywords: FTIR; MCF7; cytotoxicity chitosan; copper nanoparticle (Chi-Cu NPs); TEM.

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1- Introduction

Natural polymers, such as chitin, cellulose, and starch, have witnessed a dramatic increase in interest over the last ten years because of their numerous uses in signaling, energy storage, transportation, and structural components [1]. Shrimp, prawn, and crab shells can make chitosan, a simple polysaccharide that is the deacetylated version of chitin. Chitosan demonstrates biocompatibility and biodegradability to create a physiologically inert and adaptable environment for detecting and interacting with macromolecules and microorganisms in devices [2].

The utilization of chitosan and its derivatives as materials for high-energy battery components, biosensors, actuators, artificial muscles, environmentally sensitive membranes, wastewater treatment, and electrolytes has grown rapidly in recent years [3]. Recommended, an anticancer drug, using emulsified and cross-linked chitosan nanoparticles. Since then, extensive studies have been conducted on these systems for the delivery of medications. Additionally, many teams have created new formulations of chitosan nanoparticles with additional matrix-forming ingredients [4]. CuNPs are gaining popularity because of their beneficial properties (thermal and electrical conductivity), which make

significantly less expensive than other noble metals like silver and gold. For example, EstebanCubillo and colleagues have demonstrated the bactericidal properties of Cu NPs generated in the sepiolite matrix [5]. A biopolymer called chitosan is created when chitin is deacetylated with an alkaline agent, such as sodium hydroxide. The exoskeleton of crustaceans arthropods, the cell walls of fungus, and the cuticles of insects all contain chitin. Numerous fields, including waste water treatment, medicine, food, textiles, adhesives, and drug delivery, have found applications for chitosan. A biodegradable polymer with a porous structure that contains hydroxyl and amino functional groups is chitosan. By giving copper coordination sites, these groups improve the catalyst's stability. Additionally, they help the copper nanoparticles disperse, which raises their catalytic activity [6].

Furthermore, the possible mechanisms that underlie the interactions involving crops, pathogenic bacteria, and copper-based nanomaterials were suggested. emphasized the positive and negative aspects of using copper-based nanoparticles in agriculture, as well as the factors influencing their utilization. and nano-farming were proposed. This study aims to promote further studies and



*Corresponding Author: Email: mais.emad@sc.uobaghdad.edu.iq

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uses of copper-based nanomaterials in agriculture via offering a fresh and sustainable approach to agricultural growth [7]. Because of their special characteristics, Cu nanoparticles (CuNPs) have an extensive spectrum of uses in biomedicine. We looked into the toxicity and mode of action of CuNPs in SW480 cells because their impact on the production of oxidative stress and apoptosis in the human colorectal cancer cell line SW480 has not yet been examined.

The impact of the particles on the viability of SW480 cells was evaluated using the MTT test. Following a 24-hour treatment with CuNPs, the generation of reactive oxygen species (ROS) was measured to determine the levels of oxidative stress [8]. This study shown that copper nanoparticles (CuNPs) made using fennel extract have cytotoxic, antioxidant, and anticancer properties, particularly when it comes to non-small cell lung cancer (NSCLC). CuNPs cytotoxically affected two NSCLC cell lines, A549 and H1650, in a dose-dependent way. CuNPs inhibited cell viability to 70% in A549 cells and 65% in H1650 cells at 100 µg/ml [9].

With applications in food, health, cosmetics, and pharmaceuticals, the natural and linear biopolyamino-saccharide chitosan has attracted a lot of attention as a functional biopolymer. Shrimp shells are a major source of chitosan biopolymer. In this study, they were used as the raw material for chitosan production, which is then used to create metal nanoparticles. The production and

characterization of chitosan nanoparticles would be essential and have a wide range of applications in both commercial and medical settings, as was discussed in earlier sections.

2- Experimental work

2.1. Preparation of chitosan

The methods described in [10] are followed in this preparation. In this experiment, 1.0 g of chitosan was dissolved in 100 mL of 1% acetic acid solution.

2.2. Synthesis of CS-Cu NPs

First, a 3% (w/v) chitosan was dispersed in a 5% (v/v) acetic acid solution in 50 milliliters of room temperature distilled water, stirring consistently for 30 minutes. To generate a transparent blue solution, 25 milliliters of 1 M ascorbic acid were added to the mixture to elevate its pH to 3.5. A separate solution of 0.1 M CuSO4.5H2O was prepared in 20 cc of distilled water. After stirring continuously for 60 minutes at 60°C, this solution containing copper was added to the mixture of chitosan and ascorbic acid. The production of chitosan-copper nanoparticles was shown by using the creation of a brickbrown solution [11]. As observed in Fig. 1.

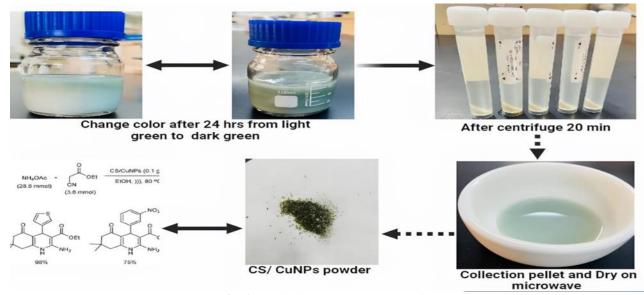


Fig. 1. Synthesis CS-Cu NPs preparation

2.3. Characterization of chitosan-copper nanoparticles

The shapes and sizes of the nanoparticles were identified using the following techniques: Field emission scanning electron microscopy (FE-SEM) was employed to graphically represent and evaluate the shape of the particle or crystal, the surface morphology, the distribution of particle sizes, the aggregation of nanoparticles, the surface functionalization, and assessments of individual particles. Atomic force

microscopy (AFM) is used to measure the diameter and height of NPs in three-dimensional vision, while UV-visible (UV- VIS) is used to examine the optical characteristics of the materials. (XRD) is a materials science technique that determines the crystallographic structure of a material. Functional groups of prepared nanostructures in the range of 4000–400 nm were examined by Fourier-transform infrared spectroscopy (FT-IR, RX I, PerkinElmer, Inc., USA) at a resolution of

4 cm1 and Zeta Potential (ZP) and energy dispersive X-ray (EDX) [12].

2.4. Cells, cell culture, and CS-Cu NPs preparation

The human breast cancer cell line MCF7 was employed in this investigation. The cells were cultivated in Roswell Park Memorial Institute-1640 media (RPMI-1640) supplemented with L-glutamine (Capricorn Scientific GmbH), as detailed in a prior work. 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution (100X; Euroclone S.p.A.) were added to the RPMI-1640 medium to make a complete medium. As previously mentioned, [13], the cells were maintained at 37°C in an incubator with 5% CO2 and 95% humidity.

The dissolved the cells were cultured in Roswell Park Memorial Institute-1640 medium (RPMI-1640) supplemented with L-glutamine (Capricorn Scientific GmbH). for in vitro tests. From the stock solution, the necessary concentrations for the ensuing experiments were made in full medium.

2.5. Cytotoxicity assay

An vitro 3-(4,5-dimethylthiazol-2-yl)-2,5in diphenyltetrazolium bromide (MTT) test was used to evaluate cytotoxic effects [14]. To guarantee cell adherence, 7,000 cells were sown in each well of a 96well plate and incubated for the whole night. After that, cell lines (MCF7) were treated to substances at progressively higher CS-Cu NPs concentrations (6.25, 12.5,25,50 and 100 μg/ml); three replicate wells were employed for each treatment. After 24 hours of incubation, the media was taken out of the plate, and each well received 20 µl of MTT solution (5 mg/ml) from Shanghai Macklin Biochemical Co., Ltd. The wells were then incubated for 3 hours at 37°C in the dark. After adding 50 µl of DMSO (Bio Basic Inc.), the MTT was shaken for 10 minutes to dissolve it [15]. Absorbance at 490 nm was measured using a microplate reader (BioTek Instruments, Inc.). From the raw absorbance data, the proportion of live cells was calculated using the Eq. 1.

[Viability (%) = (Absorbance of treated cells / Absorbance of control cells) x 100] (1)

"A." GraphPad Prism software version 6 (Dotmatics) was used to create the dose-response curve, and the same curve was used to calculate the growth inhibitory concentration (GI50), which lowers viability by 50% [16].

3- Results and discussion

3.1. Synthesis of CS-Cu NPs

The physicochemical properties of the synthesized copper nanoparticles, which may be deduced by looking at their optical properties, are crucial for determining their effectiveness, resulting function, and morphology. Aqueous solution saw slight color changes during

nanoparticle formation, The milky white solution of chitosan and acetic acid did not change color when ascorbic acid was added. Chitosan's low pH made it easily soluble in water. Afterwards, the solution's color instantly turned greenish-yellow and then brick brown upon the addition of pale blue CuSO4.5H2O formation of CuNPs.

The chemical reduction of metal salt is one of the most viable and promising synthetic processes for creating metallic nanoparticles with affordable setups. This event could be regarded as a sequence of chemical events that resulted in the production of copper nanoparticles. Copper salts became a base material or precursor for this top-down approach to manufacturing nanoparticles.

Chitosan (CS), a capping agent, forms a compound with Cu2+ ions. The creation of chitosan-copper nanoparticles was shown by the emergence of a grayish-light colored solution when this copper solution was introduced to the chitosan-ascorbic acid mixture while being continuously stirred for 60 minutes at 60 °C. Complex to eventually generate Cu (0) metallic nanoparticles. Here, ascorbic acid also stops Cu2O, CuO, and Cu (OH)2 from forming unintentionally. A colloidal suspension of equally colored copper nanoparticles emerged as a combination reaction, and the particles were then dried in microwaves to form a powder.

The production of copper nanoparticles (Cu-NP) has drawn interest because of its characteristics and potential uses. The primary goal of the project is to use green synthesis to create stable Cu-NP. By adding the acidic chitosan solution to the CuSO4 solution while stirring continuously for 12 hours at 70°C, the synthesis of Cu-NP has been shown in this work. Because copper oxidizes to CuO and Cu2O, it is quite challenging to create Cu-NPs by simple reduction of copper salts in aqueous solution, as opposed to other metals like Ag, Au, or Pt. However, a carrier material, like a polymer matrix like chitosan, which has excellent copper binding qualities, can be used to achieve the size-controlled synthesis of Cu-NPs. In the creation of Cu-NPs, this polymer has been used as a stabilizing and/or reducing agent, which limits the growth of the particles by preventing agglomeration and reducing oxidation reactions [17].

3.2. Characterization

3.2.1. FTIR analysis

The presence of free electrons in the penultimate or final orbitals of copper nanoparticles, which resonate with incident light and produce the characteristic surface plasmon resonance (SPR), is responsible for the particles' unique brick-green appearance in aqueous solution. The interaction between chitosan and copper nanoparticles was evaluated via FTIR spectroscopy show Fig. 2 a FTIR spectrum of chitosan consisted of a peak at 3000 cm⁻¹ ascribed to overlap NeH and OeH stretching bonds. The absorption bands are located at 2878 cm⁻¹ and 1658 cm⁻¹. While copper sulfate consisted of a peak at 2927 cm⁻¹. Furthermore, the absorption bands located at 3196 cm⁻¹ and 3361 cm⁻¹ corresponded to S bending and Cu

stretching bonds, respectively Fig. 2 b After the formation of chitosan-copper interfaces, the intensity of some of these bands reduced and some of them shifted confirming the interaction between chitosan and copper and the synthesis of CS-Cu NPs.

Fig. 2 c shows Zetasizer experiments showed that Cs-CuNP had a narrow size distribution (polydispersity index of 24.74%) with an average size of 87–89 nm.

Furthermore, the interaction between Ch-CuNPs was assessed using FTIR spectroscopy. The CsCuNPs had a hybrid structure made up of a copper core encased in a chitosan shell, and their nanometric particle size was approximately 131 nm. The intensity of the O-H/N-H stretching and N-H amide/amine bending bands of the chitosan structure were determined by FTIR analysis [18].

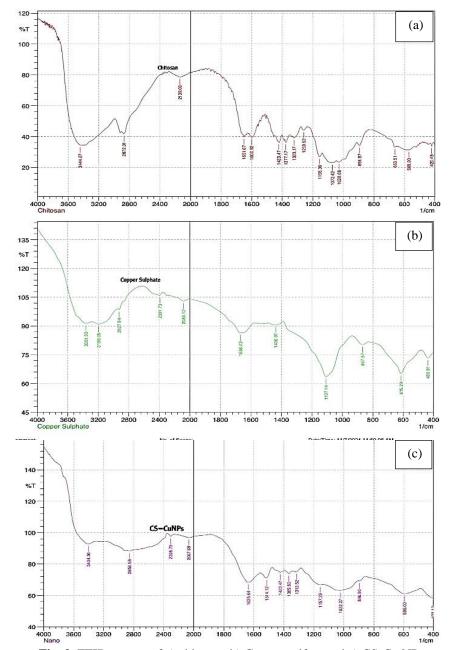


Fig. 2. FTIR spectra of a) chitosan, b) Copper sulfate and c) CS-Cu NPs

3.2.2. Ultraviolet-visible absorption spectroscopy

One essential spectroscopic approach for evaluating metal nanoparticles is UV-vis absorption spectroscopy. The results of the UV-vis analysis as shown in Fig. 3 a, b, and c which show the different absorption ranges for the nanoparticles. The spectra for the Chitosan, CuSo4, and

CS-Cu NPs nanoparticles exhibited absorption peaks at 207nm, 330nm, and 214nm, respectively, which indicates the formation of nanoparticles. This polymer has been used as a stabilizing and/or reducing agent in the creation of Cu-NPs, which restricts particle growth by minimizing oxidation reactions and preventing agglomeration.

The creation and characterization of Cs-CuNPs marked the beginning of the inquiry. Their distinctive brickbrown color in the aqueous solution was attributed to the interaction between incident light and free electrons in the outer orbitals. The spectral analysis of the generated solution revealed an absorption peak at 214 nm, which is in line with the characteristic wavelength range (500–600 nm) associated with the surface plasmon resonance (SPR) of CuNPs, as per previous studies [19]. The production of the nanoparticles was verified by measuring their

absorption spectrum using UV-Vis spectroscopy. The shape, size, and interparticle distance of the nanoparticles determine their optical characteristics; as a result, they react to UV radiation by exhibiting a particular absorption spectrum [20]. On the other hand, result by [21] show CuO presented a sharp absorbance peak at 255 nm, while chitosan showed a shift in absorbance, resulting in a broad peak at 300 nm, according to the study's absorption spectra for the nanoparticles.

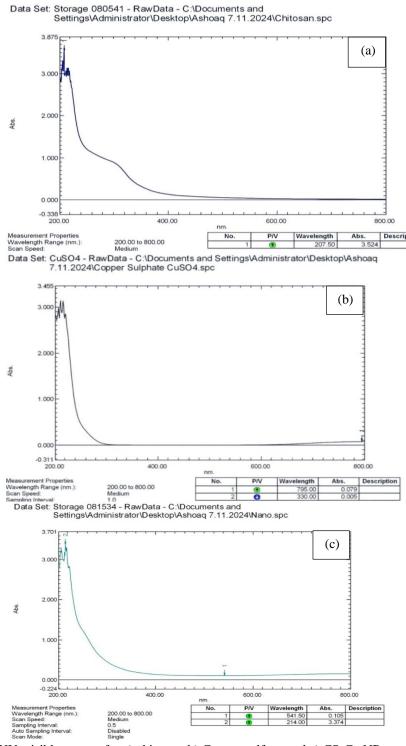


Fig. 3. UV-visible spectra for a) chitosan, b) Copper sulfate, and c) CS-Cu NPs respectively

3.2.3. Zeta Potential analysis

The produced samples 2.76 were subjected to zeta size and zeta potential measurements in the Malvern Zeta equipment 3000 (UK type of the Malvern equipment). This process involved filling the cuvette with 100 samples that had been re-suspended in 900 milliliters of milli-Q water. The experiment was performed with a scattering angle of 90° and at 25 °C.Refractive indices and material dispersion were set at 1.330 and 1.365 (viscosity (CP) 0.8872), respectively, whereas the dielectric constant was 78.5. To ascertain charges and nanoparticle stability, zeta potential measurements were made. The zeta potential of CS-Cu NPs is shown in Fig. 4 at the samples showed a negative zeta potential value of 35.36 ±0.6 mV.

The presence of carboxylic and amino groups on the surface of CuNPs was the cause of the negative potential value. One crucial factor influencing the stability of colloidal dispersion is the zeta potential. Generally

speaking, particles having zeta potential values greater than ± 30 mV are thought to produce stable dispersions 24. Increased stability in aqueous dispersion is demonstrated by high concentrations of gelatin stabilized CuNPs. Copper and chitosan's size distribution (hydrodynamic diameter) was evaluated using dynamic light scattering (DLS). The Zeta potential and size distribution investigations were conducted using the Zetasizer Nano Series from Malvern Instruments. Three runs, including 495.17 nm, were averaged to determine each value. At 25 °C, all measurements were made as shown in Fig. 4 b The stability of the colloidal solution is one important component that is necessary for the product's use. This measurement revealed that the zeta potential, which is dependent on the electric potential, surface charge, and electrostatic repulsion of nanoparticles, was +23.6 mV. The stability of the scattered particles is indicated by a zeta potential of approximately \pm 25 mV [22].

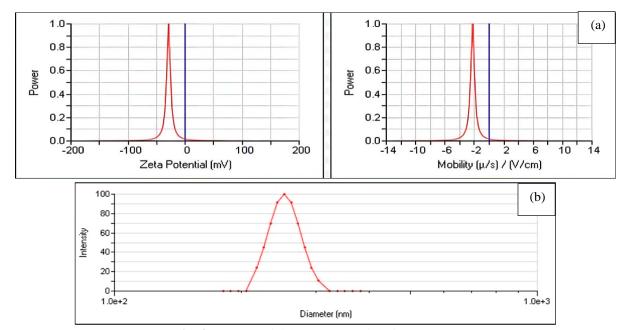


Fig. 4. a) Z potential, b) DLS potential of CS-CuNPs

3.2.4. Atomic force microscopy (AFM) analysis

CS-Cu NPs were tested by AFM examination employing CSPM to find and describe the nanoparticle distributions. The chitosan-Copper (nanocomposite microstructure was investigated utilizing atomic force microscopy (AFM). CS-Cu NPs nanoparticles were discovered to have irregular and triangle cluster morphologies, ranging in height and 62.54 nm in diameter (as verified by a particle size analyzer) according to the three-dimensional AFM, as shown in Fig. 5.

Using the AFM, one may quantify the force at the nano-Newton scale exerted by the sample surface on the AFM tip and picture the nanoparticles in a three-dimensional surface profile with atomic resolution. According to the AFM measurements, the inclusion of CuO in the system is the reason why the roughness values for the two types of samples are near together and their surface topography differs. AFM typically matches the SEM findings qualitatively. Contact angle measurements are used to assess the wettability of the solid surfaces of both hybrid copper coatings. The copper and chitosan samples have water droplet contact angles of 116° and 121° , respectively. This indicates that both coatings have poor surface wettability. However, the results show that the surface of both hybrid samples is more hydrophobic when compared to the surface of the pure copper layer (94°).

3.2.5. Field emission scanning electron microscope (FESEM)

Utilizing scanning electron microscopy (SEM), the membrane's structural features and crystallinity were examined. To comprehend the size, shape, and elemental and structural composition of the NPs samples, analysis

was done. CS-CuNPs spherical shape and diameter of 33nm are depicted as shown in Fig. 6 a, b.

at a magnification of 13000x and 50000x. The chitosan nanoparticles used in the experiment took on the appearance of white powder. Scanning electron micrographs of the Nanomagnetic chitosan revealed that they were about spherical. The groups of particles that make up the unaltered chitosan nanoparticles range in size to 33 nm. While SEM images a specimen's surface by using a concentrated, high-energy electron beam. Samples

are evaluated after drying, except for an environmental SEM, which is not covered here. No requirement for conducting samples, SEM analysis was done to determine the size, morphology, elemental composition, and structural makeup of the NPs samples. The size, shape, and degree of sphericality of nanoparticles can all be investigated using TEM. The specimen is transmitted by a concentrated high-energy electron beam in a TEM, and the picture produced is visible on a phosphor screen.

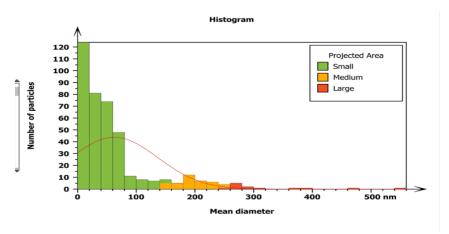


Fig. 5. Atomic force microscopy analysis of CS-Cu NPs

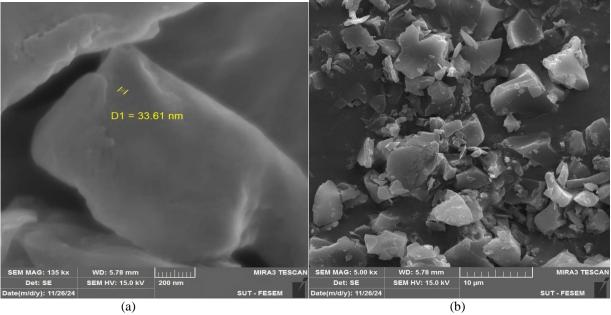


Fig. 6. FESEM images of CS-Cu NPs a) focus on 135kx bar 200 nm, b) focus on 5kx bar 10 µm

3.2.6. X-ray diffraction XRD

The XRD patterns showed that chitosan is crystalline and that CS-CuNPs nanotechnology had low intensities of their peaks, which indicates their crystallinity has decreased. With a hexagonal quartzite structure. Because the sample pattern and the standard are nearly identical, it

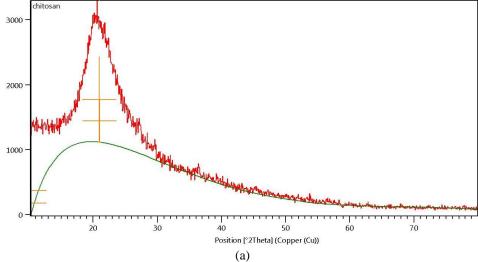
can be said that the wurtzite of CS-Cu NPs is hexagonal and has nine major peaks situated at (100), (002), (101), (102), (110), (103), (200), (112), and (201). The purpose of this research is to investigate the impact of chitosan modification on nanoparticles via the synthesis of Cu/Chitosan using different molar ratios of Cu and Ch as shown in Fig. 7 a, b When chitosan forms CNPs through

cross-linking with TPP, its distinctive broad peaks at $2\theta \approx 11^{\circ}$ and 19.6° are essentially extinguished, according to

XRD examination. as shown in Table 1 indicated the results of XRD.

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Pos. [°2Th.]	Height [cts]	FWHM	Left	d-spacing [Å]	Rel. Int. [%]	Tip Width	Matched by
		[°2Th.]					
10.2(1)	769(17)	5.0(2)		8.62906	58.24	6.0039	
10.3(1)	385(17)	5.0(2)		8.62906	29.12	6.0039	
20.94(1)	1321(11)	5.26(5)		4.23969	100.00	6.3087	
20.99(1)	660(11)	5.26(5)		4.23968	50.00	6.3087	



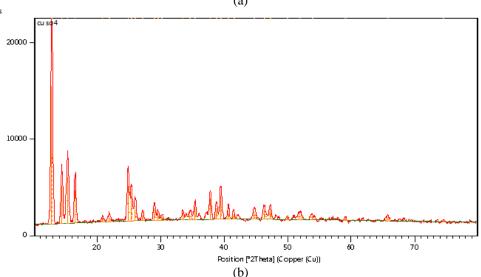


Fig. 7. XRD analysis a) Chitosan, b) CS-Cu NPs

3.2.7. Energy dispersive x-ray (EDX)

According to energy dispersive x-ray (EDX) analysis, CS-Cu NPs nanoparticles are present in the polymer matrix of the CS-Cu NPs nanocomposite film show Fig. 8. The EDX profiling was conducted on a single region of the coating's cylindrical particles. The CS-Cu NPs nanoparticles were successfully incorporated into the polymer matrix, as seen by the Cu peaks in the spectra. Furthermore, during the elemental evaluation of the nanocomposite, the CuO was discovered. According to reports, metallic nanoparticles have a wide range of uses

in agriculture and have the potential to replace dangerous chemical pesticides and fungicides by enhancing the activity of natural chemicals. Using nanotechnology in agricultural formulations has several advantages, including increased surface area, a higher size to volume ratio, maximum efficacy, and extreme precision. This study effectively manufactured copper nanobiocomposites utilizing an organic ascorbic acid and chitosan, a naturally occurring polymer as shown in Table 2.

Table 2	2. Energy	dispersive	X-ray	(EDX)

				<i>UJ</i> 1		J \ /			
Element	Line	Apparent	k Ratio	Wt%	Wt%	Atomic %	Standard	Factory	Standard
	Type	Concentration			Sigma		Label	Standard	Calibration
					_				Date
С	K series	3.29	0.03285	45.39	0.36	54.31	C Vit	Yes	
O	K series	8.85	0.02977	48.74	0.35	43.78	SiO2	Yes	
Cl	K series	0.70	0.00608	3.26	0.08	1.32	NaCl	Yes	
Cu	L series	0.25	0.00251	2.62	0.17	0.59	Cu	Yes	
Total:				100.00		100.00			

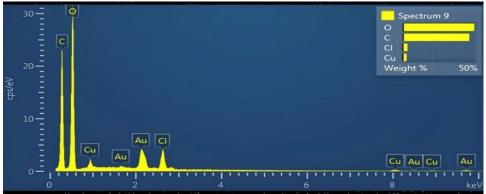


Fig. 8. EDX of CS-Cu NPs

4- The cytotoxic effect of CS-Cu NPs nanoparticles on tumor cell lines

The cytotoxic activity of chitosan-copper oxide nanoparticles on the human breast adenocarcinoma cell line MCF7 was studied as shown in Fig. 9. Morphology of control cell and treatment cell with two concentrations (50 μg/ml and 100 μg/mL). Studies have indicated that as the concentration of CS-CuNPs rose, cell viability declined. CS-CuNPs had a GI 50 value of nearly 24.5 µg/ml. These NPs exhibited toxic effects against the MCF7 cell line, and at a concentration of 25 µg/ml, the cytotoxic effects reached 65.4%. CS-CuNPs were tested for in vitro cytotoxicity against MCF7 cell lines at concentrations of 6.25-100 µg/ml; In agreement with our results, several evidence have indicated the cytotoxic potential of CS-CuNPs on different cell lines. Our finding revealed the cytotoxic of CS-CuNPs against MCF7 cell line. As observed in Fig. 10 presents the cell viability (%) MCF7cell lines when exposed to varying

concentrations of CS-CuNPs (6.25, 12.5, 25, 50, and 100 μg/ml.In every instance, as the concentration of the nanocomposite increases, the ratio of cell viability drops. The result agrees with [23] the biological applications of CS-Cu NPs, such as their anticancer (adenocarcinoma) properties, were also evaluated. It had notable cytotoxic effects on major human bladder cancer cell lines, including TCCSUP (Grade IV, transitional cell carcinoma), SCaBER (squamous cell carcinoma), and UM-UC-3 (transitional cell carcinoma). Lung cancer cell with lung well-differentiated bronchogenic adenocarcinoma (HLC-1), lung moderately differentiated adenocarcinoma (LC-2/ad), and lung poorly differentiated adenocarcinoma (PC-14) were treated with the nanocomposite using the MTT assay in the cytotoxicity and anti-human lung studies. The malignant lung cell line's cell survival declined in a dose-dependent manner when the Fe3O/CS/Cu (II) nanocomposite was present

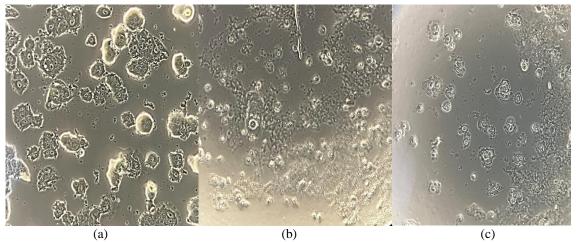


Fig. 9. The cytotoxic effect of CS-Cu NPs a) Control MCF7 cells, at concentration b) 50 ml c) 100 μg/ml

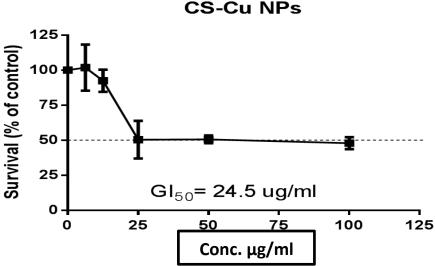


Fig. 10. The cytotoxic effect of CS-Cu NPs on MCF7 cells

5- Conclusion

Chitosan nanoparticles are convenient carriers for drugs and active ingredients, many researchers have been interested in these structures because of the benefits of nanoparticles, including their capacity to transport medications, lower toxicity, provide controlled drug release, and deliver drugs specifically to the target area. the limitations of the study, such as the use of a single cell line, and suggest future research directions, such as in vivo studies, investigations into the mechanisms of cytotoxicity, and optimization of nanoparticle formulation These characteristics make nanotechnology a promising cancer treatment option that can be applied from a research lab to a patient's bedside. The toxicity of some nanoparticles is one potential issue that restricts their use in cancer treatment; this needs more research. Thereafter, the cytotoxicity and anticancer properties of the prepared nanostructures was investigated against breast cancer cell lines.

References

- [1] B.Ates, S.Koytepe, A.Ulu, C.Gurses, & V.K.Thakur. Chemistry, structures, and advanced applications of nanocomposites from renewable resources. *Chemical Reviews*, 120(17), 9304-9362. 2020. https://doi.org/10.1021/acs.chemrev.9b00553
- [2] A.Khan, & K.A.Alamry. Recent advances of emerging green chitosan-based biomaterials with potential biomedical applications: A review. *Carbohydrate Research*, 506, 108368. 2021. https://doi.org/10.1016/j.carres.2021.108368
- [3] S. N. Banitaba, S. V. Ebadi, P.Salimi, A. Bagheri, A. Gupta, W. U. Arifeen, V. Chaudhary, Mishra, Y. K. Kaushik, & E. Mostafavi. Biopolymer-based electrospun fibers in electrochemical devices: a versatile platform for energy, environment, and health monitoring. *Materials Horizons*, 9(12), 2914-2948. 2022. https://doi.org/10.1039/D2MH00879C

- [4] A. Detsi, E. Kavetsou, I. Kostopoulou, I. Pitterou, A. R. N. Pontillo, A. Tzani, P. Christodoulou, A. Siliachli, & P. Zoumpoulakis. Nanosystems for the encapsulation of natural products: The case of chitosan biopolymer as a matrix. *Pharmaceutics*, 12(7), 669. 2020. https://doi.org/10.3390/pharmaceutics12070669
- [5] Z. M. Romi and M. E. Ahmed, "Influence of biologically synthesized copper nanoparticles on biofilm formation by Staphylococcus haemolyticus isolated from seminal fluid," *Iraqi Journal of Science*, vol. 1948–1968, 2024. https://doi.org/10.24996/ijs.2024.65.4.15
- [6] Akbar, S., Qureshi, M. N., & Khan, S. A. Fabrication of chitosan supported copper nano catalyst for the hydrogen gas production through methanolysis and hydrolysis of NaBH4. *International Journal of Hydrogen Energy*, 101, 313-322. 2025. https://doi.org/10.1016/j.ijhydene.2024.12.462
- [7] Su, C., Chen, A., Liang, W., Xie, W., Xu, X., Zhan, X., ... & Peng, C. Copper-based nanomaterials: Opportunities for sustainable agriculture. *Science of The Total Environment*, 171948. 2024. https://doi.org/10.1016/j.scitotenv.2024.171948
- [8] Ghasemi, P., Shafiee, G., Ziamajidi, N., & Abbasalipourkabir, R. Copper nanoparticles induce apoptosis and oxidative stress in SW480 human colon cancer cell line. *Biological Trace Element Research*, 201(8), 3746-3754. 2023. https://doi.org/10.1007/s12011-022-03458-2
- [9] Huang, T., Ma, K., & Wang, Y. Characterization and evaluation of the cytotoxic, antioxidant, and antihuman lung cancer properties of copper nanoparticles green-synthesized by fennel extract following the PI3K/AKT/Mtor signaling pathway. *PloS one*, 20(1), e0309207. 2025. https://doi.org/10.1371/journal.pone.0309207

- [10] S. Mallick, S. Sharma, M. Banerjee, S. S. Ghosh, A. Chattopadhyay & A. Paul. Iodine-stabilized Cu nanoparticle chitosan composite for antibacterial applications. *ACS applied materials & interfaces*, 4(3), 1313-1323. 2012. https://doi.org/10.1021/am201586w
- [11] M. R. Mehta, H. P. Mahajan, & A. U. Hivrale. Green synthesis of chitosan capped-copper biocomposites: Synthesis, characterization, and biological activity against plant pathogens. BioNanoScience, 417-427. 11, 2021. https://doi.org/10.1007/s12668-021-00823-8
- [12] N. H. H. Faiq and M. E. Ahmed, "Inhibitory effects of biosynthesized copper nanoparticles on biofilm formation of Proteus mirabilis," *Iraqi Journal of Science*, pp. 65–78, 2024. https://doi.org/10.24996/ijs.2024.64.1.7
- [13] Z. H. Kadhim, M. E. Ahmed, and I. Şimşek, "Biologically synthesized copper nanoparticles from S. epidermidis on resistant S. aureus and cytotoxic assay," *Bionatura*, vol. 8, no. 1, 2023. https://doi.org/10.21931/RB/CSS/2023.08.01.54
- [14] A. H. Alhammer, S. I. Al-junior, and S. A. Mudhafar: APR-246 enhances the anticancer effect of doxorubicin against p53-mutant AsPC-1 pancreatic cancer cells. *Baghdad Science journal* 21: 2551-2560, 2024. https://doi.org/10.21123/bsj.2024.10135
- [15] Z. J. S. Hassan, M. K. Hamid, & M. E. Ahmed. Synthesized Zinc Oxide Nanoparticles by the Precipitation Method on Streptococcus spp. from Dental Carries and Cytotoxicity Assay. *International Journal of Drug Delivery Technology*. 12, 1327-1330. 2022. https://doi.org/10.25258/ijddt.12.3.65
- [16] S. M. Mahdi, A. F. Abbas, M.E. Ahmed. Cytotoxicity of zinc oxide nanoparticles to lymphocytes using Enterococcus faecium bacteriocin and assessment of their antibacterial effects. *Nanomedicine Research Journal*. 2024.9(2): 180-194. 2024. https://doi.org/10.22034/NMRJ.2024.02.007
- [17] A. Regiel, S. Irusta, A. Kyziol, M. Arruebo, J. Santamaria. Preparation and characterization of chitosan silver nanocomposite films and their antibacterial activity against Staphylococcus aureus. Nanotechnology- IOPscience. 2013. https://doi.org/10.1088/0957-4484/24/1/015101
- [18] C. Covarrubias, D. Trepiana & C. Corral. Synthesis of hybrid copper-chitosan nanoparticles with antibacterial activity against cariogenic Streptococcus mutans. *Dental Materials Journal*, 37(3), 379-384. 2018. https://doi.org/10.4012/dmj.2017-195

- [19] T. T. Hanh, L. Chi & N. N. Duy. Fabrication of copper nanoparticles/diatomite nanocomposite by irradiation method for antibacterial application. *Aquaculture Reports*. 27, Article ID 101342. 2022. https://doi.org/10.1016/j.aqrep.2022.101342
- [20] M. H. Sarfraz, M. Zubair, B. Aslam, A. Ashraf, M. H. Siddique S. Hayat, J. N. Cruz, S. Muzammil, M. Khurshid, M. F. Sarfraz, A. Hashem, T. M. Dawoud, G. D. Avila-Quezada & E. F. Abd_Allah. Comparative analysis of phyto-fabricated chitosan, copper oxide, and chitosan-based CuO nanoparticles: antibacterial potential against Acinetobacter baumannii isolates and anticancer activity against HepG2 cell lines. Antimicrobials, Resistance and Chemotherapy. 14:1188743. 2023. https://doi.org/10.3389/fmicb.2023.1188743
- [21] I. Khan, K. Saeed, & I. Khan. Nanoparticles: properties, applications and toxicities. *Arabian Journal of Chemistry*. 12, 908–931. 2019. https://doi.org/10.1016/j.arabjc.2017.05.011
- [22] M. R. Mehta, H. P. Mahajan, & A. U. Hivrale. Green synthesis of chitosan capped-copper biocomposites: Synthesis, characterization, and plant biological activity against pathogens. BioNanoScience, 11, 417-427. 2021. https://doi.org/10.1007/s12668-021-00823-8
- [23] Z. Hongfeng, A. El-Kott, A. E. Ahmed, & A. Khames. Synthesis of chitosan-stabilized copper nanoparticles (CS-Cu NPs): Its catalytic activity for CN and CO cross-coupling reactions and treatment of bladder cancer. *Arabian Journal of Chemistry*, *14*(10), 103259. 2021. https://doi.org/10.1016/j.arabjc.2021.103259
- [24] Z. Shi, Y. Mahdavian, Y. Mahdavian, S. Mahdigholizad, P. Irani, M. Karimian & M. M. Zangeneh. Cu immobilized on chitosan-modified iron oxide magnetic nanoparticles: Preparation, characterization and investigation of its anti-lung cancer effects. Arabian Journal of Chemistry, 14(8), 103224.

https://doi.org/10.1016/j.arabjc.2021.103224

توصيف جسيمات من الكيتوسان - النحاس نانوية المصنعة بالطريقة الكيميائية والتحري عن السمية الخلوبة

اشواق فاضل عباس '، ميس عماد احمد '' *

١ قسم علوم الحياة ، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

إن الخصائص المميزة للبوليمر الحيوي الكيتوزان مثل خصائص التغطية والاستقرار الممتازة والتوافق البيولوجي والتحلل البيولوجي والود البيئي والتعدد الكاتيوني وعدم السمية - جعلته مادة داعمة حاسمة لتخليق الجسيمات النانوية المعدنية .(MNPs) حدد عملية كيميائية مناسبة، مثل الترسيب المشترك، أو هلام السول، أو تقنيات الحرارة المائية، لإنشاء جسيمات نانوبة من الكيتوزان القائمة على النحاس. فيما يتعلق بالتجانس والشكل وحجم الجسيمات، فإن كل طريقة لها فوائد. أنتجت هذه الدراسة ووصفت وفحصت الخصائص البكتريولوجية للجسيمات النانوية المعدنية القائمة على الكيتوزان، بما في ذلك الجسيمات النانوية النحاسية القائمة على الكيتوزان، بما في تم إنشاء جسيمات نانوية معدنية قائمة على الكيتوزان باستخدام تقنية الصب بالمحلول. تم توصيف الجسيمات النانوية الناتجة باستخدام فحص FTIR و AFM و UV و ZP و SEM. توضح صورة المجهر الإلكتروني النافذ أن المواد التي تم إنشاؤها هي مواد نانوبة، تتراوح في الحجم من ١ إلى ١٠٠ نانومتر. أظهرت جزيئات Ch-CuNPs، التي تظهر لونًا أحمر قرميديًا مستقرًا، ذروة امتصاص عند ٥٤١ نانومتر، مما يدل على تكوبن جسيمات نانوية أحادية التشتت ورنين بلازمون السطح. أكد حيود الأشعة السينية البنية المكعبة ذات الوجه المركزي مع قمم عند ٣٦,٧٨ درجة و٤٣,٣٨ درجة و٥٠,٥٦ درجة ومتوسط حجم الجسيمات ٦٨ نانومتر. أظهر تحليل FTIR تفاعلات بين الكيتوزان والنحاس، وخاصة حول ٣٣٧٠ -٣٢٢٦ سم⁻¹ و ۱٦٣٣ سم-1 و ٦٨٠ سم-1. كانت أفضل خصائص مضادة لخلايا سرطان الثدى البشربة MCF7 للمركبات النانوبة ضد خطوط الخلايا المذكورة أعلاه في حالة خط خلايا .MCF7 وفعًا للنتائج المذكورة أعلاه، يمكن استخدام المركب النانوي لعلاج عدة أنواع من سرطان الثدى لدى البشر. كان لدى سرطان المبيض حساسية متزايدة للجسيمات النانوية. عند تركيز ٢٥ ميكرولتر /مل، أظهرت جزيئات نانوية من Chi-Cu سمية خلوية ممتازة ونشاطًا مضادًا للسرطان، مما يجعلها مناسبة للاستخدام كعامل مضاد للسرطان.

الكلمات الدالة: السمية الخلوية، المجهر الالكتروني الماسح، كيتوسان، اوكسيد النحاس.