Original Article

Synthesis of Cus Nanoparticle and Characterization, as well as Investigation of their Anticancer Activity Against A Human Breast Cancer Cell Line

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ABSTRACT

CuS nanoparticles were prepared in a novel way, and their efficacy as an anti-cancer agent was investigated, as well as their toxicity, using a new, simple, easy, and low-cost method called chemical co-precipitation, which involved reacting dehydrated metal chloride salts of the formula MCl2.2H2O with aqueous sodium sulfide of the formula Na2S.H2O under reaction conditions 0.5 M and the best pH 6 at 35°C. CuS nanoparticles were identified using X-ray diffraction, FTIR spectroscopy, spectroscopy Energy-dispersive X-rays (EDX), scanning electron microscopy (SEM), and the activity of the prepared CuS nanoparticles was investigated using an MTT test on the Mcf7 cell line. Graphpad prism8.0 was used to perform statistical analysis on the results. CuS nanoparticles were 14.43 nm. The efficacy of CuS nanoparticles as an anti-tumor agent was compared with letrozole the efficacy has been demonstrated using different concentrations on the Mcf7 cell line, with the highest inhibition rate of 87.49 % after 24 hours and 95.67 % after 48 hours of incubation at a concentration of 400 g/ml, and the lowest inhibition rate of 1.67 % after 24hrs and 6.35 % after 48 hrs at a concentration of 1.67 g/ml. The maximum percentage of inhibition is 84.61 % after 24 hours and 96.68 percent after 48 hours of incubation at 400 g/ml, while a minimum was 9.1 % after 24hrs and 18.24 after 48 hrs at 25 g/ml.

Keywords: CuS nanoparticle, anticancer, MCF-7.

Introduction

Cancer diseases have lately surpassed heart and blood vessel diseases as the second biggest cause of death worldwide.[1] When compared to standard cancer treatment approaches such as surgery, radiotherapy, and chemotherapy.[2,3] Inorganic nanomaterials have gotten a lot of attention in both practical clinical use and scientific research. CuS NPS has also been shown in studies to be good therapeutic materials for cancer treatment.[4,5] However, these nanomaterials are frequently encounter issues such as low conduction rate due to irregular nanostructures or uneven size distribution, both of which are linked to response characteristics and biological behaviors *in vivo*.[6,7] Managing the size and shape of nanoparticles is, therefore, critical.[8,9] In the presence of alkali, the S-rich protein can seize the metal ions and form protein-metal complexes. The protein releases an active sulfur anion mainly derived from disulfide bonds, which favors the nucleation of sulfides. Finally, sulfide metal growth is trapped within the cavities of the proteinoids components, resulting in covering the protein. Metal sulfide NPs, also known as metal sulfide protein NPS,

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are particles with tiny size and good biocompatibility. The findings revealed that metal sulfide MS NPS is an excellent cancer treatment agent.[10,11] Metal-containing compounds play an essential role in cancer treatment; however, single metals have a short circulatory, low target specificity, and cause cytotoxic effects. Hepatotoxicity, gastrointestinal responses, neurotoxicity, bone marrow suppression, nephrotoxicity, ototoxicity, and hair loss have all been reported as side effects of the ions[12] that organic metal skeletons, metal carbides, metal oxides of tremendous interest in treatment for cancer.[13,14] Metal sulfide nanoparticles in particular have unique physical and chemical features, including stimulation, light conversion, radiation enhancement, and immunological activation.[12,16] These are up the abilities in the realm of cancer treatment.[17] CuSNPs, for example, exhibit a broad near-infrared (NIR) absorption spectrum. Under laser irradiation at a wavelength of 808 nm, hydro-dispersion of particles displays great optical efficiency. As a result, it can be used to treat cancers with photothermal therapy.[18, 19]. Because Metal sulfide NPs can separate hydrogen sulfide (H2S) in the acidic environment of tumors, some types of Metal sulfide NPs, such as manganese sulfide (MnS) NPs, iron sulfide (FeS) NPs, and zinc sulfide (ZnS), can be employed as a therapeutic agent.[20] Although the use of Metal sulfide NPs as therapeutic nanosystems has been studied for over a decade, only a few comprehensive studies have been published to highlight recent accomplishments as well as current obstacles.[21] In this study, the biocompatibility of copper sulfide against MCF-7 human breast cancer cells was compared to a commercial drug called letrozole used in Iraq for breast cancer treatment. Copper sulfide was prepared using a chemical co-precipitation method, and the optimized reaction conditions were ascertained.

Materials and Methods

The chemicals used in this study, copper (II) chloride hydrate $CuCl_2.2H_2O$, sodium sulfide hydrate$Na_2S.H_2O$, 96% ethanol were purchased from Alfa Assar (Germany). Polystyrene 96-well plates were purchased from Greiner Bio-One (Germany). letrozole tablets (2.5 mg) were purchased from Novartis Pharmaceuticals (UK). MCF7 cell line was obtained from (ATCC® HTB-22™, USA).

Synthesis and Characterization of CuS Nanoparticles

CuS nanoparticles were prepared in 0.5 Ma of copper chloride aqueous $CuCl_2$. 2 H_2O by dissolving 9.6 g of it in 50 ml of deionized water. The solution was placed on magnetic stirrer for 30 minutes until the substances were completely dissolved. A solution of sodium sulfide $Na_2S.H_2O$ was prepared by dissolving 2.4 g of it in 50 ml of deionized water. Twenty milliliters of 0.5 M sodium sulfide was added drop by drop and the pH was adjusted for 1 hour until a dark olive precipitate appeared. The mixture was centrifuged for 5 minutes, filtered. The precipitate was rinsed multiple times with deionized water before being dried for 4 hours at 120° C. Table 1 shows how the reaction conditions were altered in terms of concentration, pH, and optimal temperature in this experiment which were the optimum (0.5 M concentration, PH 6, and 25 oC temperature).

Table 1: Preparation of CuS at different reaction conditions

Concentration of $CuCl_2$. 2 H_2O	*Concentration of $Na_2S.H_2O$*		*Temperature*
(M)	*(M)*	*pH*	*(°C)*
1	1	4	25
0.75	0.75	6	35
0.5	0.5	8	45

Characterization Techniques CuS NPs

X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, Electron microscope (SEM), and other methods were used to analyze the CuS nanoparticles. XRD was used to assess the crystallite size of the produced nanoparticles (Shimadzu, Kyoto, Japan). Miniflex X-ray diffractometry with Cu-Ká radiations of the 2è extent (= 0.15406 nm) beyond 20°C after 80°C. Shimadzu was used to analyze the materials' FTIR spectra (Tokyo, Japan). Using a KBr pellet as a spectrophotometer. A Zeiss SEM with a voltage of 200 kV was used to examine the samples (Germany).

MTT Assay for CuS NPs

MTT dye (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) at concentration of 10 mg/ml was used in this test. CuS NPs were dissolved in 0.2% of DMSO to provide a gradient of concentrations (25, 50, 100, 200, and 400 g/ml). In 96-Microplate, an aliquot of 200 µl of cell suspension (1× 104 cell/well) prepared in RPMI media was distributed and incubated at 37 oC for 24 hours in the presence of 5% CO_2. The cells were treated with 20 µl of CuS NPs and incubated under the same conditions for 24 hours. After that, 10 µl of MTT reagent was added to each sample and incubated at 37oC for 5 hours. DANA-3200 ELISAREADER was used to measure the absorbance at 570 nm and the inhibition percentage was calculated.

Statistical Analysis

Graph Pad Prism 8.0 was used to examine the findings of CuS NPs effects on MCF-7 cells. At a significance threshold of $P < 0.05$, one-way ANOVA was used to infer the means. Under the same circumstances, all the tests were repeated at least twice with three replicates for each.

Results and Discussion

Morphological Analysis

X-ray diffraction was used to determine the crystal structure and phase purity of CuS nanocrystalline sulfide that was synthesized, as shown in Figure 1. According to JCPDS cards

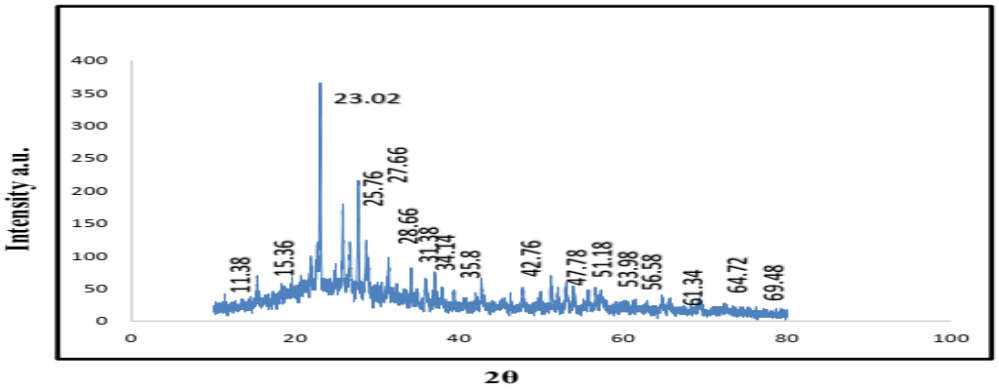

Figure 1: X-ray diffraction spectrum of CuS NPs

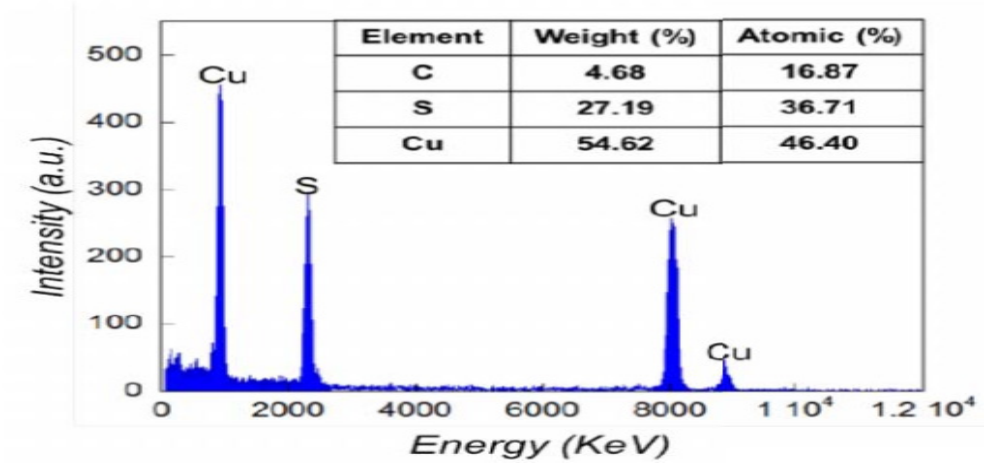

Figure 2: Energy-dispersive X-ray spectroscopy of CuS NPs

06-0464 CuS database, the X-ray diffraction spectrum of the prepared copper sulfide corresponds to the standard spectrum of CuS sulfide.[22,23] Using the Debye-Scherer formula, the mean particle size of CuS particles was 14.43 nm.

EDX analysis

Energy-dispersive X-ray (EDX) was used to determine the percentage of elements in CuS nanoparticles as shown in Figure 2. The product showed a high percentage of both copper and sulfur. This indicates a high degree of purity of copper sulfide nanoparticles, where the proportion of Cu (54.62%) and S (27.19%).

FTIR Analysis

FT-IR spectra of CuSNPs are shown in Figure 3. The wide peak intensity at 3450 cm-1 is attributed to the stretching mode of the hydroxyl ions. A vibrational peak at 613 cm-1 indicates the presence of Cu–S stretching.[24]

SEM Analysis

SEM images showed the morphological and structural characteristics of CuS nanoparticles. Figure 4 shows that some of the nanoparticles separated from each other while most of them were present in a lumpy form. This agglomeration is

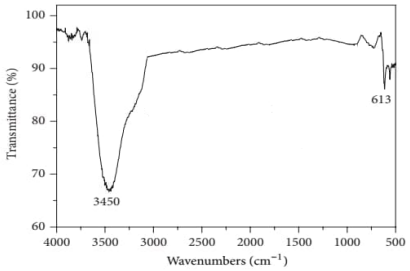

Figure 3: FTIR Spectroscopy of CuS NPs.

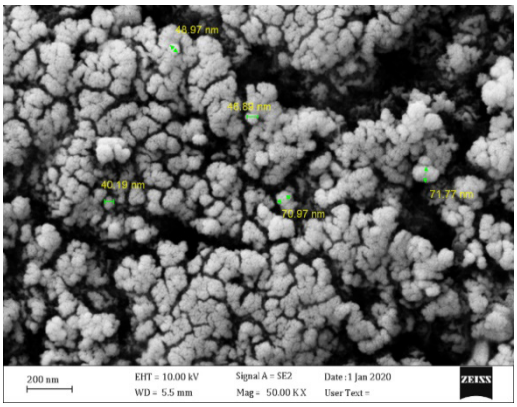

Figure 4: SEM image of CuS NPs

due to the electrostatic effects and the effect of the aqueous suspended matter, which reveals the agglomeration behavior of nanoparticles, which is consistent with similar results in previous studies. The average diameter of these particles is about 55 nm.[25]

Anticancer Activity of CuS NPs

The cytotoxicity effect of CuS NPs on MCF-7 cell lines was evaluated using the MTT test. Table 1 shows cytotoxicity of various concentrations (from 25 to 400 µg/mL) compared to the control. The survival rate of MCF-7 cells after 24 hours after the addition of copper sulfide nanoparticles at concentration of 25 µg/ml was 98.33%, indicating that the low concentration did not affect the cells. In contrast, the survival rate of MCF-7 at 400 µg/ml was 12.51%, indicating a decrease in the survival rate. As shown in Figure 5, a dropping in number of living cells by more than 87% was obtained. Half maximal inhibitory concentration (IC50) of copper sulfide nanoparticles on MCF-7 breast cancer cells after 24 hours was measured using (Graph Pad Prism 8.0) program. The value of R square is 0.9874, as shown in Figure 6. While the results in Table 2 showed the cytotoxicity after 48 hours at a concentration of 25 µg/ml which was 93.65%, indicating that there is a relationship between the time and concentration factor on the survival rate of MCF-7 cells. The results showed that the survival rate at a concentration of 400 µg/ml was 4.33%, which indicates that the cells survival rate was less than 95%, as shown in Figure 7, and the apex was (IC50= 131.1) and the apical R square was equal to 0.9891 as shown in Figure 8. CuS nanoparticles have attracted much attention due to their high drug loading and biodegradability, biocompatibility,

Table 1: Cytotoxicity of CuS NPs at 24 hours to breast cancer cells

	Cell viability (%)			
Concentration (µg/ml)	*Replicate1*	*Replicate2*	*Replicate3*	*Mean± SD*
0 (Blank)	101.96	99.47	98.68	100.04±1.71
25	97.24	99.47	98.29	98.33±1.11
50	93.30	94.22	93.96	93.83±0.47
100	69.42	74.80	72.30	72.17±2.69
200	52.62	46.58	49.08	49.43±3.03
400	14.69	11.15	11.67	12.51±1.91

Table 2: Cytotoxicity of CuS NPs after 48 hours to breast cancer cell

	Cell viability (%)			
Concentration (µg/ml)	*Replicate1*	*Replicate2*	*Replicate3*	*Mean ± SD*
0 (Blank)	100.78	102.09	100	100.96 ± 1.06
25	95.27	93.30	92.38	93.65 ± 1.47
50	83.20	85.56	83.98	84.25 ± 1.20
100	67.19	67.71	65.09	66.66 ± 1.38
200	31.10	34.77	30.70	32.19 ± 2.24
400	3.41	5.24	4.33	4.33 ± 0.19

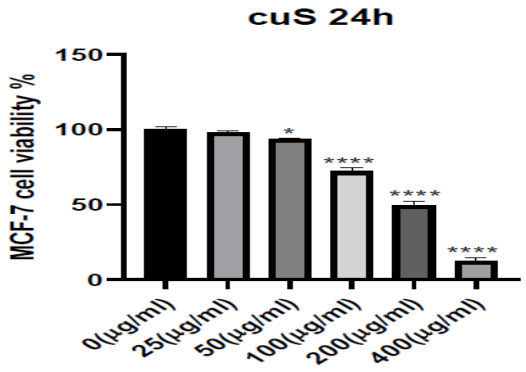

Figure 5: CuS on the survival rate of MCF-7 cells after 24 hours incubation

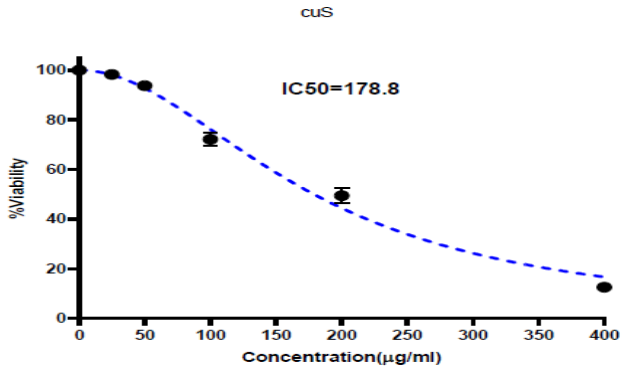

Figure 6: IC50 of CuS after 24 hours incubation

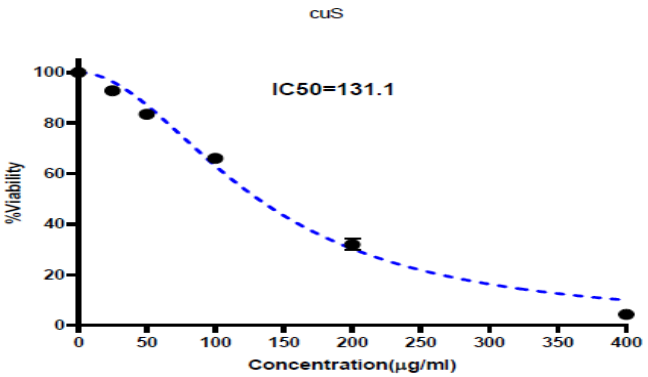

Figure 7: Effect CuS on the survival rate of MCF-7 cells after 48 hours incubation

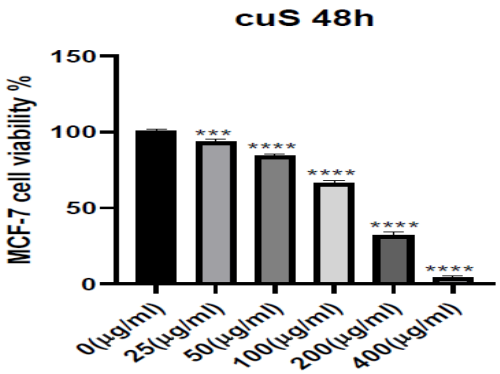

Figure 8: IC50 of CuS after 48 hours incubation

and potential to carry different types of drugs. Moreover, the proposed mechanism for this effect is the interaction of CuS nanoparticles with intracellular macromolecules such as proteins and DNA. Also, the cellular uptake of CuS nanoparticles leads to an increase in ROS resulting in the induction of apoptosis.[26]

Conclusion

In this research, we describe how to synthesize CuS-NPs using a simple and effective co-precipitation technique. CuS-NPs were characterized using FTIR, XRD, and SEM to determine their structural characteristics. Because of its capacity to slowly release heavy metal ions, CuS-NPs may have a promising therapeutic substance as anticancer agent. CuS-NPs were shown to prevent MCF-7 metastasis in a cell viability experiment. The potential value of CuS-NPs in inhibiting cell migration and invasion in breast cancer stem cells was discovered in this work, and it might be employed in breast cancer treatment.

Ethical Approval

The manuscript is written in original and all the data, results pertaining to this manuscript are original according to the research performed. The authors followed academic integrity and have not copied any content/results from another source.

Informed Consent

The authors of the manuscript agrees to publish this research in the journal if it's considerable by the editors of the journal. The authors provide full consent for reviewing and publishing this manuscript.

All the authors of this study contributed equally in terms of performing the research as well as in preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author. Any query/suggestion related to the manuscript can be reached to the corresponding author

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