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Reduce the toxicity of prepared copper sulfide nanoparticles

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ABSTRACT

Copper sulfide (CuS) nanoparticles have attracted increasing attention from biomedical researchers across the globe, because of their intriguing properties, which have been mainly explored for energy and catalysis related applications. The aim of the study is to prepare CuS NPs by BSA entrapment to reduce the toxicity, characterizing, comparative the toxicity before and after entrapment against bacteria and check the toxicity against RD and L20B cell lines. CuS-BSA NPs was an easy, low toxicity and low cost chemically synthesized. The CuS-BSA NPs was identified though UV-VIS spectrophotometer, FTIR, XRD, SEM, EDX, and Zeta potential. The antibacterial activity against different G-positive and G-negative bacterial strains have been investigated for (2 mg/ml) concentrations of CuS-BSA NPs and commercial CuS. A result showed that CuS-BSA NPs have more antibacterial activity than commercial CuS. Using different parameters of CuS-BSA NPs, its anti-cancer bioactivity for every compound synthesized in RD and L20B cell line was explored, and the result proved there was significant toxicity against RD and L20B cell lines.

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INTRODUCTION

Copper sulfide (CuS) nanoparticles have attracted increasing attention from biomedical researchers around the globe due to their intriguing properties, which have been explored primarily for applications related to energy and catalysis. In vitro, CuS nanoparticles have discovered wide-ranging applications, particularly in the identification of biomolecules, chemicals, pathogens and cancer therapy based on CuS photothermal characteristics, as well as drug delivery and theranostic applications. The progress of CuS nanoparticles has spanned a wide variety of biomedical applications (Goel *et al.*, 2014).

CuS nanomaterials, in particular, have drawn wide attention due to their low toxicity, simple preparation, low price and high stability (Zhang *et al.*, 2015). On the other side, CuS is commonly used as a material for thermoelectric cooling, optical filers, optical recording equipment, solar cells, nano-scale switches and superionic equipment (Umasankari and Anitha, 2017).

CuS nanoparticles ' ambiguous biotoxicity restricted their biological applications. Developing biocompatible CuS photothermal agents with the capacity for clinical translation is therefore extremely desirable. CuS photothermal nanoparticles using bovine serum albumin (BSA) as a model through mimicking procedures of bio materialization. The toxicity assays in vitro and in vivo showed that BSA-CuS nanoparticles had excellent bio compatibility due to the BSA's intrinsic biocompatibility. Phototherapies were conducted in vitro and in vivo,

and excellent outcomes were achieved (Zhang *et al.*, 2015). Furthermore, the results show that the CuS / BSA nanocomposites are approximately one sphere with a size distribution of 10 to 35 nm in diameter and good dispersibility, highly dependent on the concentration of BSA. In biomedical engineering and microelectronics, these protein-assisted synthesized nanocomposites have a huge prospective application. BSA is one of the most commonly researched proteins, and the synthesis of multiple nanocrystals has often been adopted. Preparation methods for CuS / BSA nanocomposites were convenient, easy, non-toxic and environmentally friendly methods for obtaining BSA solution CuS nanoparticles with controllable dimensions and sphere shapes (Huang *et al.*, 2017; M.Y.Radeef *et al.*, 2018).

The causes of chronic diseases and mortality are bacterial diseases. Because of their cost-effectiveness and strong results, antibiotics were the preferred therapy technique for bacterial diseases. However, several studies have given direct proof that extensive antibiotic use has resulted in multidrug-resistant bacterial strains emerging. In reality, owing to abuse of antibiotics, super-bacteria, which are resistant to almost all antibiotics, have lately evolved. Studies have shown that these bacteria have a gene called NDM-1 with super-resistance. Unfortunately, against each of these modes of action, bacterial resistance may grow. Mechanisms of resistance to current antibiotics have developed from pathogenic bacteria. Developing novel antibacterial therapy techniques that do not relay to traditional therapeutic regimes is urgently needed. Different therapeutic methods for antibiotic-resistant bacteria were created (Wang *et al.*, 2017).

The consideration of nanoparticles as an alternative to antibiotics is that in some instances, NPs can efficiently prevent resistance to microbial drugs. One of the accepted interactions between nanomaterials and antibacterial activity is that nanomaterials are extremely promising as antibacterial complements to antibiotics and are gaining considerable interest as they could fill the gaps where antibiotics often fail. Moreover, nanomaterials can "as a useful carrier" complement and promote traditional antibiotics (Wang *et al.*, 2017).

It is now well acknowledged that inorganic materials can extinguish bacteria without toxicating the tissue around them. Because of their antibacterial characteristics, copper and its compounds have been used as disinfectants for centuries. CuS nanoparticles ' antimicrobial activities have been studied, suggesting that the activity mainly depends on the morphology of the nanoparticles (Chakraborty *et al.*, 2016).

MATERIALS AND METHODS

Chemicals and reagents

Copper nitrate trihydrate, thioacetamide, bovine serum albumin, copper sulfide and Muller Hinton agar were obtained from Himedia (India). Nitric acid (HNO3) and Dimethyl sulfoxide (DMSO) from BDH (England).3-(Dimethylthiazol-2-yl) 2,5Diphenyltetrazoliumbromide (MTT) from Sigma (USA). Rhabdomyosarcoma (RD) cell line, as a human cell line, and a murine cell line derived from mouse L cells (L20B) cell line was provided by the Central Public Health Lab., Baghdad, Iraq.

Antibacterial activity

Antimicrobial activity of CuS-BSA nanoparticles and CuS commercial separately were tested against eight bacterial strains (Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Salmonella, Klebsiella, Pseudomonas aeruginosa, Proteus, Enterobacter feacalis) by using the agar disk diffusion (Yang *et al.*, 2015). The test samples were prepared by dissolving CuS-BSA nanoparticles and CuS in distilled water using D.W. solvent as control. Mueller Hinton agar plates were inoculated with active cultured bacteria. Then, a sterile filter paper discs (6 mm in diameter), were loaded with 20 μl of 2mg/ml concentration of CuS-BSA NPs and CuS commercial then placed on the agar surface. The Plates were incubated at 37 °C for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zone against the tested bacteria.

Determination of anticancer activity of CuS-BSA nanoparticles by MTT assay

RD and L20B cell lines were grown in RPMI-1640 medium at 37 °C with 5% CO2 in 96 – well flat-bottom culture plates at density 1×104 cells /ml for 48 hrs. The cells were treated, in duplicate, with the concentrations of 20 - 70 μg/ml of CuS-BSA NPs and incubated for 24 hrs to determine the toxicity against examined cell lines. 10 μl of MTT solution was added to each well, and the plates were incubated for 4 hrs at 37 °C. The media were then removed, and the remaining formazan crystals were dissolved in DMSO , and the absorbance was measured at 570 nm using an ELISA microplate reader to determine the toxicity of LPS, once with a tumor cell line and another with normal cells. The cytotoxicity percentage was calculated by the equation (Fresh-

ney, 2015):

$$Growth\ Inhibition\ rate\ \%\ (G.I) = \frac{A-B}{A} x\ 100$$

A = an optical density of the control.

B = an optical density of the treated sample.

RESULTS AND DISCUSSION

Characterization

UV-VIS spectroscopy analysis

A UV-VIS spectroscopy were used to study the shape and size-controlled nanoparticles in aqueous suspensions. As seen in Figure 1 Absorption spectrum at different wavelengths ranging from 190 to 900 nm were examined to detect the CuS-BSA nanoparticles. The sharp bands of the BSA are observed at 197.5 nm while the spectrum of CuS could be seen at 616 nm. These results were close to a result of (Huang *et al.*, 2010).

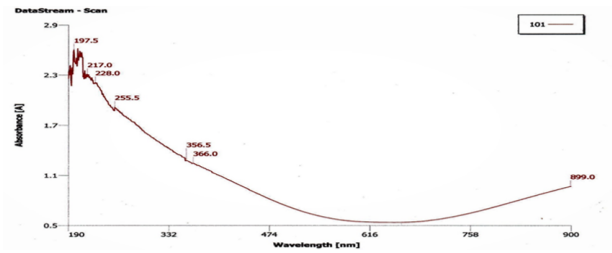


Figure 1: UV-Vis Spectroscopy scanning of CuS-BSA Nanoparticles

Scanning Electron Microscopic (SEM)

The SEM analysis was conducted to visualize the structure, morphology, and size of CuS-BSA NPs as seen in Figure 2. The SEM CuS-BSA NPs are almost plate-like, and the particle size is in the range of 30–60 nm. This is close to results obtained by (Huang *et al.*, 2010).

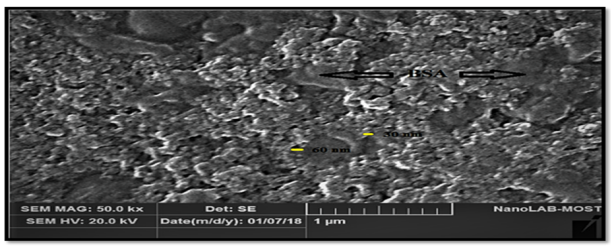


Figure 2: Scanning Electron Microscopic (SEM) image of CuS-BSA

X-Ray Diffraction (XRD) analysis

The XRD pattern of CuS-BSA NPs observed in Figure 3 that show the peaks in the 2Θ rang of 5°-80°. XRD spectrum analysis showed four different strong peaks at 29.1445°, 31.9310°, 48.0042° and 59.6812° that are indexed the planes 102, 103, 110 and 203, respectively. According to the Scherrers equation, the larger FWHM values proposed smaller particle size 25 nm and these results agreed with (Huang *et al.*, 2010, 2017; Chu *et al.*, 2018).

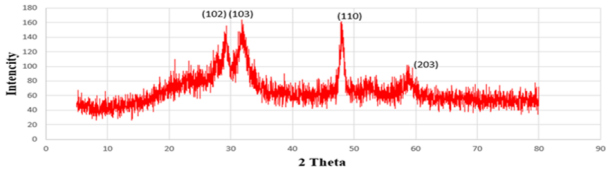


Figure 3: X-ray diffraction analysis of CuS-BSA Nanoparticles

Fourier transform infrared (FTIR) spectroscopy analysis

This technique was used to confirm the existence of functional groups in the synthesized CuS-BSA NPs. The FTIR spectra for CuS-BSA NPs were recorded in the spectral region (4000-400) cm^{-1} as given in Figure 4.

The peak at 3435.34 cm^{-1} is due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The band at 2928.04 cm^{-1} is due to alkyl C-H stretching vibration. The band at 2729.37 cm^{-1} is due to S-H stretching vibration. The FTIR spectrum peak of C=O amide stretch appeared at 1637.62 cm^{-1}. The bands at 1548.89, 1518.03 cm-1 are attributed to aromatic C=C stretch. The peak at 1386.86 cm^{-1} is attributed to the absorption of NO3-1, which was introduced by the addition of Cu (NO3)2. The band at 1105.25 $cm^{-1}$is due to C-O stretching vibration and the band at 1035.81 cm^{-1} stretching from to C-N stretching of amines. The results were closed to (Huang *et al.*, 2010; Chu *et al.*, 2018; Zhao *et al.*, 2018) when they confirm the existence of functional groups in the synthesized CuS-BSA NPs.

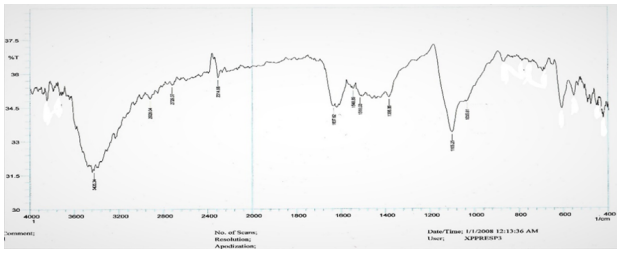
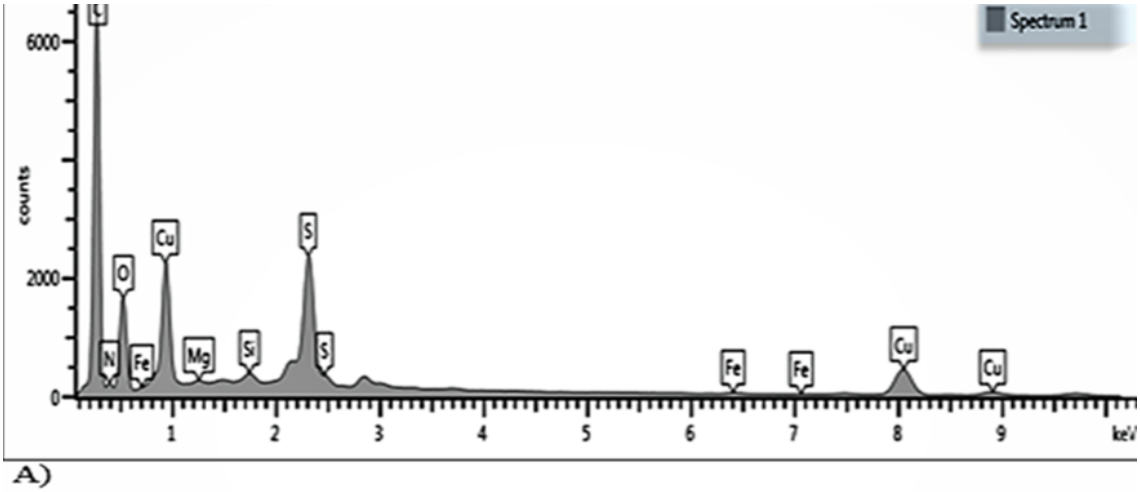
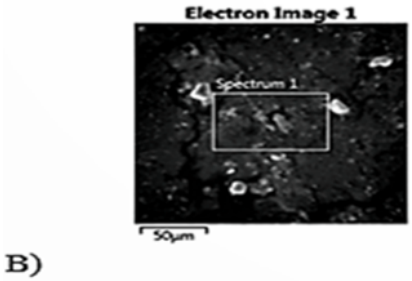


Figure 4: The FTIR spectra for CuS-BSA NPs were recorded in the spectral region

Energy dispersive X-ray spectrum (EDX)

EDX was used to verify the presence of CuS in the suspension of nanoparticles and to determine the chemical composition of CuS-BSA NPs, as shown in Figure 5.





Element	Wt%	Wt% Sigma
C	63.08	0.52
N	6.64	0.63
O	19.64	0.31
Mg	0.10	0.02
Si	0.21	0.02
S	3.74	0.06
Fe	0.14	0.03
Cu	6.45	0.13
Total:	100.00	

C)

Figure 5: Energy dispersive X-ray spectrum (EDX); (A) EDX of CuS, (B) Electron image, (C) Table of elements

The results showed the presence of carbon by a large percentage followed by oxygen, nitrogen, copper and sulfur. It should be noted that the element of silicon, magnesium and iron could be attributed to their association with carbon. This result is related to (Chu *et al.*, 2018).

Zeta potential

Zeta potential analysis is important to measure the surface charge of CuS-BSA nanoparticles as demonstrated in Figure 6 the zeta potential value carries a negative charge (-14.49 mV) which means that CuS-BSA NPs solution is stable.

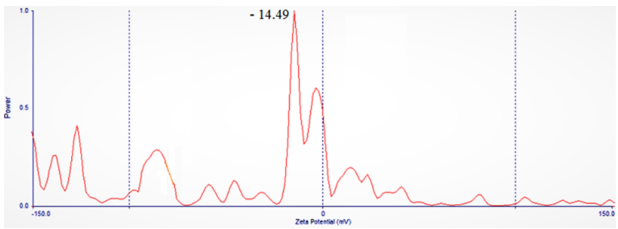


Figure 6: Zeta potential of CuS-BSA Nanoparticles

Antibacterial activity

It was studied against eight bacterial strains; E. coli, S. aureus, S. epidermidis, Salmonella, Klebsiella, Pseudomonas aeruginosa, Proteus, Enterobacter after incubation for 24 hours at 37°C. The antibacterial activity of CuS-BSA NPs, at concentration 2 mg/ml, revealed an inhibition zone with a diameter of) 10, 8, 7 and 6 (mm against S. aureus, Proteus, E. coli and Enterobacter respectively, while there is no inhibition zone against Klebsiella, P. aeruginosa, Salmonella and S. epidermidis. The antibacterial activity of commercial CuS, at concentration 2 mg/ml, revealed an inhibition zone with a diameter of 11 and 7 mm against S. epidermidis and S. aureus respectively, while there is no inhibition zone against Klebsiella, P. aeruginosa, Salmonella; E. coli, Proteus and Enterobacter.

The results shown that the antibacterial activity of CuS-BSA NPs against G-ve and G+ve strains, possibly because of the different structures of bacterial walls, as mentioned by (Huang *et al.*, 2017). The antibacterial effects of metallic nanoparticles are greater than other nanomaterials, which demonstrated increasing chemical activity owing to the crystallographic surface structure and their large surface to volume ratios (Wang *et al.*, 2017).

Anticancer

In the current study, the cytotoxic effects of different prepared compounds and complexes against Rhabdomyosarcoma (RD), as human cell line, and murine

cell line derived from mouse L cells (L20B) were determined by MTT colorimetric assay.

The cell lines were treated with different concentration of CuS-BSA NPs (20, 30, 40, 50, 60 and 70 μg/ml) for 24 hours, as shown in Figure 7. The RD cytotoxicity was 58%, at 20 μg/ml that increased gradually to reach 100% when concentrations increased to 70 μg/ml, while L20B cells revealed cytotoxicity of 62%, at 20 μg/ml, to reach whole death of cells at a concentration of 70 μg/ml.

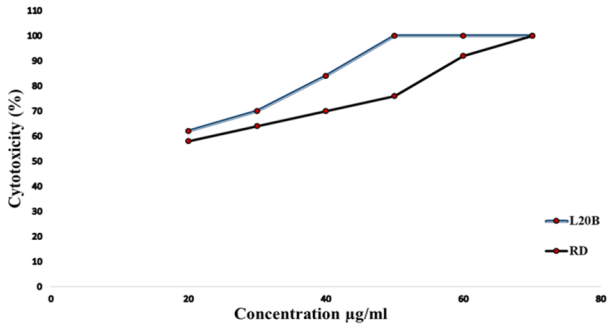


Figure 7: Cytotoxicity of RD and L20B cell lines at different concentrations determined by MTT assay

CONCLUSION

This study provides an effective way to prepare CuS-BSA NPS nanoparticles from CuS-BSA NPs, which is an easy, low-toxic and low-cost way to produce nanoparticles. From this study, CuS-BSA NPs have been found to have a large antibacterial activity with a lower concentration than commercial CuS.

The CuS-BSA NPs has proven to have significant toxicity against cancer. The results suggest that CuS-BSA NPs should be further researches for application as drug cancers for other cell lines. In addition, it should be investigated with more different biological activities.

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