RESEARCH ARTICLE

Using of Nano-poly Chitosan Cephalexin Drug to Inhibit Spread Cancer Cells

Aliaa H. Abbas, Mohammad N. Al-Baiati*

Department of Chemistry, College of Education for Pure Sciences, University of Karbala, Karbala, Iraq

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ABSTRACT

In our work, a natural chitosan nano polymer was synthesized and characterized by fourier transform infrared (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), atomic force microscopy (AFM), transmission electron microscope (TEM) and X-ray diffraction (XRD) techniques. Then the nano-chitosan was linked with cephalexin drug, which FTIR characterized, and ^{1}H-NMR techniques. The biological activity of the nano-chitosan- cephalexin drug was studied by measured cell line breast cancer. Where it showed a high rate in preventing the spread of breast cancer.

Keywords: Chitosan, Cephalexin, Nano co-polymer-drugs, Drug delivery systems.

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INTRODUCTION

Tumors are considered cancerous when they exhibit invasiveness, spread, and non-specific cell growth and division, cells that divide rapidly can invade and destroy nearby tissues or spread to distant tissues, such abilities characterize malignant tumors in contrast to benign tumors that are characterized by a specific growth and the inability to invade or move. Benign tumors can, however, sometimes develop into malignant cancers.[1] There are more than 100 types of cancer, there are several common types of cancer. The most common cancers are colon, prostate, breast, lung and rectal cancers. Lung cancer is more commonly seen in men, although breast cancer is more common in women.[2] Cancer of the breast derives from cells that line the lobules (15%) or ducts (85%) of the glandular tissue. Initially, the tumor resides within a single duct or lobule but gradually spreads throughout the body. These cancers may develop at stage zero and invade surrounding breast tissue and then spread to nearby lymph nodes or other body organs.[3] It accounted for more than 2.2 million cases in 2020, about a woman affected one in 12 women with breast cancer in their lifetime. Nearly 685,000 women died from breast cancer in 2020, making it the leading cause of cancer death in women.[4] In spite of the great advances made against cancer disease, and the diversity of available anticancer treatments, there is no complete cure for it, one of the most effective treatments is through the application of the drug delivery system, which is a pharmaceutical method or process to achieve a therapeutic effect in humans or animals aimed at delivering the drug to the place to be treated is done in several ways that differ according to the drug or the place to be treated.[5,6] Drug delivery systems using nanoparticles are an extremely common method of drug delivery. Nanoparticles have enormous potential for drug delivery as it appears that polymeric nanoparticle delivery systems can penetrate cell membranes in a minimally invasive manner. The drug reaches the target cells, thus increasing the effectiveness of the drug and reducing the toxicity associated with the drug, as the drug is released in the site and does not affect the organs, tissues and cells.[7] In our work, we used chitosan polymer as a carrier of the anticancer drug due to its interesting properties such as biocompatibility, biodegradation, easy bio absorbability, antibacterial and non-toxic.[8] The chitosan polymer also plays an important role in medicine and pharmacology, in particular, chitosan is known to have immune-enhancing effects, antitumor activities and increased protective effects against infections caused by some pathogens.[9,10]

MATERIALS AND METHODS

The chemicals were used all analytical grade.

Synthesis of Nano Chitosan Solutions[11]

About a 4% acetic acid aqueous solution, chitosan will be dissolved by sonication, and the solution will be transparent when completed. As chitosan solution is dissolved, deionized water will be added and a 0.22 mm filter used to produce a final stock solution of 1% (w/v, 1-mg/mL). In deionized water at a concentration of 0.5 mg/mL, TPP will be dissolved with deionized water.

*Author for Correspondence: mohammad.nadhum@uokerbala.edu.iq

Synthesis of Nano Co-polymer-drugs[12]

After dissolving 0.03 moles of polymer and drug in 30 mL THF with three drops of HCl, it was exposed to reflection for 24 hours. Finally, the precipitate was washed with diethyl ether and 2.0 M NaOH and left to dry for 16 hours.

Biological Activity[13,14]

Cell Cultures Maintenance

RPMI-1640 with 10% fetal bovine serum, 100 g/mL streptomycin, and 100 units/mL penicillin were used for AMJ13 cells. Passaging was performed twice a week with trypsin-EDTA, reseeding at 80% confluence twice a week, and incubating at 37°C.

Assays of Cytotoxicity

A 96-well plate was used for the MTT assay to measure the cytotoxicity of (X-SUBSTANCES). The seeding of cells per well was 1 x 104 cells. Cells were exposed to different concentrations of X-SUBSTANCES after 24 hours. when a monolayer was formed and After 48 hours of treatment, the cells were incubated for 2.5 hours at 37°C in 28 mL of 2 mg/mL MTT solution after removal of the medium. To solubilize the crystals in the wells, 130 L of DMSO (Dimethyl sulphoxide) was added after the MTT solution was removed, incubation take place at 37°C for time 15 minutes and jolting followed. Microplate readers were used to measuring the absorbency at 492 nm using triplicate tests. Using the equation below, the growth inhibition rate (cytotoxicity percentage) was determined.

$$\text{Rate of Inhibition} = A - B/A*100$$

According to this equation, the optical density of the control represented by A, while the optical density of the samples represented by B. 24-well micro-titration plates at a density of 1×10^5 cells for 1-mL. The cells were seeded in and incubated for 24 hours at 37°C to observe their form where inverted microscope was used. Cells were then exposed for 24 hours to X substances. After the cells were exposed to X substances for 24 hours, and Incubate the plates for 10–15 minutes at 37°C, where they stained with crystal violet stain. We gently removed the dye stain with tap water after it was thoroughly washed. Using an inverted microscope and a digital camera, images of the cells were captured under a magnifying glass at 100* magnification.

Statistical Analysis

Graph Pad Prism 6 was used to analyze the obtained data using the unpaired t-test. Values were presented as means + SDs of three measurements.

RESULTS AND DISCUSSION

Synthesis of Chitosan Solutions

Nano chitosan's FTIR spectrum, Figure 1, shows a weak broad absorption band at (3069.93 cm^{-1}) due to the bond (O-H) alcoholics together with the appearance of an absorption band 3004.93 cm^{-1} and an absorption band (2970.22 cm^{-1}) attributed to C-C-H amide and C-C-H aliph, The C=O ester has an absorption band at 1671.77 cm^{-1} and the C=C aromatic has an absorption band at (1402.47) cm^{-1} and C-N has an absorption band at 1277.68 cm^{-1}. This band appears as an absorption bond substation aromatic ring at 736.49 cm^{-1}.

Figure 2 shows ^{1}H-NMR spectrum of nano chitosan, single signal is disappeared at 13 ppm for (OH) acid and appearance at 5.70 for (C=C-H) amid and re-appearance at 7.58 for (C=C-H) aromatic and appearance at 9.52 ppm for (CHO), and re-appearance at 3 with ($C-H_3$) and re-appearance at 2.5 with (C=O) ester.

Atomic force microscopy (AFM) was used to quantify the size of the nano chitosan particles; Figure 3 A, B and C shows the nano chitosan exterior surface. The surface roughness of nano chitosan was calculated to be 0.827 nm and its square root square to be 0.955 nm. In addition, Figure 3 A, shows that the particles had an average height of 3.30 nm. Chitosan had a molecular size of 69.42 nm, according to the results. Figure 4, represent the distribution of nano chitosan particle sizes and Table 1, report on atomic force microscopy results.

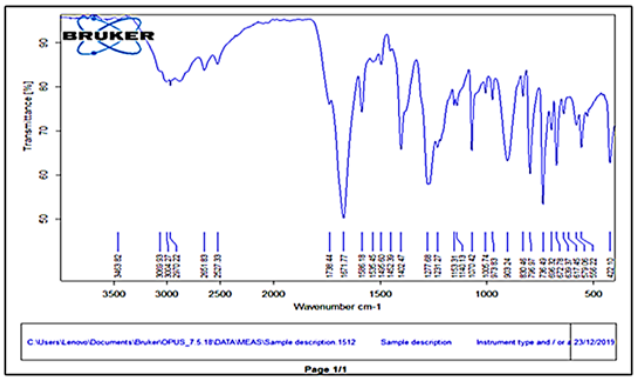

Figure 1: Infrared spectra of Nano Chitosan

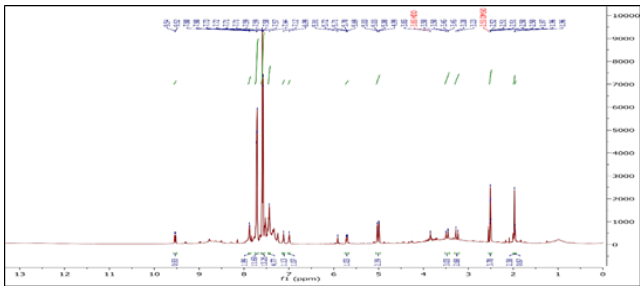

Figure 2: The ^{1}HNMR spectrum of Nano Chitosan

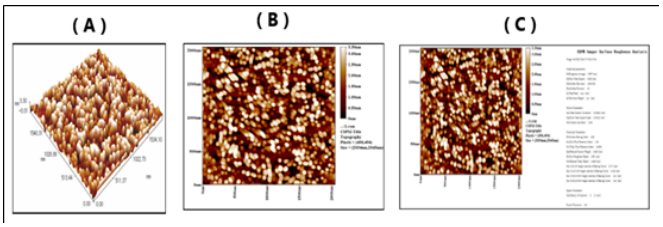

Figure 3: AFM Spectrometer (A) A photograph that has three dimensions, (B) A photograph that has two dimensions and (C) Two dimensions' photograph with all detail.

Table 1: Report on atomic force microscopy results.

Avg. Diameter: 69.42 Nm	<=Diameter 10%: 50.00 Nm
<=Diameter 50%: 65.00 Nm	<=Diameter 90%: 85.00 Nm

Diameter (nm)<	*Volume (%)*	*Cumulation (%)*	*Diameter (nm)<*	*Volume (%)*	*Cumulation (%)*	*Diameter (nm)<*	*Volume (%)*	*Cumulation (%)*
45.00	2.16	2.16	75.00	12.67	67.12	105.00	0.81	97.57
50.00	7.82	9.97	80.00	10.24	77.36	110.00	0.81	98.38
55.00	7.82	17.79	85.00	8.89	86.25	115.00	0.54	98.92
60.00	10.78	28.57	90.00	5.12	91.37	120.00	0.54	99.46
65.00	12.94	41.51	95.00	3.23	94.61	125.00	0.27	99.73
70.00	12.94	54.45	100.00	2.16	96.77	130.00	0.27	100.00

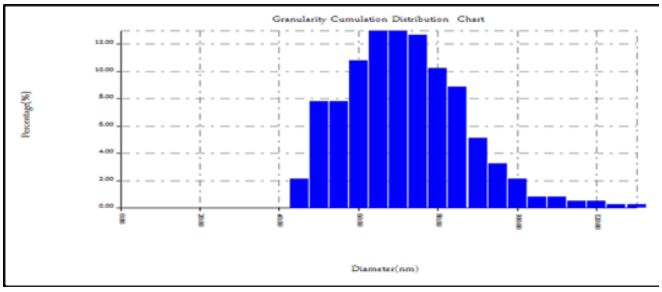

Figure 4: Distribution of Nano Chitosan particle sizes.

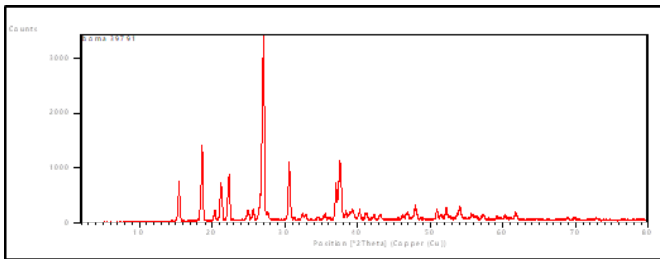

Figure 5: Chitosan nanoparticles x-ray diffraction

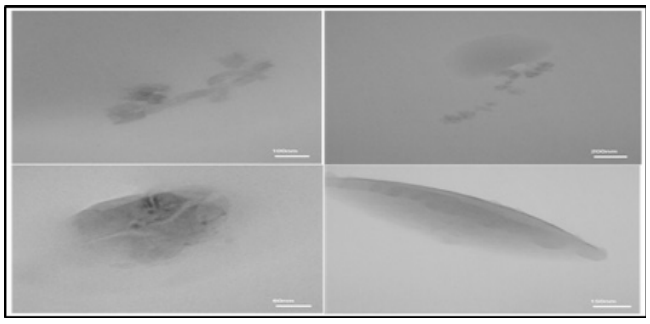

Figure 6: Micrographs of chitosan nanoparticles using TEM

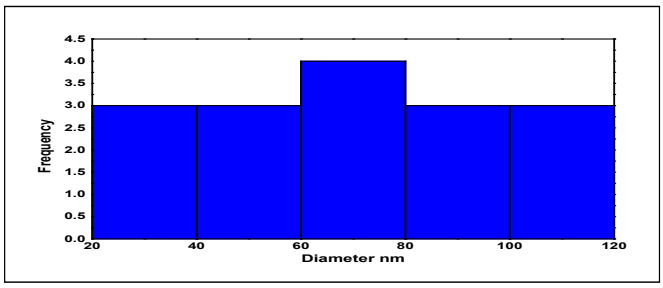

Figure 7: Histogram showing the distribution of nano chitosan particle sizes

The XRD pattern of microparticle chitosan in Figure 5 shows peaks at 2θ values of (15.4, 18.6, 22.3, 27.0, 30.5 and 37.0°). Based on Bragg's law, the average interplaner spacing between atoms (dhkl) was 0.385 nm using origin

$n\lambda = 2d\sin\theta$ ------- Bragg's law software

According to Scherrer's equation, the total average crystallite size was 69.04 n

$D = \frac{k\lambda}{\beta cos\theta}$ ---------- Scherrer's equation

Figure 6 represent the TEM micrographs for the nanoparticles chitosan, these images show the different shapes such as spherical shape with random arrangement, rods shape, annular disk shape, and semi-spherical shape on the basis of low and high resolution. In Figure 7 is shown the histogram for the distribution of the different proportions of particle sizes for the nano chitosan, and results show the distribution of diameters, angles, and standard deviations of the nano chitosan and their particles were found to have an average particle size of 69.81 nm.

Synthesis of Nano Chitosan Drug

The nano chitosan-cephalexin drug polymer was synthesized according to the following reaction Equation (1), and characterized by FTIR and ^{1}HNMR. Figure 8 FTIR (KBr, cm^{-1}): ν (3114, OH), (2989, aliphatic C-H), (3024, aromatic C-H), (1751, ester C=O), (1691, amide C=O), (3230, NH2), (1471, bend NH) (1600, C=C), (1440, bend CH2), (1377, bend CH3), (1234, C-O), Figure 9 ^{1}H NMR (600 MHz, (CD3)3SO) 8.72 (s, 1H of drug primary amine), 8.85 (s, 2H of the polymeric amine), 8.74 (s, 1H of drug amide), 7.75-7.16 (m, J = x Hz, aromatic hydrogen's), 5.72 (d, methylene group 4), 5.05 (s, hydrogen of atom 19), 5.00 (s, hydrogen of atom 25), 3.5 (s, 2H, hydrogen of atom 9), 1.43 (s, 1H, hydrogen of atom 20) (Table 2).

Biological Activity

It was found that the biological activity of cephalexin-linked chitosan nano polymer is highly effective in inhibiting the

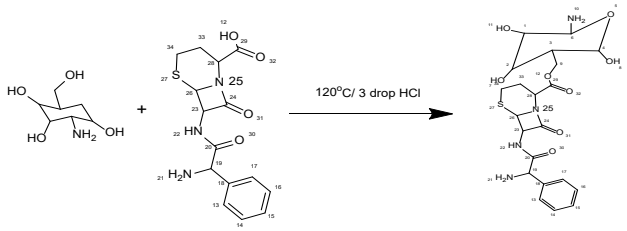

Figure 8: FTIR spectra of the nano chitosan-cephalexin drug

Table 2: Chitosan nanoparticles, crystallite sizes and atomic distances (d-spacing) within the nanoparticle

2 θ	*θ*	*FWHM*	*D nm*	*d_{hkl} nm*	*D (Av.) nm*	*d_{hkl} (Av.) nm*
15.4675	7.733765	0.1004039	79.849600	0.5724151	69.0494	0.3851
18.6201	9.31007	0.1136509	70.833825	0.4761484		
22.3068	11.15343	0.1176784	68.808052	0.3982175		
27.0317	13.51585	0.1302705	62.719995	0.3295902		
30.5960	15.29801	0.1189843	69.220243	0.2919576		
37.0535	18.52677	0.1332784	62.864653	0.2424250		

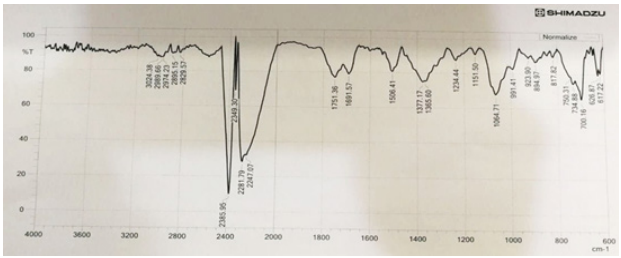

Figure 9: The ^{1}HNMR spectrum of co nano polymer- cephalexin

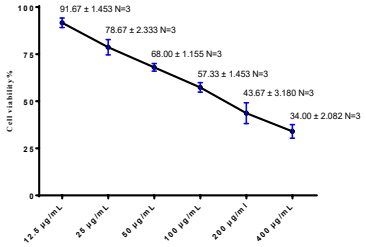

Figure 10: Cytotoxicity of Chitosan in AMJ13 cells IC50 =183.72 µg/mL.

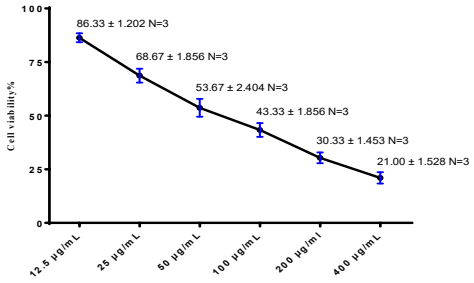

Figure 11: Cytotoxicity of Chitosan- Cephalexin in AMJ13 cells IC_{50} =117.03 µg/mL.

proliferation of breast cancer cells as shown in Figures 10 and 11. Where the effectiveness of the two compounds appeared as follows:

Nano Chitosan with drug > Nano Chitosan without drug

Increase biological efficacy

CONCLUSION

The FTIR conducted synthesis of chitosan nano polymer and evaluation of its properties, ^{1}HNMR, AFM, XRD and TEM. and ester bypass ligation with cephalexin and diagnosed by FTIR, and ^{1}HNMR techniques, where the biological activity of the drug bound polymer was studied by studying it in inhibiting breast cancer cells.

Nano Chitosan with drug > Nano Chitosan without drug

Increase biological efficacy

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